DETAILED STRUCTURAL ASPECTS OF THE HERPES SIMPLEX VIRUS GENOME

BY

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SUMMARY

This thesis describes the derivation of restriction endonuclease maps for HSV-1 and HSV-2 DNA; a study of the homologous regions between the genomes of HSV-1, HSV-2, EHV-1 and PRV, and the effect of homology upon recombination between HSV-1 and HSV-2; nucleotide sequences of the L-S joint regions in HSV-1 and HSV-2 DNA; and an analysis of two HSV-1/HSV-2 intertypic recombinants which fail to invert normally in one or both segments of the genome.

Restriction endonuclease maps were already available of HSV-1 DNA for XbaI, HindIII, EcoRI, BglII and HpaI, and of HSV-2 DNA for these enzymes and KpnI (Wilkie, 1976; Skare and Summers, 1977; Morse et al., 1977 ; Cortini and Wilkie, 1978). Maps of HSV-1 DNA for KpnI, BamHI, XhoI and PvuII, and of HSV-2 DNA for BamHI, were determined using the techniques of simultaneous digestion with two endonucleases, recleavage of isolated restriction fragments, and blot hybridisation. Previously reported size heterogeneity at the L terminus and L-S joint of the two genomes was confirmed. The results suggested that HSV-2 DNA is 1-2 x 10^6 larger in molecular weight in the S segment than HSV-1 DNA.

HSV-1 and HSV-2 DNA share approximately 50% homology, and PRV DNA possesses not greater than 8% homology with HSV-1 DNA (Kieff et al., 1972; Bronson et al., 1972; Rand and Ben-Porat, 1980). Hybridisation of ^{32}P-labelled recombinant plasmids containing HSV-1 or HSV-2 DNA inserts to blot strips of restriction endonuclease digests of HSV-1 or HSV-2 DNA showed that the two genomes are colinear, within the resolution attained in these experiments. Hybridisation of ^{32}P-labelled HSV-1 or HSV-2 DNA to similar blot strips allowed seven regions of the genome to be identified which

are more homologous than neighbouring regions. EHV-1 and PRV DNA also showed greater homology to these regions of the HSV genome than to others, but no significant homology between HSV DNA and HCMV DNA was detected. Homologous regions probably reflect greater conservation of the structures of polypeptides encoded by them. Five good candidates for conserved HSV-1 polypeptides are the major DNA-binding protein, the major capsid protein, the DNA polymerase, and two immediate-early polypeptides, V_{MW} IE 175 and V_{MW} IE 136'(143). Hybridisation of cloned HSV DNA fragments to blot strips of EHV-1 or PRV restriction endonuclease digests showed that homologous regions in the L segment of the EHV-1 genome are colinear with the L segment of the HSV-1 genome in the I_L arrangement. Homologous regions between HSV and PRV DNA were shown not to be arranged in a simple colinear fashion.

The genome structures were analysed of more than a hundred HSV-1/HSV-2 intertypic recombinants produced by marker rescue of HSV-1 tsD with HSV-2 restriction fragments spanning the L-S joint. The recombinants possessed crossovers preferentially in homologous regions. At least two of the genome arrangements (P and I_L) recombined, a result which disproves the earlier proposition that only one of these two arrangements is able to take part in the generation of viable recombinants.

HSV DNA is terminally redundant, possessing a directly repeated sequence of 250-500 base pairs (the a sequence) at the termini which is also present in inverse orientation at the L-S joint (Sheldrick and Berthelot, 1974; Grafstrom et al., 1974 and 1975; Wagner and Summers, 1978). Fine structure restriction endonuclease maps of the L-S joint region of HSV-1 and HSV-2 DNA were determined, and nucleotide sequences were derived using cloned DNA fragments. Relative to these

sequences the genome termini are in both cases located close to a short direct repeat of 17-21 base pairs at the b-a and a-c junctions. The HSV-1 L-S joint region contains three separate tandem direct reiterations of short sequences (12, 16 and 17 base pairs), and the size heterogeneity of virion DNA in the a and c sequences is almost certainly due to variable copy numbers of these repeated units. A second type of size heterogeneity was found to be due to insertion of an additional a sequence immediately adjacent to the a sequence at the L-S joint. The region coding for the 3' terminus of HSV-1 V_{mw} IE 175 mRNA was identified approximately 800 base pairs from the a sequence, but it is likely that the a sequence and immediately adjacent regions do not code for polypeptide.

The HSV-1/HSV-2 intertypic recombinant Bx1(28-1) possesses a majority of virion DNA molecules with the L segment in one orientation (fixed in L), whereas the S segment inverts normally (Preston et al., 1978). An analysis of Bx1(28-1) and twenty subclones thereof, some of which are fixed and some of which invert normally in L, showed that fixed genomes are totally heterologous for TR_L and IR_L, and that genomes which invert normally possess additional crossovers which generate regions of type-specific homology between TR_L and IR_L. Homology between a sequences is sufficient to allow normal inversion of L, suggesting that segment inversion occurs by a site-specific event in or near the a sequence. A less extensive analysis of RE4, a recombinant which is fixed in L and S, suggests that a similar requirement applies to the S segment. As predicted, fixed Bx1(28-1) subclones were able to give rise to normally inverting progeny, but the reverse conversion was not observed. The analysis was extended to

include a number of other recombinants which were initially thought to be heterologous for TR_L/IR_L or TR_S/IR_S, and yet inverted normally. Each of these was found to possess type-specific homology between repeat regions by virtue of previously undetected crossovers. Investigation of the immediate early polypeptides induced by Bxl(28-1) subclones, including two which lack part of IR_L or TR_L, showed that expression of only one of the two repeats is necessary for growth of HSV *in vitro*.

ABBREVIATIONS

Abbreviations for media and solutions are given in the Materials and those for the genome regions of HSV are given on page 7 and in Figure A1.1. All temperatures are given in degrees centigrade.

A	adenine-containing moiety
ACV	acyclovir
ATP	adenosine triphosphate
BHK	baby hamster kidney cells
BMV	bovine mammilitis virus
bp	base pairs
C	cytosine-containing moiety
CCV	channel catfish virus
cDNA	complementary DNA
CMV	(human) cytomegalovirus
c.p.e.	cytopathic effect
c.p.m.	counts per minute
dATP	deoxyadenosine triphosphate
dCTP	deoxycytidine triphosphate
dGTP	deoxyguanosine triphosphate
DMSO	dimethyl sulphoxide
DNase	deoxyribonuclease
dPyK	deoxypyrimidine kinase
dTTP	thymidine triphosphate
EBV	Epstein-Barr virus
EDTA	ethylenediamine tetra-acetic acid
EHV-1	equid herpesvirus 1
EHV-2	equid herpesvirus 2
G	guanine-containing moiety
GC content	moles per cent deoxyguanosine plus deoxycytidine moieties

HCMV	human cytomegalovirus
hr	hours
HSV	herpes simplex virus
HSV-1	herpes simplex virus type 1
HSV-2	herpes simplex virus type 2
HVA	herpesvirus ateles
HVP	herpesvirus papio
HVS	herpesvirus saimiri
HVT	herpesvirus of turkeys
IE	immediate early
kb	kilobases
kbp	kilobase pairs
LHV	Lucké herpesvirus
MCMV	murine cytomegalovirus
MCP	major capsid protein
MDBP	major DNA-binding protein
MDV	Marek's disease virus
min	minutes
m.o.i.	multiplicity of infection
mRNA	messenger RNA
m.wt.	molecular weight
n	nucleotides
N	unspecified nucleotide (A, C, G or T)
NP40	Nonidet P40
oz	ounces
PAA	phosphonoacetic acid
p.f.u.	plaque-forming units
p.i.	post infection
PRV	pseudorabies virus
Pu	purine
Py	pyrimidine
RNase	ribonuclease

r.p.m.	revolutions per minute
rRNA	ribosomal RNA
SCMV	simian cytomegalovirus
SDS	sodium dodecyl sulphate
SV40	simian virus 40
syn	syncytial
syn$^+$	non-syncytial
T	thymidine-containing moiety
T_m	melting temperature
tRNA	transfer RNA
ts	temperature-sensitive
U	units, or uracil moiety
UV	ultraviolet
vol/vol	volume/volume
VZV	varicella-zoster virus
wt	weight
wt/vol	weight/volume
X	unspecified nucleotide (A, C, G or T)

Arrangement of data

Tables and then Figures have been included after each Part or Section, and additional copies of key Figures have been included at the inside back cover.

PART A

INTRODUCTION

Knowledge of herpesviruses has expanded considerably during the past few years, and therefore the following pages stress herpesvirus genome structure and the expression of the HSV genome in the lytically infected cell. Necessarily, other aspects of equal interest and importance are dealt with only briefly.

THE HERPESVIRUSES

Classification and pathogenicity

Herpesviruses are characterised by virion morphology, genome size and site of virus replication. Particles with herpesvirus morphology have been observed in a wide variety of organisms: fungi (Kazama and Schornstein, 1972), fish (e.g. channel catfish virus, CCV; Wolf and Darlington, 1971), amphibia (e.g. Lucké herpesvirus, LHV; Stackpole, 1969), birds (e.g. Marek's disease virus, MDV; Churchill and Biggs, 1967), marsupials (e.g. Parma wallaby virus; Finnie et al., 1976) and a large number of mammals. Several herpesviruses have been isolated from non-human primates (e.g. herpesvirus saimiri, HVS, and herpesvirus ateles, HVA; Barahona et al., 1974; herpesvirus papio, HVP; Falk et al., 1976), and five from humans (herpes simplex virus types 1 and 2, HSV-1 and HSV-2; human cytomegalovirus, HCmV; Epstein-Barr virus, EBV; varicella-zoster virus, VZV).

The number of members of the family Herpesviridae has increased apace during recent years and with it the labour in assigning an international nomenclature (Roizman et al., 1978; Mathews, 1979). Three subfamilies have been suggested which are distinguished by host range, features of the replicative cycle, and, to some extent, genome organisation.

The alphaherpesvirinae possess a variable host range and a relatively short replicative cycle, and include HSV-1, HSV-2, equid herpesvirus 1 (EHV-1), suid herpesvirus 1 or pseudorabies virus (PRV), and bovid herpesvirus 2 or bovine mammalitis virus (BMV). A number of other cytomegaloviruses as well as HCMV constitute the betaherpesvirinae, which have a narrow host range and a relatively long reproductive cycle, and usually grow best in fibroblasts. EBV, HVP, HVS and HVA are counted among the gammaherpesvirinae, which have a narrow host range and replicate in lymphoblastoid cells. These viruses are specific for either B- or T-lymphocytes.

Biological aspects of the human herpesviruses have been studied in some detail. HSV-1 and HSV-2 are related genetically and antigenically, but form two distinct serotypes (Plummer, 1964; Pauls and Dowdle, 1967). HSV-1 is responsible for vesicular lesions of the lips and mouth and for ocular herpetic keratitis. More severe symptoms, and also generalised fatal infection, have been described. HSV-1 is maintained in a latent form in the trigeminal, and sometimes other, sympathetic ganglia (Baringer and Swoveland, 1973; Lonsdale et al., 1979). The virus may be reactivated periodically leading to recurrent symptoms. The mechanisms by which latency is established and maintained, and by which reactivation and recurrence take place, are not known. Virus latency is a feature of several, if not all, herpesviruses and their pathogenesis.

HSV-2 is sexually transmitted, lesions occurring in the genital region. There is an association between HSV-2 and cervical carcinoma in women (Naib et al., 1966; Rawls et al., 1968). Tumour tissue has been shown, in at least some cases, to express HSV-2 specific antigens and to contain

RNA complementary to HSV-2 DNA (Royston and Aurelian, 1970; Frenkel et al., 1972; Eglin et al., 1981). Both HSV-1 and HSV-2 cause morphological transformation of rodent cells in vitro, either as debilitated virus or as DNA fragments (Duff and Rapp, 1971; Darai and Munk, 1973; MacNab, 1974; Camacho and Spear, 1978; Reyes et al., 1979; Jariwalla et al., 1980; Galloway and McDougall, 1981; I. Cameron, personal communication). Tumours can be induced in experimental animals by inoculation with in vitro transformed cells, but not by infection with virus particles. The HSV-1 transforming region has been mapped at 0.31-0.42 fractional genome units, and that of HSV-2 at either 0.43-0.58 or 0.58-0.62, depending on the transformation assay employed. The nature of transformation by HSV at the molecular level remains obscure.

HCMV infection is usually mild, although it may be severe in neonates, and this virus has been associated with Kaposi's sarcoma (Giraldo et al., 1975). VZV causes chicken pox, usually in childhood, and, in adulthood, a painful local vesicular condition known as shingles. VZV has been reported to transform cells in vitro (Gelb et al., 1980). EBV is B-lymphocyte specific and causes infectious mononucleosis and is involved in the aetiology of two tumours: Burkitt's lymphoma, perhaps promoted by endemic malaria, and nasopharyngeal carcinoma, in association with a genetic predisposition (Old et al., 1966; Henle et al., 1968 and 1969).

PRV is a parasite of swine and is usually inapparent, but gives rise to the alarming symptoms of "mad itch" and then death in cattle. EHV-1 causes pregnant mares to abort. These two viruses have not yet been implicated in tumourigenesis in vivo, in contrast with several other non-human herpesviruses. MDV causes a highly contagious

neurolymphomatosis in chickens, now controlled by immunisation with the related herpesvirus of turkeys (HVT; Marek, 1907; Purchase et al., 1971). LHV produces a renal adenocarcinoma in frogs, the tumour and virus growth demonstrating opposed seasonal dependence (Lucké, 1934; Fawcett, 1956). Herpesvirus silvilagus causes a lymphoproliferative disease in cotton-tail rabbits (Hinze, 1971). The T-lymphocyte specific New World simian herpesviruses, HVS and HVA, invariably produce malignant lymphomas in certain heterologous primate hosts (Meléndez et al., 1969 and 1972).

Structure of the virion

The herpesvirion comprises four morphologically distinct structures: the core, capsid, tegument and envelope. The core contains the double-stranded DNA genome (Epstein, 1962a; Ben-Porat and Kaplan, 1962) toroidally arranged around a central proteinaceous matrix (Chai, 1971; Furlong et al., 1972; Nazerian, 1974; Heine and Cottler-Fox, 1975). Pleomorphic forms, perhaps representing different developmental stages, have also been observed (Nii and Yasuda, 1975; Okada et al., 1979). The icosahedral capsid which surrounds the core is approximately 100 nm in diameter, and consists of 162 capsomeres in 5:3:2 axial symmetry (Wildy et al., 1960). The capsomeres are hollow elongated prisms, and intercapsomeric fibrils have been observed (Wildy et al., 1960; Vernon et al., 1974). A fibrous layer known as the tegument surrounds the capsid (Roizman and Furlong, 1974), the width of which is determined at least in part by the virus (McCombs et al., 1971). The fragile virion envelope consists of a trilamellar membrane with spikes projecting from the outer surface (Wildy et al., 1960).

Herpesvirions contain 18-33 polypeptides of m.wt. 11,000-290,000 (Spear and Roizman, 1972; Heine et al., 1974; Perdue et al., 1974; Stevely, 1975; Strnad and Aurelian, 1976; Kim et al., 1976a and b; Dolyniuk et al., 1976; Dixon and Farber, 1980). About half of the structural proteins of HSV-1 have been assigned relative positions in the virion on the basis of experiments involving selective removal of polypeptides by detergent treatment (Roizman and Furlong, 1974), differential chemical treatment (Olshevsky and Becker, 1972; Roizman and Furlong, 1974) and virus neutralisation (Powell et al., 1974; Cohen et al., 1978).

The envelope contains most or all of the virus-coded structural glycoproteins (Spear and Roizman, 1972), cell lipids (Asher et al., 1969), an ATPase probably of cellular origin (Epstein and Holt, 1963), and spermidine (Gibson and Roizman, 1971). In contrast to other virion proteins, electrophoretic profiles of capsid proteins from various herpesviruses are similar. Cohen et al. (1980b) identified seven major capsid polypeptides in HSV-1, ranging in m.wt. from 12,000 to 155,000, and Zweig et al. (1979) showed that the major capsid protein (155,000) participates in disulphide bonding in the integral virion structure. Gibson and Roizman (1972 and 1974) presented evidence for the processing of a phosphorylated capsid protein to a less tightly bound form during virus maturation. A cyclic nucleotide-independent protein kinase is capable of phosphorylating about seven structural polypeptides, including a capsid protein (Rubenstein et al., 1972; Lemaster and Roizman, 1980). The significance in virus maturation and infectivity of these observations is not known. Polypeptides which have not been located in the capsid or envelope are thought to reside in the tegument. Only one protein has been suggested as a

structural component of the core, in addition to the polyamine, spermine (Gibson and Roizman, 1971).

Structure of the HSV genome

DNA extracted from HSV-1 virions is a linear duplex with a m.wt. of approximately 100 x 10^6 as determined by sedimentation, electron microscopic, and restriction endonuclease analyses (Becker et al., 1968; Kieff et al., 1971; Wilkie, 1973; Grafstrom et al., 1974; Wadsworth et al., 1975; Clements et al., 1976; Wilkie, 1976; Skare and Summers, 1977). It has a deoxyguanosine plus deoxycytidine (GC) content of 67% (Kieff et al., 1971) and, unlike cell DNA, does not have a scarcity of the nucleotide doublet CpG (Subak-Sharpe et al., 1966). Both single and double stranded DNA are infectious in cell monolayers (Lando and Ryhiner, 1969; Graham et al., 1973; Sheldrick et al., 1973; Wilkie et al., 1974; Farber, 1976; Stow and Wilkie, 1976).

The lability of HSV-1 DNA in alkali has been attributed to ribonucleotides covalently bound to the DNA, detected by the incorporation of radioactive uridine into mature DNA (Hirsch and Vonka, 1974; Muller et al., 1979a). This conclusion is in support of the work of Gordin et al. (1973) who reported that formamide denaturation resulted in intact single strands. On the other hand, Hyman et al. (1977) found that the majority of alkali-labile sites could be repaired by DNA polymerase and DNA ligase, and Ecker and Hyman (1981) claimed that single strands were fragmented even using gel electrophoresis systems which leave ribose moieties intact. These authors concluded that most or all alkali-sensitive sites are due to nicks or gaps in the single strands. This conclusion, however, depends on the assumption that the DNA was not damaged upon release from virions.

Frenkel and Roizman (1972a) concluded from renaturation studies that HSV-1 DNA contains uniquely situated nicks on only one strand of the duplex, rather like bacteriophage T5. Wilkie (1973) concluded from similar experiments that interruptions are located on both strands with equal distribution, and moreover showed by comparison with bacteriophage T5 DNA that they are randomly situated on both strands (Wilkie et al., 1974). The work of Ecker and Hyman (1981), with the caveat mentioned above, is in support of this view. Ben-Porat et al. (1979) reported that interruptions in PRV DNA are located at random in the single strands.

HSV-1 DNA is not circularly permuted (Hirsch et al., 1975). Renaturation of HSV-1 DNA which has been treated with processive exonucleases, such as 3'-exonuclease III or lambda 5'-exonuclease, results in the formation of double stranded circles (Sheldrick and Berthelot, 1974; Grafstrom et al., 1974 and 1975; Wadsworth et al., 1976; Hyman et al., 1976; Kudler and Hyman, 1979). This is interpreted as the presence of a direct repetition at the two genome termini, the size of which for various strains of HSV-1 has been estimated to be 400-7500 bp, although the more reliable estimates place it between 400 and 1600 bp.

Upon examination of annealed intact single strands of HSV-1 DNA in the electron microscope, Sheldrick and Berthelot (1974) found structures consistent with the following model. The genome consists of two covalently joined segments (L and S), each of which comprises a unique region (U_L and U_S) bounded by inverted repetitions (TR_L and IR_L, TR_S and IR_S), as summarised in Figure A1.1. They suggested that the two segments might invert by intramolecular recombination, thereby generating four distinct genome arrangements. Although these workers supposed that TR_L/IR_L and TR_S/IR_S

are identical, further electron microscopic, denaturation, and restriction endonuclease analyses showed that the two sets of repeats are different (Wadsworth et al., 1975; Hayward et al., 1975b; Delius and Clements, 1976; Clements et al., 1976; Wilkie and Cortini, 1976). These results also confirmed that virion DNA contains four equimolar genome arrangements resulting from the ability of L and S to invert relative to each other. The establishment of restriction endonuclease maps showed that half- and quarter-molar fragments map at the termini of L and S and their mutual joint, as predicted by the model and illustrated in Figure A1.1 (Wilkie, 1976; Wilkie et al., 1977a; Skare and Summers, 1977).

The terminal repetition of HSV-1 DNA, or a sequence, is also present in inverse orientation at the joint between L and S (Figure A1.1). Wagner and Summers (1978) obtained detailed restriction maps of this region, and estimated the size of the a sequence in HSV-1 strain KOS to be 265 bp. Two types of size heterogeneity are associated with the termini and joint region. The first corresponds to insertions of approximately 300 bp, thought to contain the a sequence, at the L terminus and joint (Wilkie, 1976; Wagner and Summers, 1978; Locker and Frenkel, 1979b). This has also been observed for the HSV-2 genome (Wilkie et al., 1977a). The second type of heterogeneity consists of variable insertions of 10-50 bp within the a sequence and the adjacent c (TR_S/IR_S) sequence (Wagner and Summers, 1978).

Renaturation of exonuclease-treated HSV-1 DNA results, in some instances, in a small loop at one terminus, interpreted as the presence of part of the whole of the a sequence in inverse orientation a few hundred bp away from the terminus (Wadsworth et al., 1976; Hyman et al., 1976). Wilkie and

Cortini (1976) suggested from experiments involving end-labelling of HSV-1 DNA that this structure is present at the L terminus. Miller et al. (1980) have observed a wide variety of foldback and stem-loop structures in reannealed single strands, some of which correspond to recognised repeats within the genome, and others of which are of unknown significance.

Kudler and Hyman (1979) observed that partially deproteinised DNA is more resistant to 3'-exonuclease than to 5'-exonuclease, and suggested that this was due to a protein bound at the 3' termini. Indeed, four specific proteins remain non-covalently associated with HSV-1 DNA following detergent treatment (Hyman, 1980). Wu et al. (1979) attached dinitrophenyl (DNP) groups to the bound proteins and visualised them in the electron microscope by treatment with an anti-DNP immunoglobulin. They found preferential binding close to the two termini and at two positions corresponding to the a sequence at the joint, and proposed that a protein specifically interacts with the a sequence. The significance of this observation has yet to be elucidated.

HSV-2 DNA has a 2% higher GC content than HSV-1 DNA (Goodheart et al., 1968; Kieff et al., 1971; Halliburton, 1972). Approximately 50% of HSV-1 and HSV-2 DNA sequences are homologous with 65-85% matching of nucleotides (Kieff et al., 1972; Frenkel et al., 1973; Sugino and Kingsbury, 1976). The genomes of the two serotypes are structurally very similar, but possess distinctive arrangements of restriction sites (Hayward et al., 1975a and b; Skare et. al., 1975; Cortini and Wilkie, 1978). These differences have been exploited as a diagnostic tool for identification of HSV-1 and HSV-2 isolates and for studying epidemiological aspects of overt and latent infections in man (Linneman et al., 1978;

Buchman et al., 1978 and 1980; Lonsdale et al., 1979 and 1980; Lonsdale, 1979). Restriction fragments representing the majority or entirety of the HSV-1 and HSV-2 genomes have recently been cloned into bacterial plasmids, both in Glasgow and elsewhere (Post et al., 1980; Galloway and Swain, 1980; Goldin et al., 1981).

Structures of other herpesvirus genomes

Although a number of genome structures have been demonstrated among the herpesviruses, in each case a linear duplex, these may be represented in circular form in order to show the four basic organisations found among those so far analysed (Figure A1.2). GC contents of herpesvirus DNAs are given in Figure A1.3.

Two subgroups are included in group I because, although they are distinct, it is possible that they reflect a basic similarity. The first is common to HSV-1, HSV-2, BMV and HCMV. BMV DNA possesses 14% homology with HSV-1 DNA (Sterz et al., 1974) and has a m.wt. of approximately 90×10^6, possessing smaller inverted repeats than HSV-1 DNA (Buchman and Roizman, 1978a). The BMV genome has a terminal repetition of approximately 1000 bp (Buchman and Roizman, 1978b). The genome of HCMV has a m.wt. of 150×10^6 (Demarchi et al., 1978; Geelen et al., 1978; Stinski et al., 1979; Weststrate et al., 1980), although it was earlier reported that HCMV DNA has a m.wt. of 100×10^6 (Sarov and Friedmann, 1976; Kilpatrick et al., 1976; Huang et al., 1973). Kilpatrick and Huang (1977) detected both size classes, and Lakeman and Osborn (1979) a further class of 130×10^6. It is clear that the size class of 150×10^6 represents the full length genome, both from determinations of its infectivity and from restriction maps (Geelen et al., 1978; Lakeman and Osborn, 1979; Weststrate et al., 1980). The smaller sizes of DNA

molecules are thought to result from defective virions. The HCMV genome is structurally similar to that of HSV-1 (Weststrate et al., 1980). U_L and U_S are larger than in HSV-1, and the region of least homology between two closely related strains is in TR_S/IR_S (comparison of the results of Pritchett, 1980 with the restriction maps of Weststrate et al., 1980). Jean et al. (1978) have postulated from indirect evidence that HCMV DNA is terminally redundant. Restriction profiles of simian CMV (SCMV) isolates differ significantly from those of HCMV (Kilpatrick et al., 1976), and there has been at least one case of a cytomegalovirus isolated from a human patient who died from clinical encephalopathy which bore almost total nucleic acid homology with SCMV strains but none with HCMV (Huang et al., 1978). Mosmann and Hudson (1973) reported that the DNA of murine CMV (MCMV) has a m.wt. of 132×10^6. Taking into account their underestimate of the size of the HSV-1 genome, this is equivalent to 150×10^6. However, genome structures of MCMV, SCMV and the cytomegalovirus EHV-2 (Wharton et al., 1981) have not been published. Nevertheless, it is clear that the HSV type of genome organisation is found in representatives of both alpha- and beta-herpesvirinae.

The second subgroup of group I contains the PRV and EHV-1 genomes, which are similar in size to that of HSV-1 but lack inverted repeats flanking the L segment, and in which only S inverts (Stevely, 1977; Powell, 1979; Ben-Porat et al., 1979; Soehner et al., 1965; Whalley et al., in press). There is no direct evidence that either PRV or EHV-1 DNA is terminally redundant, as it has not proved possible to circularise either after exonuclease III treatment (Ben-Porat et al., 1979; P. Sheldrick, personal communication).

Group II contains EBV and its simian counterpart, HVP.

The EBV genome has a m.wt. of approximately 110 x 10^6 (Pritchett et al., 1975; Given and Kieff, 1978 and 1979). The termini are redundant and of variable size, containing up to 12 direct repeats of a 500 bp sequence (Given and Kieff, 1978; Given et al., 1979; Kintner and Sugden, 1979), and, as with HSV-1, the linear genome can be circularised in vitro by annealing following exonuclease treatment (Kintner and Sugden, 1979). Circular DNA produced by representatives of group I has so far been detected only in this fashion or in replicating DNA during lytic infection, but EBV-transformed cells have been shown to contain EBV genomes as episomal covalently closed circles (Lindahl et al., 1976). The two unique segments of the linear genome do not invert (Given and Kieff, 1979; Hayward et al., 1980) and are separated by approximately 10 direct repeats of 3 kbp (IR), which are GC-rich and not homologous to the terminal repeats (Sugden, 1977; Rymo and Forsblum, 1978; Given and Kieff, 1979; Hayward et al., 1980). Given and Kieff (1979) detected internal homology within IR, and concluded that the 3 kbp sequence itself contains a repeated unit. They also detected homology between IR and two locations in L. The various strains of EBV differ in that some lack sequences possessed by others (Pritchett et al., 1975; Sugden et al., 1976; Given and Kieff, 1978; Rymo and Forsblum, 1978; Raab-Traub et al, 1978 and 1980; Hayward et al., 1980; Bornkamm et al., 1980; Heller et al., 198la). Some strains are unable to transform cells, but it remains to be shown precisely which regions of the genome are essential for transformation.

HVP exhibits approximately 40% DNA homology with EBV, as do the gammaherpesvirinae of other Old World primates such as the orangutan (herpesvirus pongo), the chimpanzee (herpesvirus pan) and the gorilla (herpesvirus gorilla)

(Gerber et al., 1976; Falk et al., 1976; Rabin et al., 1978; Neubauer et al., 1979; Lee et al., 1980). Restriction mapping of HVP DNA and hybridisation, using in one case cloned EBV DNA fragments, have shown that the two genomes are structurally identical and colinear (Lee et al., 1981; Heller et al., 1981b; Heller and Kieff, 1981).

The genomes of HVS and HVA, which are in group III, both consist of a single unique region of 35% GC bounded on both sides by multiple direct repeats of a sequence of approximately 1500 bp containing 70% GC (Fleckenstein and Wolf, 1974; Fleckenstein et al., 1975 and 1978; Bornkamm et al., 1976). As with EBV, HVS-transformed cells contain episomal closed circular HVS genomes, although such molecules have at least in one case been shown to comprise rearrangements of the HVS genome (Werner et al., 1977). In the linear genome, the terminal repeats are approximately constant in number, accounting for 30% of the genome size, but can be distributed asymmetrically at the two termini. The HVS and HVA genomes have a m.wt. of 90 x 10^6, and defective genomes composed entirely of reiterations of the terminal repeat have been characterised.

CCV is the only identified member of group IV. Chousterman et al. (1979) presented restriction maps of CCV DNA, showing that the genome consists of a unique region of 62 x 10^6 in m.wt., bounded on each side by a direct repeat of 12 x 10^6. No minor bands were detected and, unlike HVS and HVA, the termini were not highly reiterated.

The DNA of two other herpesviruses has to some extent been characterised, but determination of their genome structures has been hampered by difficulties in clonally

purifying these viruses from possible defective virus. VZV DNA has a m.wt. of 80-100 x 10^6 and restriction profiles of several clinical isolates are very similar (Oakes et al., 1972; Iltis et al., 1977; Rapp et al., 1977; Richards et al., 1979; Dumas et al., 1980). Rapp et al. (1977) failed to detect repeated sequences by kinetic hybridisation, but submolar restriction fragments have been detected. MDV DNA has a m.wt. similar to that of HSV-1, and submolar restriction fragments have been detected (Lee et al., 1971; Bachenheimer et al., 1972; Hirai et al., 1979).

THE LYTIC CYCLE OF HERPES SIMPLEX VIRUS

Interaction of virus with the cell

After entry of HSV into the permissive cell, the DNA enters the nucleus, where it is transcribed and the mRNA transported to the cytoplasm. Most synthesised polypeptides are transported into the nucleus where DNA replication and virus assembly take place. In the course of infection, HSV inhibits host cell macromolecular synthesis. Virus may spread by release of infectious particles from the cell or by cell fusion.

Herpes simplex virions will adsorb to the cell surface at 4°, but penetration requires a higher temperature (Farnham and Newton, 1959; Holmes and Watson, 1963; Huang and Wagner, 1964). The route by which virions enter the cell has raised considerable furore. It was concluded from several earlier electron microscopic studies that the virus is taken up by pinocytosis into vacuoles (Holmes and Watson, 1963; Hummeler et al., 1969; Dales and Silverberg, 1969). On the other hand, Morgan et al. (1968) insisted that virus entry occurs by direct fusion with the cell membrane.

Subsequent work is consistent with the presence of specific virus receptors on the cell surface (Hochberg and Becker, 1968; Blomberg, 1979; Vahlne et al., 1979). Moreover, evidence that virion glycoproteins function in adsorption and penetration makes direct fusion the more tenable hypothesis at present (Cohen et al., 1978; Sarmiento et al., 1979). The virion envelope is most probably required for infectivity (Smith, 1964; Spring and Roizman, 1968; Stein et al., 1970; Rubenstein et al., 1972). After entry the virion structure is degraded in the cytoplasm and the DNA transported rapidly by an unknown mechanism into the nucleus (Hochberg and Becker, 1968; Hummeler et al., 1969). Knipe et al., (1981) have recently described a ts mutant which complements other ts mutants poorly, and which expresses no polypeptides at the nonpermissive temperature. The lesion may map in a gene coding at late times in infection for a structural protein, and the authors propose that the defect is in uncoating the virus.

Infection of cells in tissue culture with HSV results in cessation of mitosis (Stoker and Newton, 1959; Wildy et al., 1961), and initiation of the infectious cycle is independent of the cellular phase of DNA synthesis (Cohen et al., 1971). Soon after infection, HSV causes an inhibition of cell DNA synthesis which is dependent on protein synthesis (Roizman and Roane, 1964). The fact that some HSV-2 ts mutants either fail to inhibit, or stimulate, host cell DNA synthesis at the nonpermissive temperature suggests that virus functions are involved in inhibition of host DNA synthesis (Halliburton and Timbury, 1973; Yamanishi et al., 1975). It is not known whether the inhibition is a result of inhibition of cell RNA and protein synthesis (Roizman et al., 1965), rather than a direct effect on DNA synthesis.

Inhibition of protein synthesis involves disaggregation of host polyribosomes and their reassembly (Sydiskis and Roizman, 1966). The total protein synthesising capacity decreases throughout infection, a fact which Silverstein and Engelhardt (1979) interpreted as the accumulation of non-functional polyribosomes. Fenwick and Walker (1978) have shown that UV irradiation of HSV-2 does not affect inhibition of host cell macromolecular synthesis, and concluded that inhibition is due to a structural component of the virus. The same authors (1979) have noted the specific phosphorylation of a host ribosomal protein upon infection, but concluded that this is not the cause of inhibition of host protein synthesis. The HSV-2 function responsible for inhibition has been mapped at 0.56-0.57 fractional genome units (Fenwick et al., 1979; H. S. Marsden, personal communication).

HSV-1 infection leads to the expression of virus-specified RNA, and cell RNA synthesis is rapidly suppressed (Hay et al., 1966; Flanagan, 1967). The particularly rapid suppression of rRNA synthesis may be attributed to two inhibitory steps (Wagner and Roizman, 1969b). Firstly, there is a decrease in the rate of synthesis of 45 S precursor RNA, perhaps mediated by a virus-induced inhibitor of RNA polymerase I (Sasaki et al., 1974). Secondly, processing of precursor rRNA into mature 18 S and 28 S rRNA is inhibited. Talley-Brown and Millette (1979) have noted changes in the patterns of small cytoplasmic RNA synthesis early in infection of Xeroderma pigmentosum cells, including the appearance of a novel 380 nucleotide species.

The activity of RNA polymerase II declines on infection, a feature which Preston and Newton (1976) attributed not only to inhibition of cell protein synthesis but also to a virus-induced polypeptide which may interact either with the

enzyme or with the chromatin complex. Using a cell line which expresses a high level of globin mRNA, Nishioka and Silverstein (1977 and 1978) showed that HSV-1 infection causes degradation of pre-existing cell mRNA. Unlike dissociation of polyribosomes, this is dependent upon genome expression. Nevertheless, a low level of cell mRNA is synthesised even late in infection (Stringer et al., 1977). Inglis and Newton (1981) reported that HSV-1 mRNA is translated in vitro with a lower efficiency than cell mRNA, and suggested that, if this applies in vivo, inhibition of host cell RNA synthesis would be instrumental in efficient virus growth.

Cell chromosomal breakages which occur early in HSV infection are thought to be due to a virus-induced enzyme, since virus DNA is not physically associated with the breakages (Waubke et al., 1968). Although chromosomal breakage seems not to be caused by integration of virus DNA into cell DNA, it has been reported that about 200 HSV genome equivalents per cell are covalently associated with host DNA late in infection (Biegeleisen and Rush, 1976; Biegeleisen et al., 1977; Yanagi et al., 1979). The significance of these observations in the infectious cycle remains to be seen.

The pathway by which virus is assembled in the nucleus is poorly understood. Electron microscopic studies of normally infected cells show condensation and margination of host chromatin followed by the appearance of capsids of no, low or high electron-density, as well as more bizarre granular, crystalline, filamentous and tubular structures (Morgan et al., 1959; Nii et al., 1968a; Miyamoto and Morgan, 1971; Smith and de Harven, 1973). Other potentially useful attempts to unravel what is undoubtedly a complex process have included the use of

metabolic inhibitors (Nii et al., 1968b; Friedmann et al., 1975; Marciano-Cabral et al., 1977), and studies of HSV-2 ts mutants (Cabral and Schaffer, 1976; Atkinson et al., 1978). Unlike HSV-1, HSV-2 produces tubular structures in the nuclei of infected cells (Murphy et al., 1967). This late event seems not to require entire genome expression, and kinetic studies using inhibitors suggest that such structures are intermediates in virus assembly rather than results of aberrant capsid formation (Iwasaka et al., 1979; Oda et al., 1979). Hexagonal and pentagonal intranuclear crystals of capsids have frequently been observed (Morgan et al., 1958; Melnick et al., 1968). It is not known how DNA is encapsidated into virions, but Pignatti and Cassai (1980) isolated nucleoprotein complexes which included non-nucleosomal DNA with capsid-like structures at one terminus. It is not clear, however, whether these represent true intermediates in the encapsidation process, or breakdown products.

HSV infection is accompanied by extensive reduplication of the nuclear membrane, at which capsids acquire envelopes as they pass into the perinuclear cisterna (Falke et al., 1959; Epstein, 1962b; Darlington and Moss, 1968; Nii, 1971). Atkinson et al. (1978) reported that envelopment may occur by de novo synthesis of membrane in the nucleus. Envelopment at cytoplasmic membranes may occur following disintegration of the nucleus. Mature virus particles leave the cell by reverse phagocytosis (Darlington and Moss, 1968), or via cytoplasmic ducts (Schwartz and Roizman, 1969), or by both mechanisms (Nii, 1971). Observations of virus egress are dependent on cell type (Schlehofer et al., 1979).

Productive infection kills the host cell, and cellular components leak into the medium (Wagner and Roizman, 1969b; McCormick, 1978). Virus spreads from cell to cell via the

medium, and also by direct intercellular passage (Stoker, 1958). Wild-type virus usually causes rounding up of infected cells, but some mutant strains (syn) cause extensive cell fusion and the formation of syncytia (Gray et al., 1958; Hoggan and Roizman, 1959). The recruitment of cells into syncytia seems largely dependent upon the recruiting rather than the recruited cell (Lee and Spear, 1980; Bzik and Person, 1981). Cell fusion is dependent on virus expression, since several inhibitors or UV irradiation abolish syncytia formation (O'Donovan and Roizman, 1961; Kousoulas et al., 1978). Inhibition by 2-deoxy-D-glucose or tunicamycin strongly indicates that virus glycoproteins are involved in cell fusion (Gallaher et al., 1973; Keller, 1976; Knowles and Person, 1976; Pizer et al., 1980). Indeed, using recombinants between a virus defective in glycoprotein C (gC) and one with a ts lesion in glycoprotein B (gB), Manservigi et al., (1977) concluded that gB is a fusion promoter and gC a fusion suppressor. However, multiple viral and cellular factors are involved in cell fusion (Yamamoto et al., 1975; Yamamoto and Kabuta, 1977; Ruyechan et al., 1979; Lee and Spear, 1980).

DNA synthesis

Much of the more conclusive work on herpesvirus DNA synthesis has been done with PRV, so the following discussion includes both HSV and PRV.

Rice et al. (1976) reported that, soon after infection, input DNA becomes specifically associated with a cell nuclear protein. In contrast, they did not find newly synthesised DNA in such a complex, and DNA sythesis does not involve the normal cell nucleosomal apparatus (Mouttet et al., 1979; Leinbach and Summers, 1980). Jacob and Roizman (1977) reported that only a small fraction of parental DNA enters

the replicative pool, whereas the majority of parental and progeny PRV DNA can replicate (Jean and Ben-Porat, 1976; Ben-Porat et al., 1976a). Density-shift experiments have shown that HSV and PRV DNA are replicated semiconservatively (Kaplan and Ben-Porat, 1964; Kolber, 1975; Khan et al., 1978). Most single strand breakages in parental DNA are repaired prior to DNA replication (Ben-Porat et al., 1976a), but nascent DNA contains single stranded regions and covalently linked RNA (Biswal et al., 1974; Murray and Biswal, 1974; Hirsch et al., 1976; Shlomai et al., 1976; Muller et al., 1979a). Pulse-chase experiments indicate that newly synthesised DNA sediments as large heterogeneous molecules, and is rapidly processed to a form sedimenting with the properties of unit-length molecules (Hirsch et al., 1976; Shlomai et al., 1976; Jacob and Roizman, 1977; Ben-Porat and Tokazewski, 1977). A single round of PRV DNA replication takes 25 or 45 min, depending on whether replication is uni- or bi-directional (Ben-Porat et al., 1977). Most systems for in vitro DNA synthesis allow completion of nascent DNA but not reinitiation (Bell, 1974; Biswal and Murray, 1974; Shlomai and Becker, 1975; Bolden et al., 1975; Kolber, 1975; Becker and Asher, 1975; Shlomai et al., 1977; Jongeneel and Bachenheimer, 1980). Promising attempts have been made to extract the physiological replication complex from infected cell nuclei (Pignatti et al., 1979; Knopf, 1979).

Temperature-sensitive mutants in about half of the identified complementation groups of HSV and PRV are deficient in DNA synthesis to a greater or lesser extent, and therefore it is likely that many gene products are involved in this process, including the virus-coded DNA polymerase (Halliburton and Timbury, 1973 and 1976; Pringle et al., 1973; Schaffer et al., 1973; Esparza et al., 1974; Benyesh-Melnick et al., 1974;

Purifoy and Benyesh-Melnick, 1975; Aron et al., 1975; Courtney et al., 1976; Bone et al., 1978).

Electron microscopic examination of replicating DNA has revealed linear molecules smaller than, equal in size to, or larger than virion DNA, circles of unit size or smaller, and large tangled molecules. Such molecules may contain single stranded regions, forks, eyes, loops or collapsed regions (Shlomai et al., 1976; Jean and Ben-Porat, 1976; Ben-Porat et al., 1976b; Rixon, 1977; Jean et al., 1977; Hirsch et al., 1977; Friedmann et al., 1977; Jacob and Roizman, 1977). The following replication model has been derived from these and other data. Observations that HSV-1 DNA is terminally redundant, that terminal restriction fragments of HSV-1 DNA are reduced in abundance in infected cell nuclei (Jacob et al., 1979), that a proportion of input PRV DNA acquires single stranded ends (Jean and Ben-Porat, 1976) and a proportion becomes circular, and that PRV DNA in the first round of replication is circular (Ben-Porat and Veach, 1980), are in support of the hypothesis that linear input DNA circularises after infection, probably involving the action of an exonuclease. Head-to-tail concatemers are then formed by an unknown mechanism, but which may be a rolling circle (Jean and Ben-Porat, 1976; Jean et al., 1977; Ben-Porat and Tokazewski, 1977; Ben-Porat and Rixon, 1979; Jacob et al., 1979). The question of how unit-length DNA molecules result from concatemers, with four equimolar arrangements of L and S, is considered in the Discussion. Models for DNA synthesis involving independent replication of L and S (Skare and Summers, 1977) or replication of linear molecules from a terminal hairpin loop (Rixon, 1977; Jacob and Roizman, 1977) failed to find subsequent support. Ben-Porat and Kaplan (1963) showed

that although a minority of mature PRV DNA is encapsidated, it is selected at random from the pool. Similarly a minority of HSV-1 DNA is encapsidated (Russell et al., 1964), and Frenkel and Roizman (1972a) concluded that molecules with fewer nicks are preferentially packaged, although it is equally likely that nicks are repaired during packaging.

Two approaches have indicated that HSV-1 and PRV DNA contain an origin of replication in the region of the S terminus, and perhaps another in U_L. Shlomai et al. (1976) and Friedmann et al. (1977) described two origins of replication from electron microscopic studies of replicating HSV-1 DNA, one corresponding to TR_S and IR_S, and the other to a position 40% from one terminus. Hirsch et al. (1977) detected greater incorporation of isotope in a pulsed label in restriction fragments containing the joint region. Jean et al. (1977) obtained results similar to those described above from their electron microscopic analysis of replicating PRV DNA, although 10% of molecules with replication eyes apparently exhibited multiple replication sites. Ben-Porat and Veach (1980) concluded from blot hybridisation of PRV DNA thought to have been isotopically labelled during the first round of replication that the main origin of replication is at or near the S terminus, from which DNA synthesis proceeds unidirectionally. They suggested that the other origin 40% from one terminus is used relatively infrequently during the first round of replication.

As a second approach, structural analysis of defective virus genomes has provided some evidence for the locations of origins of DNA replication. High multiplicity passage of HSV or PRV results in a cyclic pattern of particle to infectivity ratio and often the appearance of DNA of novel

buoyant density (Bronson et al., 1973; Wagner et al., 1974; Ben-Porat et al., 1974; Murray et al., 1975; Stegman et al., 1978). DNA from the defective interfering particles is approximately the same size as, but has a lower kinetic complexity than, standard DNA (Wagner et al., 1974; Bookout et al., 1979). Partial denaturation mapping has shown that defective DNA consists of multiple head-to-tail reiterations of a basic unit (Rubenstein and Kaplan, 1975; Frenkel et al., 1975).

Two types of repeat unit have been described from restriction endonuclease analysis of HSV-1 defective DNA (Kaerner et al., 1979; Frenkel et al., 1980). That of class I contains approximately 8.5 kbp of DNA comprising TR_S and an adjacent portion of U_S. (Frenkel et al., 1975, 1976 and 1980; Locker and Frenkel, 1978 and 1979a; Graham et al., 1978; Vlazny and Frenkel, 1981). Whereas one end of the defective genome is unique and probably possesses an a sequence, the other terminus is thought to be random in nature. Such a structure suggests that maturation and packaging of DNA occur by a specific recognition of the S terminus and an approximate length measurement. This is unlikely to be the case for maturation of standard HSV-1 DNA because both termini are unique. It is not yet clear whether the a sequence is present in each repeat unit of class I defective genomes. Size heterogeneity of the repeat unit in different defective genomes of this class is due to variation in the length of U_S sequences included therein. The kinetics of defective DNA synthesis, its sensitivity to the virus DNA polymerase inhibitor phosphonoacetic acid (PAA), and the fact that coinfection with separated repeat units generates

full length defective genomes, argue that defective DNA contains the HSV-1 replication origin which is functional in the replication of defective DNA (Frenkel et al., 1980; Vlazny and Frenkel, 1981). The observation that single defective DNA molecules contain only one size of repeat unit makes it tenable that they are synthesised by a rolling circle mechanism from a circular repeat unit. In support of this hypothesis, Becker et al. (1978) observed circular and circular-linear molecules of various sizes by electron microscopy of replicating defective DNA.

The repeat unit of class II defective DNA consists of the S terminal region linked to a region of U_L mapping at 0.34-0.40 fractional genome units. The greater sensitivity of this class than class I to PAA supports the presence of a separate replication origin in U_L. Indeed, it has been noted that attempts to clone into plasmids fragments from this region of U_L have consistently led to the deletion of about 100 bp at a specific location, perhaps indicating the presence of an inverted repeat in virus DNA which proves unstable in plasmids (Post et al., 1980; B. Matz, personal communication).

RNA synthesis

HSV-1 RNA synthesis is as sensitive as cell RNA synthesis to inhibition by α-amanitin, an inhibitor of RNA polymerase II (Alwine et al., 1974; Ben-Zeev et al., 1976), and all stages of viral transcription are equally sensitive (Costanzo et al., 1977). Moreover, HSV-1 RNA synthesis is resistant to the drug in cells possessing an α-amanitin resistant RNA polymerase II (Costanzo et al., 1977; Ben-Zeev and Becker, 1977). Lowe (1978) was unable to detect RNA polymerase activities in the infected cell other than those normally

present in the uninfected cell. These observations strongly indicate that cell RNA polymerase II is responsible for HSV-1 RNA synthesis at all stages of infection, although modification of the transcriptional specificity of this enzyme during infection is not ruled out.

HSV-1 mRNAs are polyadenylated after transcription (Bachenheimer and Roizman, 1972). It has been reported that a significant proportion of mRNA is not polyadenylated (Silverstein et al., 1973; Stringer et al., 1977), but Silverstein et al. (1976) showed that most or all virus mRNAs are polyadenylated, in general possessing 30, 50 or 155 adenylic acid residues at the 3' terminus. The compartmentalisation of shorter polyadenylic acid tracts in the nucleus, and the longer in the cytoplasm, was interpreted by the latter authors as two separate processing steps in polyadenylation. HSV-1 mRNAs also possess a 7-methylguanosine 5'-triphosphate cap and internally methylated nucleotides (Bartkoski and Roizman, 1976; Moss et al., 1977). Jacquemont and Huppert (1977) showed that HSV-1 replication is reversibly blocked by 5'-S-isobutyladenosine, an inhibitor of methylation, especially of the cap. Assuming that this is the significant inhibitory action of the drug, this result suggests that methylation is essential for translation of mRNA. Cessation of methylation of internal nucleotides at late times may be due to expression of an early gene (Bartkoski and Roizman, 1978).

HSV transcription is a complex process under tight temporal control. Early work using liquid hybridisation showed that most of the HSV-1 genome is expressed at early (before the onset of DNA synthesis) and late times (Frenkel and Roizman, 1972b; Wagner, 1972; Frenkel et al., 1973; Swanstrom and Wagner, 1974; Stringer et al., 1977). Early mRNA

comprises a subset of late mRNA, and each group consists of scarce and abundant transcripts. Wagner et al. (1972) proposed a very early class which forms a subset of early species. The evidence for three major classes of transcripts was supported by experiments involving the use of metabolic inhibitors. Initially it was reported that mRNA made in the absence of de novo protein synthesis was similar to that made at early times (Frenkel et al., 1973; Swanstrom et al., 1975), but it soon became clear that immediate early (IE) mRNA produced in the presence of cycloheximide forms a more restricted class (Kozak and Roizman, 1974; Roizman et al., 1974). Inhibition of DNA synthesis causes the production of mRNA very similar to that made at early times (Wagner et al., 1972; Murray et al., 1974; Swanstrom and Wagner, 1974; Swanstrom et al., 1975).

Hybridisation of IE mRNA to nitrocellulose strips containing restriction fragments of HSV-1 DNA allowed IE genes to be mapped on the genome (Clements et al., 1977; Jones et al., 1977). The directions of transcription of these non-contiguous genes were established by hybridisation using short cDNA synthesised using polyadenylated mRNA (Clements et al., 1979; Anderson et al., 1980a; J. B. Clements, personal communication). Isolation of mRNAs from methylmercuric hydroxide agarose gels followed by their translation in vitro has allowed the correlation of mRNA size, genome location, and polypeptide size (Watson et al., 1979; Talley-Brown and Millette, 1979; Anderson et al., 1980a). Clements et al. (1979) proposed a model for transcription of IE genes utilising a single promoter in TR_S/IR_S, but subsequent work has shown that each IE gene has its own promoter (Easton and Clements, 1980; Millette and Klaiber, 1980; Mackem and Roizman, 1980;

F. J. Rixon, personal communication). Data concerning the HSV-1 IE genes are summarised in Figure A1.4. Although Frenkel et al. (1973) reported that the transcriptional program of HSV-2 is significantly different from that of HSV-1, Easton and Clements (1980) have clearly shown by blot hybridisation, mRNA isolation and in vitro translation that the two viruses share very similar IE transcription patterns.

Early mRNA forms a more complex class mapping throughout the HSV-1 genome (Clements et al., 1977; Stringer et al., 1978). Some 16 species have been mapped, most of which seemingly have individual promoters (Holland et al., 1979; Millette and Klaiber, 1980). Anderson et al. (1979) located on the genome approximately 40 late mRNAs ranging in size from 1.5 to greater than 8 kb. Costa et al. (1981) have mapped the major late mRNA, which is probably not spliced and codes for the major capsid protein of m.wt. 155,000. It is obvious that dissecting the complexities of these transcriptional patterns will occupy investigators for not a few years.

Regarding the temporal control of the transcriptional program of HSV-1, Ward and Stevens (1975) showed that when transcription was blocked by actinomycin D, earlier mRNAs tended to be more short-lived than later ones, suggesting a control mechanism operating at this level. A second type of control, acting at the level of processing of initial transcripts in the nucleus and translocation of mature mRNAs to the cytoplasm, is likely to be operative especially in later stages of infection. Transcripts isolated from nuclei at late times are larger than cytoplasmic mRNAs, and some nuclear species are under-represented in the cytoplasm (Wagner and Roizman, 1969a and b; Wagner, 1972; Kozak and

Roizman, 1974; Clements et al., 1977; Jones and Roizman, 1979; Jacquemont et al., 1980). However, similar claims that most or all of the genome is transcribed in the nucleus under IE conditions, and that most transcripts accumulate in the nucleus so that only certain species enter the cytoplasm (Kozak and Roizman, 1974; Jones and Roizman, 1979) have been countered by others who showed that nuclear and cytoplasmic IE RNAs are of similar complexities (Clements et al., 1977; Easton and Clements, 1980; Anderson et al., 1980a). Liquid hybridisation studies showed that a significant proportion of the genome is symmetrically transcribed at late times (Ben-Zeev and Becker, 1975; Jacquemont and Roizman, 1975a and b; Kozak and Roizman, 1975; Miovic and Pizer, 1980).

The most important level of control is thought to be that of transcriptional initiation. The HSV-1 mutants tsK, tsD and tsT have independent lesions in the gene coding for IE mRNA 3, which is translated into the IE polypeptide V_{mw} IE 175 (ICP4) (Preston et al., 1978; Stow and Wilkie, 1978; Preston, in press). Although tsK complements tsD and tsT in some tests (Crombie, 1975), it is now overwhelmingly likely that the three lesions are in one gene. The mutation in tsK is due to a single base pair change resulting in the replacement of an alanine by a valine residue (M-J. Murchie, personal communication). These mutants produce at non-permissive temperature mRNA and polypeptide profiles more or less similar to the IE patterns (Marsden et al., 1976; Watson and Clements, 1977, 1978 and 1980; Dixon and Schaffer, 1980). The properties of these mutants show that at least one IE function (i.e. V_{mw} IE 175) is continuously required for the transition from IE to early transcriptional patterns. Preston (1979) showed that this function is directly involved in the activation at the transcriptional level of an early

gene (deoxypyrimidine kinase, dPyK). Inhibition of DNA synthesis leads to an early transcriptional pattern and much reduced production of late polypeptides (Honess and Roizman, 1974; Honess and Watson, 1977a; Clements et al., 1977; Watson and Clements, 1977; Holland et al., 1980). Although Wolf and Roizman (1978) interpreted this as a requirement for both parental and progeny DNA templates for a normal level of late polypeptide synthesis, it seems more likely that virus DNA synthesis is required for the transition from early to late patterns of transcription.

Protein synthesis and modification

Approximately 50 HSV-induced polypeptides have been detected in infected cells by SDS-polyacrylamide gel electrophoresis (Honess and Roizman, 1973; Marsden et al., 1976). Two-dimensional gel electrophoresis has allowed 230 polypeptides to be distinguished, although undoubtedly many of these result from post-translational modification of primary translation products (Haarr and Marsden, 1981).

Virus proteins are synthesised in the cytoplasm and most, with the exception of glycosylated polypeptides, are then transported to the nucleus (Sydiskis and Roizman, 1968; Spear and Roizman, 1968 and 1970). Initial studies of the kinetics of synthesis of virus-induced polypeptides demonstrated temporal control of protein synthesis, and control of protein abundance (Honess and Roizman, 1973). Enzymatic functions tend to be produced early in infection, and most structural proteins later (Russell et al., 1964). The subsequent use of metabolic inhibitors suggested the existence of three kinetic classes of polypeptides, α, β, and γ, which correspond in general to the IE, early and late mRNA species (Honess and Roizman, 1974 and 1975a). α polypeptides are

synthesised immediately after release of a cycloheximide block at least one of this class is required for the production of β polypeptides. β functions are required for the inhibition of α polypeptide synthesis and the stimulation of γ polypeptides, which in turn inhibit β synthesis.

The threefold classification of virus-induced polypeptides is a simplistic model, since Pereira et al. (1977) identified two classes of β polypeptide (β_1 and β_2), each of which is probably dependent upon an α function for expression (Preston, 1979). Studies of polypeptide synthesis in the presence of an inhibitor of DNA synthesis have led to the identification of at least two γ classes (Honess and Watson, 1977a;Pederson et al., 1981). The latter authors defined six classes of polypeptide on the basis of their expression in the presence of an inhibitor of DNA synthesis, and Marsden et al. (1976) described nine classes of dependence relationship between polypeptide synthesis and the DNA phenotype of HSV-1 ts mutants. The properties of a dominant lethal early mutant isolated by Jofre et al. (1981) are in support of the existence of as least two β and γ classes. Post et al. (1981) established a cell line containing a β gene (dPyK) under α (V_{mw} IE 175) transcriptional control and found that dPyK activity was induced by infection with dPyK$^-$ virus. This effect was shown not to be due to a virus α function, so they interpreted it as the stimulation of transcription from the α promoter by a virion structural polypeptide, that is, a pre-α function. It is clear that a more or less complex set of cascade controls is operative in the temporal expression of HSV-induced polypeptides, probably acting at the transcriptional level.

Treatment of HSV-infected cells with protease

inhibitors failed to show rapid post-translational cleavage of virus-induced polypeptides (Honess and Roizman, 1973; Pereira et al., 1977). In contrast, exposure of virus-infected cells to the inhibitor for longer periods of time showed altered processing of several polypeptides (D. MacDonald, M. Suh and H.S. Marsden, unpublished data).

Approximately 20 phosphorylated polypeptides have been identified in HSV-infected cells (Marsden et al., 1978; Bookout and Levy, 1980; Wilcox et al., 1980). The phosphorylation pathway may differ between polypeptides and the level of phosphorylation may play a part in the binding affinity of certain phosphoproteins for DNA (Fenwick et al., 1980; Wilcox et al., 1980). The processing of the phosphorylated α polypeptide V_{mw} IE 175 (ICP4) has been studied in some detail. This polypeptide exists in three forms differing in apparent molecular weight, and it is thought that ICP4a is passively transported into the nucleus, where it is processed into ICP4b then ICP4c (Pereira et al., 1977; Fenwick et al., 1978). The nature of these processing events has not been determined, although a mutant with a lesion in the ICP4 gene has been studied which accumulates ICP4a and fails to transport it into the nucleus (Courtney and Powell, 1975; Courtney et al., 1976; Cabral et al., 1980). The phosphate moiety is stably associated with ICP4b but cycles on and off ICP4a and ICP4c (Wilcox et al., 1980). Bookout and Levy (1980) have recently described four forms of ICP4, only two of which are found in the cytoplasm.

A number of HSV-induced polypeptides are glycosylated, probably by host cell enzymes (Keller et al., 1970). Glycosylation of polypeptides occurs in situ, soon after polypeptide synthesis and their binding to cell membranes

(Spear and Roizman, 1970). Five major glycoproteins (gA, gB, gC, gD and gE) have been identified in the virion envelope and infected cell membrane (Spear et al., 1970; Spear, 1976; Marsden et al., 1976; Baucke and Spear, 1979). gA is rare in virions, and gB functions in virus penetration and cell fusion (Manservigi and Spear, 1977; Sarmiento et al., 1979). These two glycoproteins are thought to originate from the same precursor polypeptide (Eberle and Courtney, 1980b). gC has an inhibitory effect upon cell fusion, and is not essential for virion infectivity (Manservigi and Spear, 1977; Ruyechan et al., 1979). The least studied of the glycoproteins, gE, is essential for virion infectivity and acts as an Fc receptor for γ-globulin, a function which may inhibit the host immume cytolytic response mediated by viral glycoproteins exposed at the infected cell surface (Baucke and Spear, 1979; Para et al., 1980; Machtiger et al., 1980).

Glycosylation of precursor polypeptides is a β or γ event occurring in two stages: rapid addition of an oligomannosyl core followed by slower addition of fucose and sialic acid residues (Honess and Roizman, 1975b; Eisenberg et al., 1979; Cohen et al., 1980a). Attempts have been made to identify precursor forms by immunological analysis, the use of drugs which block the glycosylation pathway, treatment of processed polypeptides with glycosidases, and by pulse-chase experiments. Conclusions have been criticised on the grounds that breakdown products and true precursors are difficult to distinguish. Nevertheless, two-dimensional electrophoretic analysis strongly indicates that 16 species are involved in the formation of gB, 15-20 in gC, and 11 in gD (Cohen et al., 1980a; Haarr and Marsden, 1981).

HSV-1 glycoproteins are also sulphated, and a sulphated

breakdown product of gD is excreted from the infected cell (R.G. Hope and H.S. Marsden, personal communication). The major glycoprotein of PRV-infected cells is sulphated subsequent to glycosylation and then excreted (Erickson and Kaplan, 1973; Kaplan and Ben-Porat, 1976; Pennington and McCrea, 1977).

Much of the present understanding of HSV-specified functions, and the location of essential genes in the genome, has come from analyses of mutants. In this respect the commonest and most generally useful type is the ts mutant, which was first described in HSV by Subak-Sharpe (1969). Since then a large number of ts mutants have been isolated. 23 complementation groups in HSV-1 and 20 in HSV-2 were identified in a collaborative study by Schaffer et al. (1978), and the recent use of specific mutagenesis of DNA fragments has added at least three more to HSV-1 (Chu et al., 1979; Conley et al., 1981). Linear genetic linkage maps for ts, plaque morphology and drug resistance markers have been deduced (Brown et al., 1973; Schaffer et al., 1974; Subak-Sharpe et al., 1974; Benyesh-Melnick et al., 1974; Timbury and Calder, 1976; Brown and Jamieson, 1977).

Recombination in HSV was first noted by Wildy (1955), and intertypic complementation and recombination by Timbury and Subak-Sharpe (1973). Repeated rounds of mating and recombination occur during infection, and recombinants seem to be produced by the formation of partial heterozygotes (Brown and Ritchie, 1975; Ritchie et al., 1977). Excellent use has been made of intratypic (marker rescue) and intertypic recombination to physically map HSV mutations and functions on the genome (Wilkie et al., 1977b; Marsden et al., 1978; Morse et al., 1978; Preston et al., 1978; Stow and Wilkie,

1978; Stow et al., 1978; Halliburton, 1980; Parris et al., 1980; Chartrand et al., 1981; Sandri-Goldin et al., 1981). HSV-1/HSV-2 intertypic recombinants have proved especially powerful in assigning specific polypeptides and biochemical functions to regions of the genome, since restriction endonuclease mapping allows crossovers to be accurately defined, and the polypeptide profiles of the two serotypes are sufficiently different to distinguish the origin of many of the polypeptides induced by recombinants. A summary of the mapping positions of several polypeptides is shown in Figure A1.5.

Enzyme activities induced by HSV

Several enzymatic activities are induced in HSV-infected cells. These include pyrimidine deoxynucleoside kinase (dPyK) (Kit and Dubbs, 1963a), DNA polymerase (Keir and Gold, 1963), alkaline deoxyribonuclease (Morrison and Keir, 1968), ribonucleotide reductase (Ponce de Leon et al., 1977; Huszar and Bacchetti, 1981), adenylic acid:deoxythymidine 5'-phosphotransferase (Jamieson et al., 1976a; Falke et al., 1981), deoxyribopyrimidine triphosphatase (Wohlrabb and Francke, 1980), and a protein kinase (Blue and Stobbs, 1981). The induction of deoxycytidine deaminase (Chan, 1977; B. Dutia, personal communication) and deoxycytidylate deaminase (Rolton and Keir, 1974; Langelier et al., 1978) is disputed. A DNA gyrase has been sought but not yet demonstrated (Francke and Margolin, 1981).

The isolation of an HSV-2 ts mutant which also has a non-lethal mutation in the structural gene for the alkaline deoxyribonuclease has demonstrated that this enzyme is at least in part coded by the virus genome (Francke et al., 1978). The purified enzyme has a m.wt. of 90,000, but its function

in vivo is a matter of speculation (Strobel-Fidler and Francke, 1980). Moss *et al.* (1979) mapped the nuclease gene to 0.12-0.21 fractional genome units.

The virus-coded DNA polymerase has a m.wt. of 150,000 and seems to be active *in vitro* as a monomer (Powell and Purifoy, 1977). This enzyme is readily distinguished from the cell DNA polymerase by immunological (Keir *et al.*, 1966), genetic (Aron *et al.*, 1975) and inhibitor studies. The HSV DNA polymerase is very sensitive to inhibition by phosphonoacetic acid (PAA; Mao and Robishaw, 1975; Hay and Subak-Sharpe, 1976) and by acycloguanosine (acyclovir, ACV) in the triphosphate form (Furman *et al.*, 1979; Darby *et al.*, 1980). Mutant viruses resistant to PAA possess a PAA-resistant DNA polymerase (Jofre *et al.*, 1977; Purifoy and Powell, 1977), suggesting that a single locus is involved in PAA-sensitivity. ACV-sensitivity, on the other hand, is associated with two distinct loci corresponding to the DNA polymerase and dPyK (Crumpacker *et al.*, 1980). This difference is due to direct interaction of PAA with the polymerase, whereas ACV is first phosphorylated by dPyK, thence to the triphosphate which inhibits the polymerase (Elion *et al.*, 1977). It is possible that two cistrons are involved in DNA polymerase expression, since *ts* mutants from two complementation groups, which are closely associated in the region of 0.4 fractional genome units (Chartrand *et al.*, 1980), both express a *ts* DNA polymerase (Purifoy and Powell, 1981).

The virus-coded dPyK, as its name suggests, is able to phosphorylate both thymidine and deoxycytidine, and seems also to exhibit a nucleoside phosphotransferase activity (Jamieson *et al.*, 1974 and 1976a; Jamieson and Subak-Sharpe, 1974). Strong evidence that this enzyme is virus-coded comes from

immunological, biochemical and genetic studies (Klemperer et al., 1967; Buchan and Watson, 1969; Buchan et al., 1970; Thouless, 1972; Aron et al., 1973). The active polypeptide induced in vivo or produced in vitro by translation of mRNA has an apparent m.wt. of 40,000 (Honess and Watson, 1974; Summers et al., 1975; Thouless and Wildy, 1975; Preston, 1977; Cremer et al., 1977 and 1978). Two-dimensional gel electrophoresis of HSV-induced polypeptides showed that this polypeptide may exist in three differently charged forms (Haarr and Marsden, 1981). Preston and McGeoch (1981) identified a slightly smaller polypeptide expressed in vitro and in vivo, concluding that this was the result of false initiation of translation from a single mRNA species. The results of experiments using mixed infections of dPyK and dPyK$^-$ viruses suggest that the active enzyme is probably a multimer (Jamieson and Subak-Sharpe, 1978).

A number of nucleoside analogues inhibit virus growth because they are phosphorylated by dPyK, and thence bind to the DNA polymerase or become lethally incorporated into newly synthesised virus DNA. Among these are ACV, bromodeoxycytidine, bromodeoxyuridine and 1-β-arabinosyl thymine (Kit and Dubbs, 1963b; Cooper, 1973; Schildkraut et al., 1975; Miller et al., 1977; Elion et al., 1977; Muller et al., 1979b). Such inhibitors have been used to select viable dPyK$^-$ mutants. Although dPyK activity is not required for virus replication in actively dividing cells, it is an essential function for growth in resting cells, for full neurovirulence, and for normal establishment of latency (Jamieson et al., 1974; Field and Darby, 1978 and 1980; Darby et al., 1981).

The construction of bacterial plasmids containing the

HSV-1 dPyK gene (HSV-1 BamHI p mapping at approximately 0.3 fractional genome units) has opened the way to understanding the molecular genetics of this function (Wilkie et al., 1979a; Enquist et al., 1979; Colbère-Garapin et al., 1979). The DNA sequence of the gene has been determined (McKnight, 1980; Wagner et al., 1981; D. McGeoch, personal communication), and viable dPyK$^-$ deletion mutants have been constructed in vitro (Wilkie et al., 1980; Smiley, 1980). The dPyK is a β polypeptide which requires for its production in the infected cell an α function which acts at the transcriptional level (Garfinkle and McAuslan, 1974; Leung et al., 1980). The gene is transcribed from its own promoter leftwards with respect to the P orientation of the genome, and the mRNA is not spliced (Wilkie et al., 1980; Smiley et al., 1980).

The HSV-1 dPyK gene has been used as a separate entity from the genome from which it was derived. Cell lines lacking the cell thymidine kinase have been biochemically transformed using inactivated HSV or DNA fragments (Munyon et al., 1971; Jamieson et al., 1976b; Maitland and McDougall, 1977; Wigler et al., 1977). Using a cloned DNA fragment containing the dPyK gene, Pellicer et al. (1978) have reported that the virus DNA is integrated into the cell DNA, although not at a specific site. Other exogenous DNA sequences have been stably introduced into cells by employing the dPyK gene as a selectable marker, in some experiments covalently linked to the exogenous DNA (Mantei et al., 1979; Wigler et al., 1979)

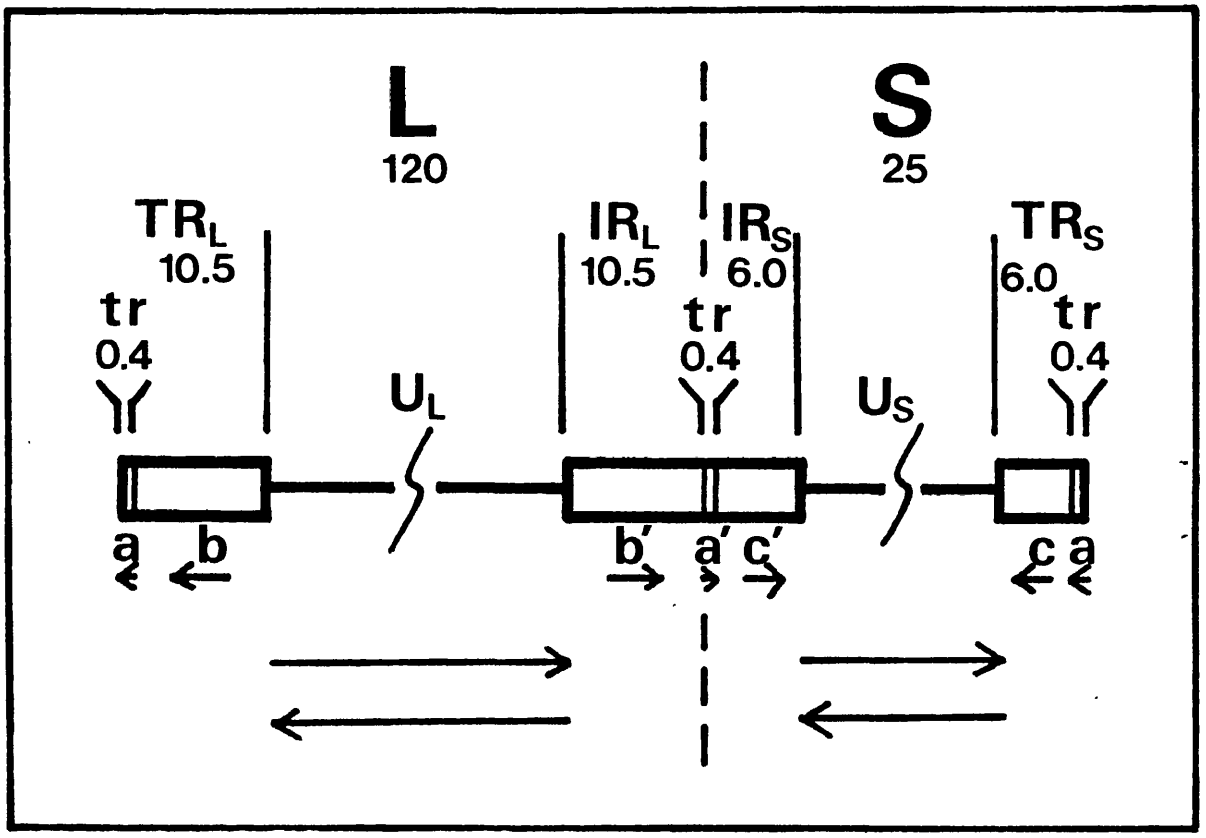
Approximately 20 HSV-1-induced DNA binding proteins have been identified, including the α polypeptides, the DNA polymerase, and probably the alkaline deoxyribonuclease and dPyK (Bayliss et al., 1975; Powell and Purifoy, 1976; Bookout and Levy, 1980; Becker et al., 1980; Hay and Hay, 1980).

Figure A1.1

UPPER PANEL Structural arrangement of the HSV-1 genome. The genome comprises two covalently linked segments (L, S), each of which consists of a unique sequence (U_L, U_S) bounded by inverted repetitions (TR_L, IR_L, TR_S, IR_S). U_L and U_S are shortened in this diagram to emphasise the locations and orientations (arrowed) of the a sequences, or terminal repetitions (tr) at the genome termini and L-S joint. Also shown are the b and c sequences ($TR_L = b + a$, $IR_L = b' + a'$, $TR_S = c + a$, $IR_S = c' + a'$). Sizes are given in kbp. A single DNA molecule contains each segment in either orientation with equal probability, as shown by the larger arrows, allowing four permutations of sequence arrangement. The genome structure of HSV-2 is essentially identical.

LOWER PANEL Generation of submolar restriction endonuclease fragments from HSV DNA. A hypothetical endonuclease cleaves the DNA in three locations. As a consequence of inversion of the two segments, four quarter-molar fragments spanning the L-S junction (a + e, c + e, a + b, c + b), four half-molar fragments from the termini (a, b, c, e), and one molar fragment (d) are generated. Extension of this argument shows that if one set of repeats also contains a restriction site, half-molar but not quarter-molar fragments would be generated. If both sets of repeats also contained a restriction site, only molar fragments would result.

In this Figure the four arrangements have been termed P, I_L, I_S, I_{SL} in accordance with the nomenclature proposed by Roizman et al. (1979). Restriction maps of HSV DNA are conventionally displayed in the P arrangement.


L
120
S
25
TR_L
10.5
IR_L
10.5
IR_S
6.0
TR_S
6.0
tr
0.4
tr
0.4
tr
0.4
U_L
U_S
a
b
b'
a'
c'
c
a

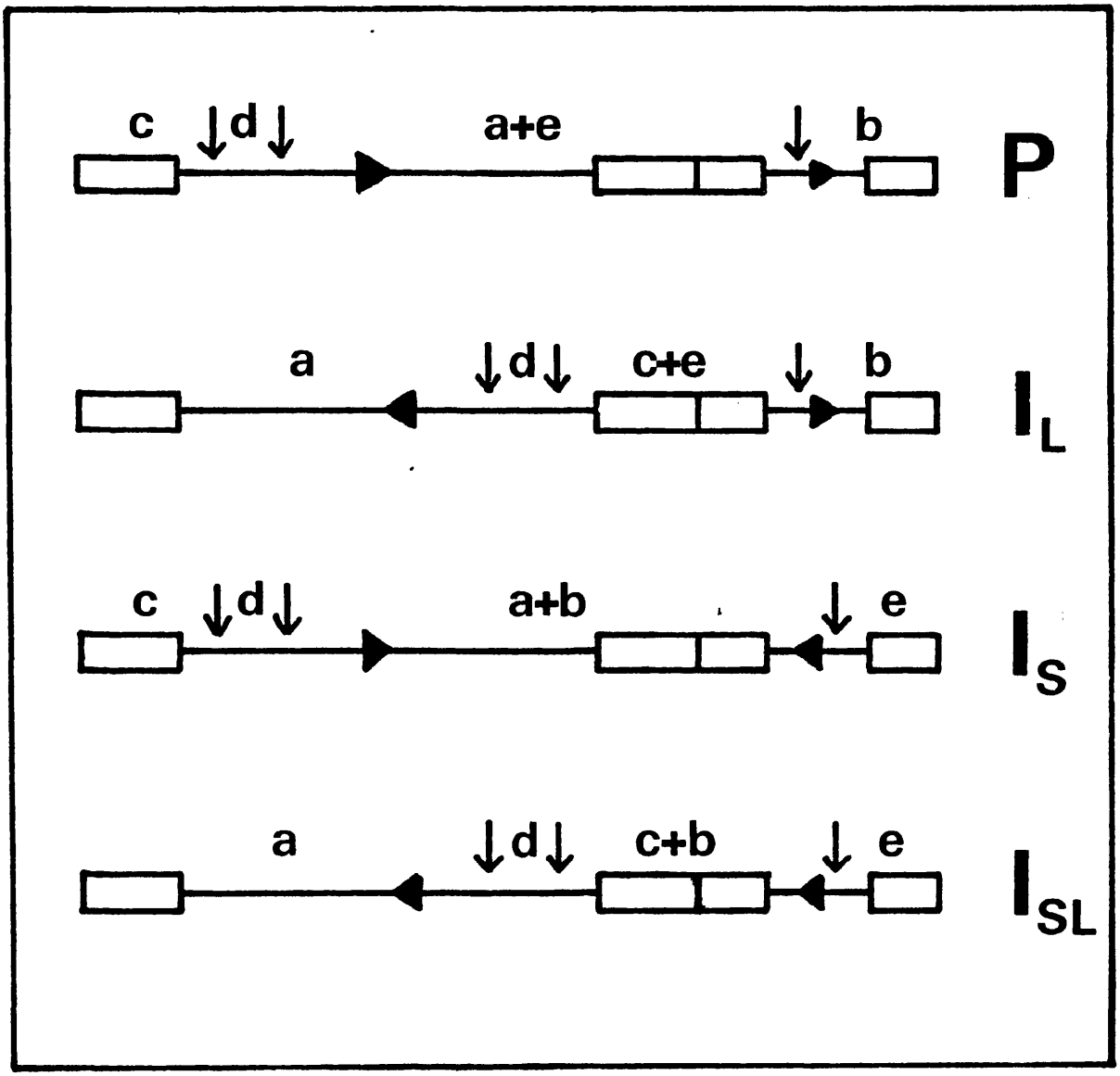
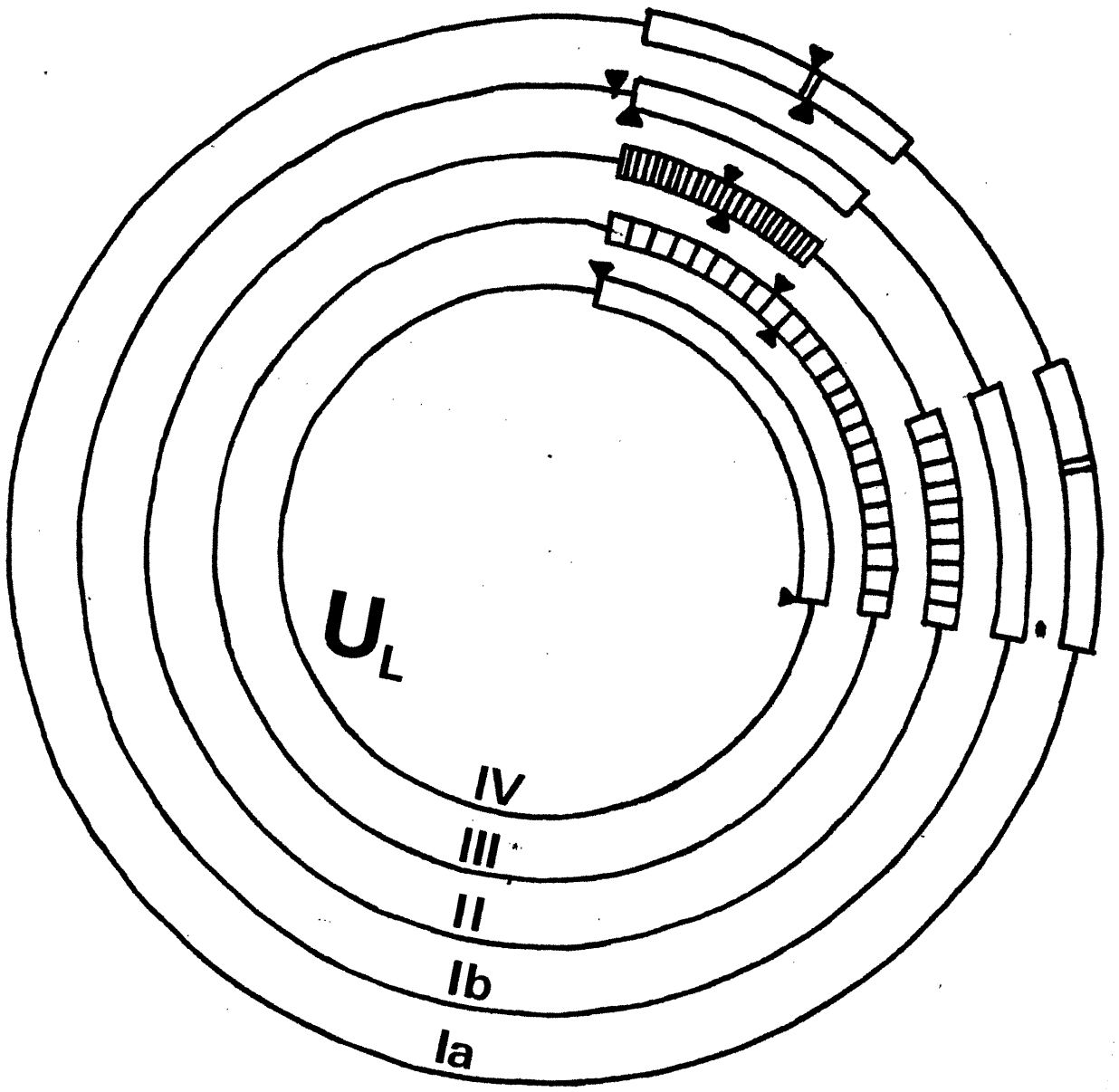

c
d
a+e
b
P
a
d
c+e
b
I_L
c
d
a+b
e
I_S
a
d
c+b
e
I_SL

Figure A1.2 (overleaf)

Structures of herpesvirus genomes and general model for interconversion of linear and circular forms.

Linear genome structures of the four main groups (I-IV) are shown to scale on the right hand page. Two subgroups have been tentatively included in group I. In each case the m.wt. ($\times 10^{-6}$) and observed orientations (filled arrowheads) of U_L and, where appropriate, U_S, are indicated. Repeated sequences are shown as rectangles, and their relative orientations by arrows and by use of the letters a, b, c and their complement a', b', c' (⟿ and a_n, b_n signify multiple tandem repeats). The use of letters does not denote similarity of sequence or function in these regions between different genomes.

It is known that the genomes illustrated are terminally redundant, although those of PRV and EHV-1 have not yet been directly shown to be so. Nevertheless, this feature makes it possible, at least in principle, to circularise herpesvirus genomes by ligation subsequent to the action of an exonuclease. The four groups of putative circular forms are shown on the left hand page, not drawn to scale. Orientations of repeated sequences (rectangles) have been omitted for clarity. Positions of ligation on opposite strands of the duplex are indicated (▼). Clearly, the reverse process, single stranded cleavage on each side of the terminal repeat within a circular DNA molecule followed by repair of the single stranded termini, would generate a linear genome. Cleavage may occur at several possible locations within the tandem reiterations of groups II and III, generating the heterogeneous termini observed in virion DNA.


U_L
IV
III
II
Ib
Ia

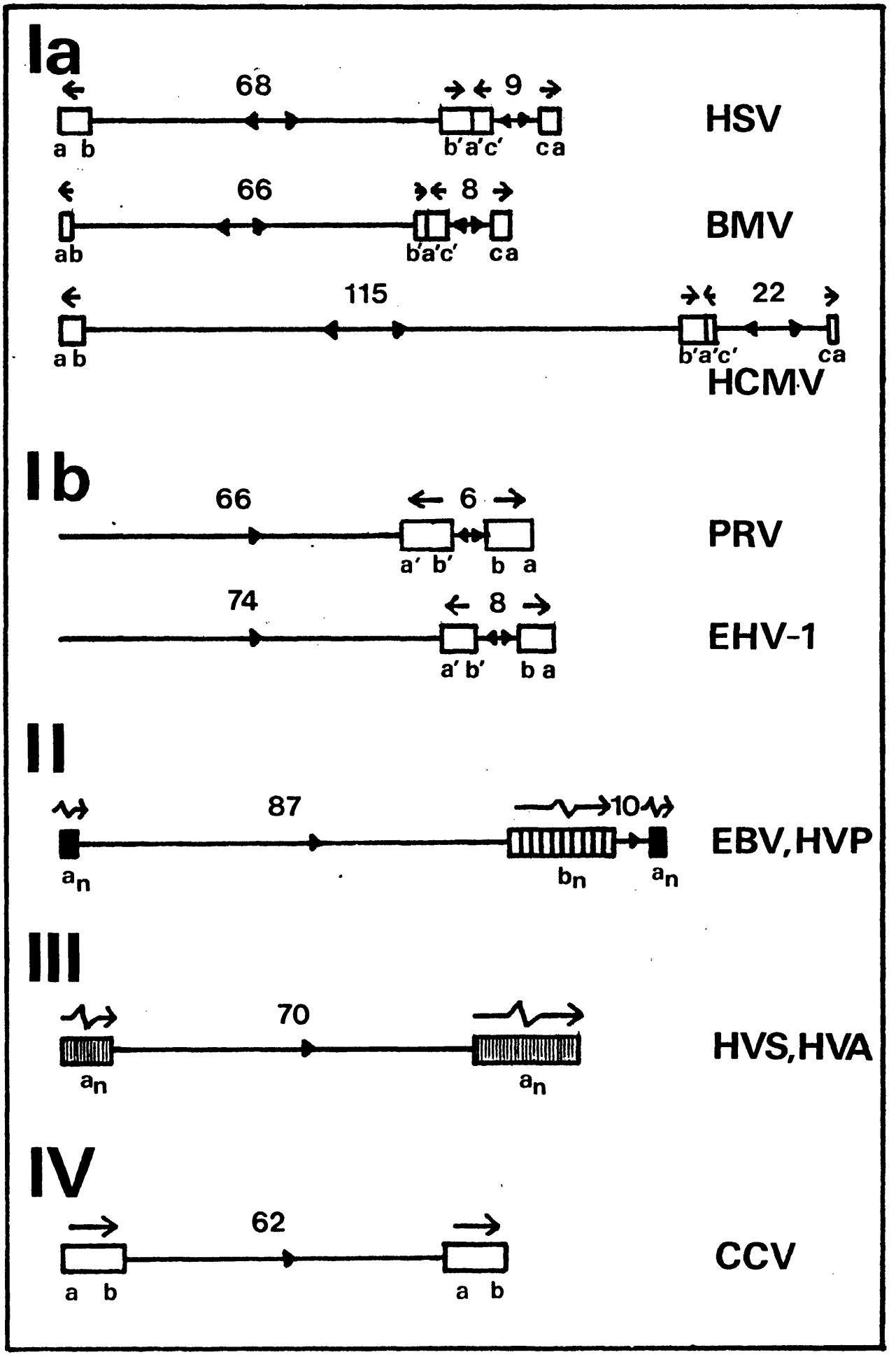

Ia
68
9
a b
b'a'c'
c a
HSV
66
8
ab
b'a'c'
c a
BMV
115
22
a b
b'a'c'
c a
HCMV
Ib
66
6
a' b'
b a
PRV
74
8
a' b'
b a
EHV-1
II
87
10
an
bn
an
EBV,HVP
III
70
an
an
HVS,HVA
IV
62
a b
a b
CCV

Figure A1.3

Mean GC contents of herpesvirus DNAs, taken from Honess and Watson (1977b). Filled circles indicate human herpesviruses.

Abbreviations of virus nomenclature other than the obvious are as follows.

SpM	-	spider monkey herpesvirus (cebid herpesvirus 3)
IBR	-	infectious bovine rhinotracheitis virus
SqM	-	squirrel monkey herpesvirus (herpesvirus tamarinus)
GCMV	-	guinea pig cytomegalovirus
MaCMV	-	marmoset cytomegalovirus
SA6	-	simian cytomegalovirus
RhCMV	-	rhesus cytomegalovirus
VeCMV	-	vervet cytomegalovirus
FeILT	-	feline infectious laryngotracheitis virus
AvILT	-	avian infectious laryngotracheitis

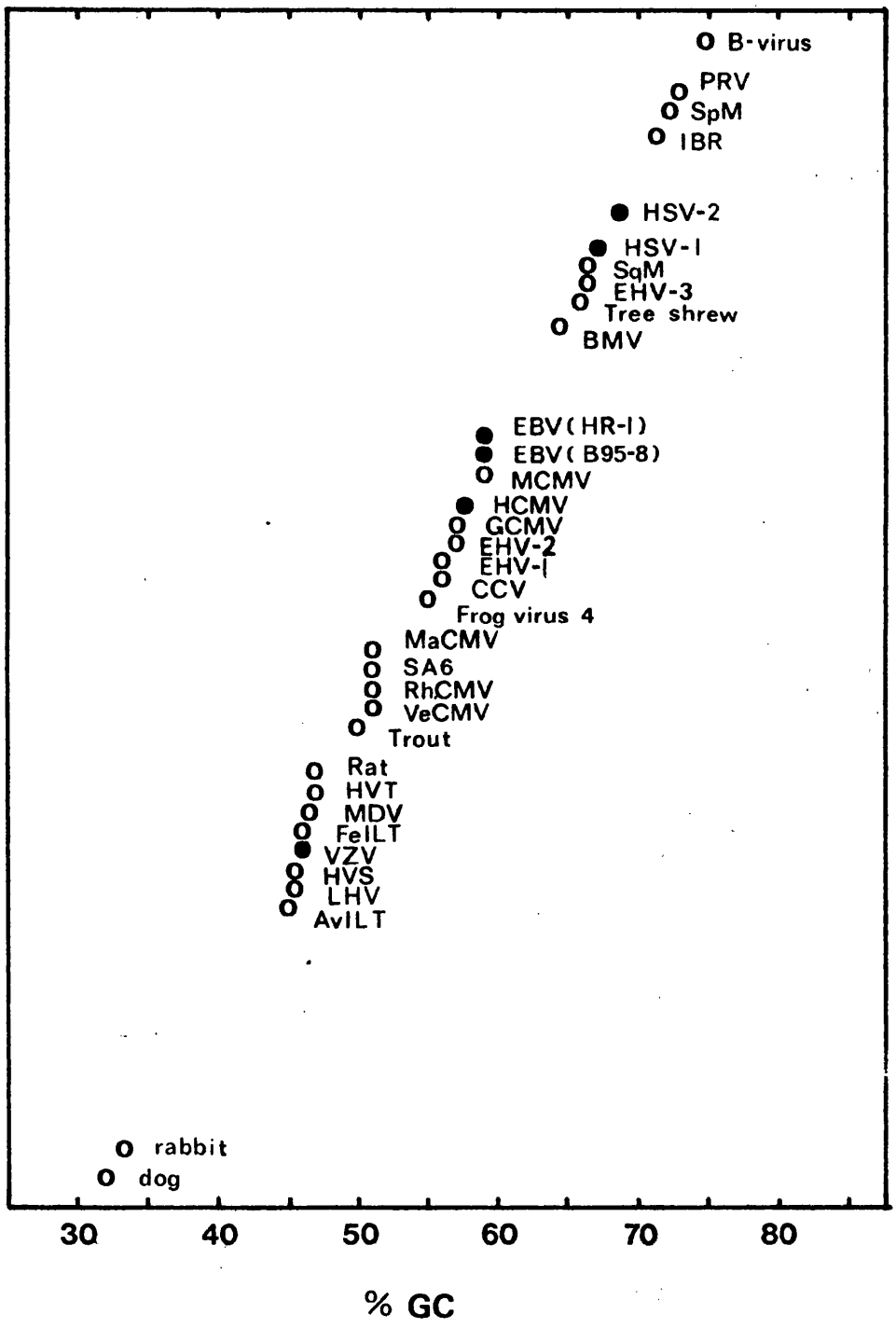

B-virus
PRV
SpM
IBR
HSV-2
HSV-1
SqM
EHV-3
Tree shrew
BMV
EBV(HR-1)
EBV(B95-8)
MCMV
HCMV
GCMV
EHV-2
EHV-1
CCV
Frog virus 4
MaCMV
SA6
RhCMV
VeCMV
Trout
Rat
HVT
MDV
FeILT
VZV
HVS
LHV
AvILT
rabbit
dog
30
40
50
60
70
80
% GC

Figure A1.4 (overleaf)

Immediate early (IE) transcriptional program of HSV-1. The genome is shown to scale in the conventional manner, except that two regions in U_L have been omitted for clarity (∿). The scale at the top of the Figure shows coordinates of fractional genome length.

The locations and directions of transcription of IE mRNAs 1-5 are shown (Clements et al., 1977; Jones et al., 1977; Clements et al., 1979; Anderson et al., 1980a; F.J. Rixon, personal communication). Also included are similar data for the 5.0 kb mRNA coding for V_{mw} IE 136'(143) (Anderson et al., 1981; J. McLauchlan and J.B. Clements, personal communication). The position of the intron common to the genes for IE mRNAs 4 and 5 is indicated (Watson et al., 1981; F.J. Rixon, personal communication). It is suspected that the gene for IE mRNA 1 also contains an intron, because the mRNA is considerably smaller than the region of DNA encoding it, whereas the genes for IE mRNAs 2 and 3 contain no detectable introns (F.J. Rixon, personal communication). The regions in the DNA encoding the 5' termini of IE mRNAs 1, 3, 4 and 5 have been located (Mackem and Roizman, 1980) and nucleotide sequences determined for these regions of IE mRNAs 3, 4 and 5 (M-J. Murchie, personal communication). Similarly the regions encoding the 3' termini of IE mRNAs 1, 2 and 5 are known (Section 3 of Results; M-J. Murchie and F.J. Rixon, personal communications). The sequence coding for the translated region of IE mRNA 5, which lies completely within U_S, has been determined (M-J. Murchie, personal communication). The 5.0 kb mRNA coding for V_{mw} IE 136'(143)

is expressed minimally under IE conditions and therefore there is some hesitation in its inclusion in the IE class. A 1.2 kb mRNA 3'-coterminal with the 5.0 kb mRNA encodes a different polypeptide, the coding region for which lies outside that for V_{mw} IE 136'(143) (J. McLauchlan and J.B. Clements, personal communication).

Below each mRNA is indicated the size in kb of the polyadenylated mRNA determined by methylmercuric hydroxide agarose gel electrophoresis (Watson et al., 1979; Talley-Brown and Millette, 1979; Anderson et al., 1980a and b). In vitro translation of isolated mRNAs performed in the studies mentioned above, and the mapping of polypeptides utilising HSV-1/HSV-2 intertypic recombinants (Preston et al., 1978; Marsden et al., 1978; Morse et al., 1978), have allowed identification of the polypeptide encoded by each IE mRNA. At the foot of the Figure the equivalent polypeptides in the nomenclature of Honess and Roizman (1973 and 1974) are given.

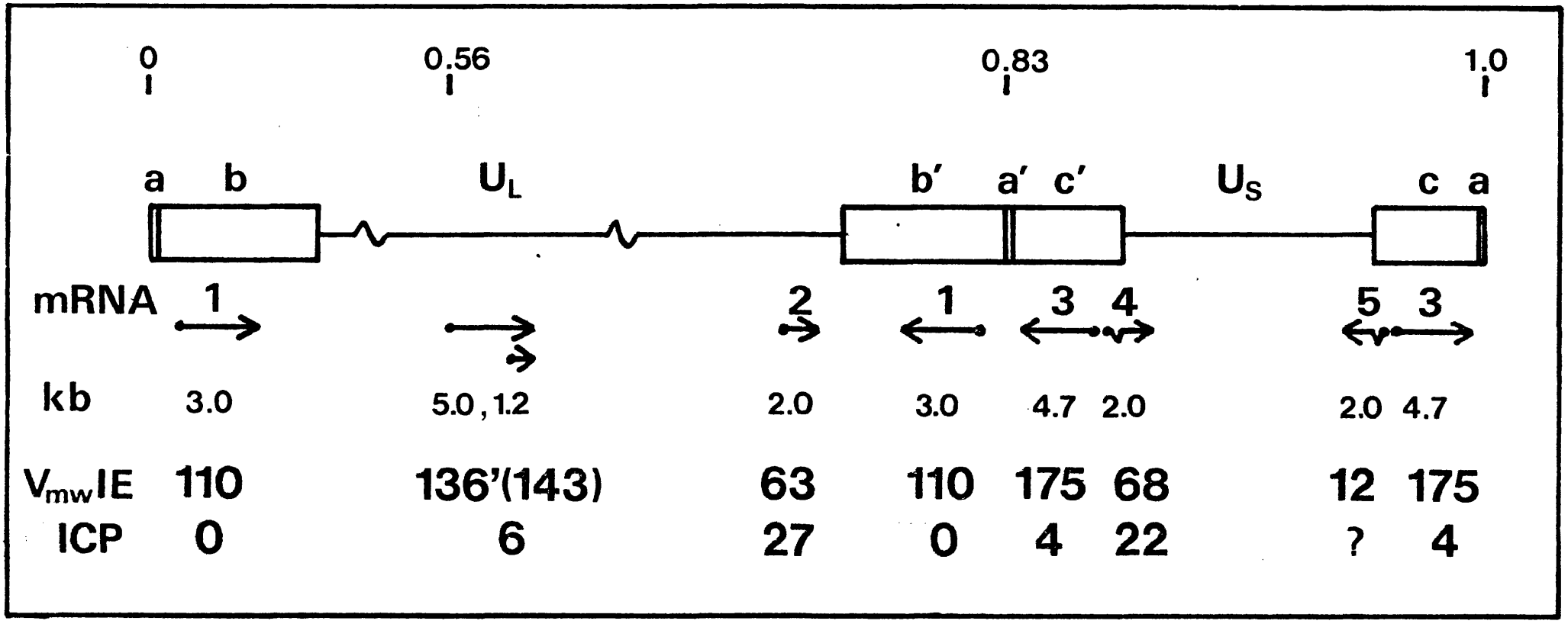
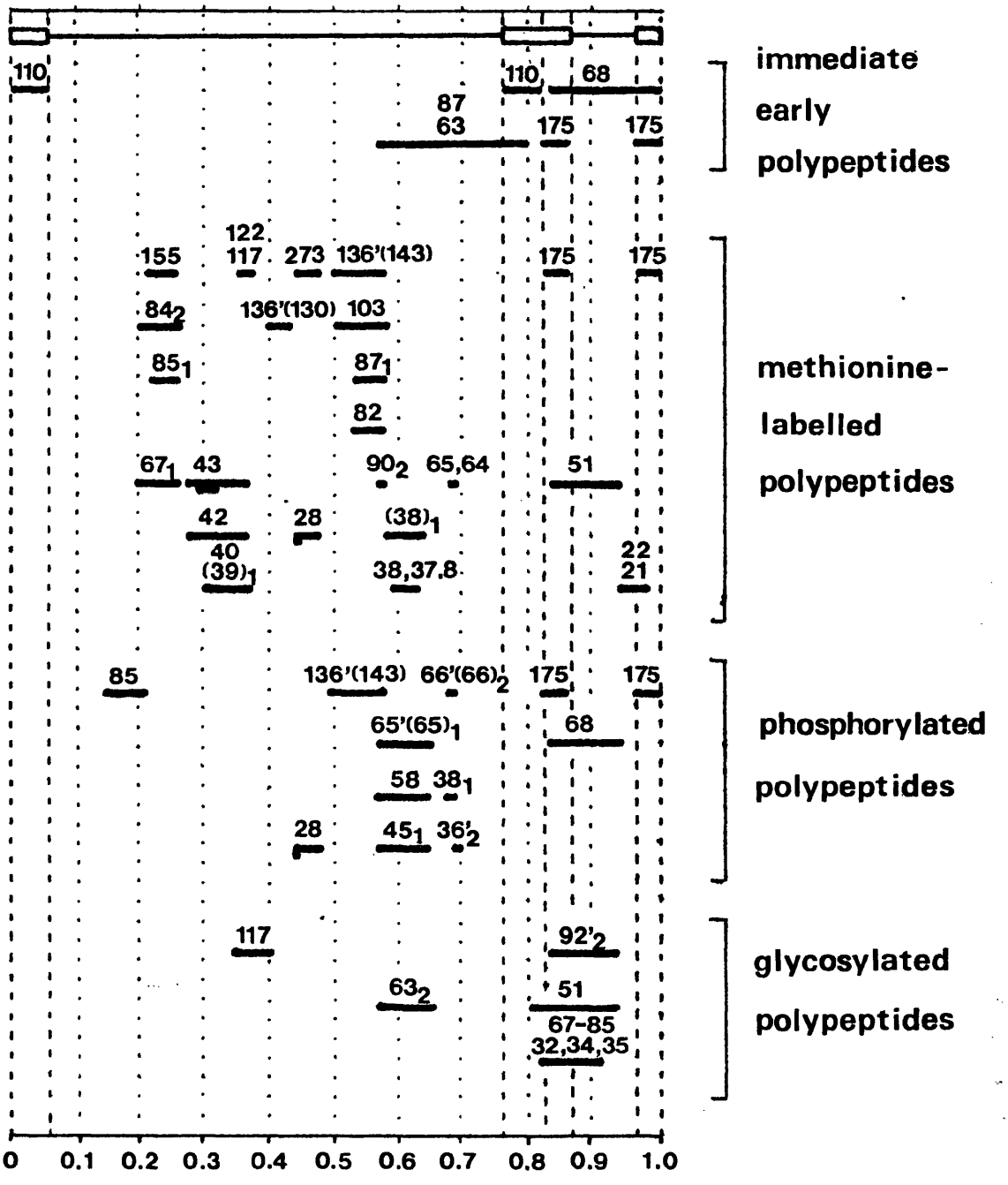

0
0.56
0.83
1.0
a b
U_L
b' a' c'
U_S
c a
mRNA
1
2
1
3 4
5 3
kb
3.0
5.0, 1.2
2.0
3.0
4.7 2.0
2.0 4.7
V_{mw}IE
110
136'(143)
63
110
175 68
12 175
ICP
0
6
27
0
4 22
?
4

Figure A1.5

Map locations of HSV-induced polypeptides (Marsden et al., 1978; Preston et al., 1978; H.S. Marsden, personal communication).

Each number (X) indicates the apparent m.wt. ($\times 10^{-3}$); full nomenclature is V_{mw} X or, in the case of immediate early (IE) polypeptides, V_{mw} IE X. Polypeptides with the subscript "1" have no readily recognisable type 2 equivalent, and those with the subscript "2" have no readily recognisable type 1 equivalent. Other polypeptides are indicated by the m.wt. of the HSV-1 form and have no subscript. Accurate locations of IE polypeptides from transcript mapping data are shown in Figure A1.4.


110
110
68
87
63
175
175
immediate early polypeptides
122
155
117
273
136'(143)
175
175
84 2
136'(130)
103
85 1
87 1
82
67 1
43
90 2
65,64
51
42
28
(38) 1
40
(39) 1
38,37.8
22
21
methionine-labelled polypeptides
85
136'(143)
66'(66) 2
175
175
65'(65) 1
68
58
38 1
28
45 1
36' 2
phosphorylated polypeptides
117
92' 2
63 2
51
67-85
32,34,35
glycosylated polypeptides
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1.0

PART B

MATERIALS AND METHODS

MATERIALS

Viruses

HSV-1 Glasgow strain 17 *syn*$^+$ was isolated in Glasgow by Dr C.A.P. Ross, and mutants *syn*$^+$ *ts*B and *syn*$^+$ *ts*D were indirectly derived therefrom (Brown *et al.*, 1973).

HSV-1 strain USA-8 *syn*$^+$ was isolated in Philadelphia from the left trigeminal ganglion of a female who died from lymphocytic lymphoma (Lonsdale *et al.*, 1979).

HSV-2 strain HG52 was isolated from an anal lesion of a female patient in Edinburgh, and mutants *ts*1 and *ts*6 were derived from this strain (Timbury, 1971).

HSV-1/HSV-2 intertypic recombinant viruses are described in Figure C4.3.

The pseudorabies virus strain used was that originally isolated by Dr. A.S. Kaplan (Kaplan and Vatter, 1959).

Cells

The continuous cell line used was baby hamster kidney clone 13 (BHK C13; MacPherson and Stoker, 1962).

Bacteria

Escherichia coli K12 strain HB101 was used as host to plasmids (Boyer and Roúlland-Dussoix, 1969).

Escherichia coli *met*$^-$ *r*$^-$ *m*$^-$ (cI_{857}S7) used for the growth of bacteriophage lambda was given by Dr D. Ritchie.

Bacteria for the production of restriction endonucleases are described in Table B1.1.

Tissue culture media and solutions

Cells were grown in the Glasgow modification of Eagle's medium (Busby *et al.*, 1964) supplemented with 100 units/ml

penicillin, 100 µg/ml streptomycin, 0.2 µg/ml antimycotic agent n-butyl-p-hydroxybenzoate, and 0.002% wt/vol phenol red. Calf serum and pooled human serum were prepared in the Institute of Virology.

The following composite solutions were used.

ECx — Eagle's medium containing x% vol/vol calf serum;

ETCx — ECx containing 10% vol/vol Difco tryptose phosphate broth (TP);

EHux — Eagle's medium containing x% vol/vol human serum;

ECl-Pi — Eagle's medium lacking orthophosphate containing 1% vol/vol calf serum.

Phosphate buffered saline (PBS) contained 0.17 M NaCl, 0.0034 M KCl, 0.001 M Na_2HPO_4, 0.002 M KH_2PO_4 in distilled water, pH7.2.

PBSCa was PBS containing 5% vol/vol calf serum.

Versene consisted of 0.006M EDTA in PBS containing 0.0015% wt/vol phenol red.

Tris saline was 0.14 M NaCl, 0.03 M KCl, 0.00028 M NaH_2PO_4, 1 mg/ml dextrose, 0.0015% wt/vol phenol red, 0.025 M Tris-HCl, pH 7.4 supplemented with 100 units/ml penicillin and 100 µg/ml streptomycin.

Trypsin-versene comprised one volume of 0.25% wt/vol Difco trypsin in Tris saline plus four volumes of versene.

Giemsa stain consisted of 1.5% vol/vol suspension of Giemsa in glycerol, heated to 56° for 90-120 min and diluted with an equal volume of methanol.

Radiochemicals

The following radioisotopes were obtained from The Radiochemical Centre, Amersham, Bucks.

deoxynucleoside (α-^{32}P) triphosphates (dATP, PB164; dCTP, PB165; dGTP, PB166; dTTP, PB167; each at >14.8 TBq/mmol);

adenosine (γ-^{32}P) triphosphate (PB10218; >185 TBq/mmol);

^{32}P orthophosphate (PBS11).

Chemicals

The following firms supplied the listed chemicals.

Agarose (type 1), Trizma base, ethidium bromide, 5% vol/vol sterile bovine serum albumin, polyvinylpyrollidone, bovine serum albumin (RIA grade), ribonucleases A and T_1, yeast RNA and lysozyme - Sigma (London) Chemical Company, Kingston-upon-Thames, London.

N,N,N',N'-tetramethylethylenediamine (TEMED), hydroxylapatite, ammonium persulphate and Biorex 70 (100-200 mesh) - Biorad Laboratories, Richmond, California.

Dextran sulphate, Ficoll 400 and Sephadex G50 Fine - Pharmacia Fine Chemicals, Uppsala, Sweden.

Ultrogel AcA 34 - LKB, Bromma, Sweden.

Diaminoethyl cellulose (DE52) -Whatman Ltd., Maidstone, Kent.

Brain-heart infusion - Oxoid Ltd., Basingstoke, Hants.

2,5-diphenyloxazole (PPO), 1-4-di-(2-(5-phenyloxazolyl)) benzene (POPOP), trichloroacetic acid and boric acid - Koch-Light Laboratories, Colnbrook, Berks.

Dimethyl sulphate (DMS) - Aldrich Chemical Co. Ltd., Gillingham, Dorset.

Hydrazine - Eastman-Kodak, Rochester, New York.

Piperidine - Fluorochem Ltd., Glossop, Derbyshire.

Mithramycin ("Mithracin") - Pfizer Ltd., Sandwich, Kent.

Ampicillin - Beecham Research Laboratories, Brentford, Middlesex.

Other chemicals, where not stated, were obtained where possible in Analar grade from BDH Chemicals Ltd., Poole, Dorset.

DNA

Calf thymus DNA and salmon sperm DNA were obtained from Sigma Ltd., and ØX174 RF I DNA from New England Biolabs, Beverly, Massachussetts.

Professor D. Sherratt kindly provided pAT153 DNA.

Human cytomegalovirus DNA (strain AD169) was given by Dr M. Weststrate.

DNA was prepared and generously supplied by Dr J. M. Whalley from a plaque-purified Australian isolate (HVS 25) of equid herpesvirus type I obtained from Dr M. Sabine of the Department of Veterinary Medicine, University of Sydney.

Enzymes

Restriction endonucleases not prepared from bacteria in this study, and other enzymes acting upon DNA (T4 DNA ligase, bacterial alkaline phosphatase, *E. coli* DNA polymerase I and the Klenow fragment thereof) were purchased from New England Biolabs, or from Bethesda Research Laboratories, Rockville, Maryland, or from the Boehringer Corporation (London) Ltd., Lewes, East Sussex.

Deoxyribonuclease I was obtained from the Worthington Biochemical Corporation, Freehold, New Jersey.

T4 polynucleotide kinase was supplied by P-L Biochemicals Inc., Milwaukee, Wisconsin.

Restriction endonucleases XhoI and SmaI were the generous gifts of Dr J. Arrand, and SstI of Dr P. Rigby.

Other materials

Petri dishes - Flow Laboratories, Irvine, Scotland.

Kodirex X-ray film, X-Omat H X-ray film and DX80 developer - Kodak Ltd., London.

Amfix - May and Baker Ltd., Dagenham.

Teflon tape - the Minnesota Mining and Manufacturing Company, USA.

Nitrocellulose membrane filter in sheet (BA85) and gridded disc forms - Schleicher and Schüll, Dassel, West Germany.

Commonly used solutions and buffers

SSC	0.15 M NaCl, 0.015 M sodium citrate, pH 7.5;
NTE	0.01 M Tris-HCl, 0.1 M NaCl, 0.001 M EDTA, pH 7.4;
scintillation fluid	5 g PPO and 0.3 g POPOP per litre of toluene.

METHODS

Growth of cells

BHK cells were grown to confluence in 80 oz roller bottles at 37° in 200 ml ETC10 under 5% CO_2 in air. Cells were harvested by washing twice with trypsin-versene and reseeded at 3 x 10^7 cells/roller bottle in ETC10. 50 mm diameter plastic Petri dishes were seeded at 2 x 10^6 cells/dish in 4 ml ETC10, the monolayers attaining confluence after 16-24 hr at 37°.

Production of virus stocks

80 oz roller bottles containing almost confluent BHK cells were infected with virus at a m.o.i. of 1 p.f.u./ 300 cells. Virus was added in 40 ml EC5 per bottle and incubated at 31°. After 2-4 days infected cells were shaken into the medium, pelleted at 2000 r.p.m. for 10 min in an MSE Coolspin centrifuge, and resuspended in 5 ml PBSCa. The cells were ruptured using a Cole-Palmer ultrasonic bath and centrifuged at 2000 r.p.m. for 10 min to remove cellular debris. The supernatant was stored in sterile vials at -70°. Sterility checks were carried out using blood agar plates and tryptose phosphate broth.

Virus titration

Virus was diluted serially at tenfold dilutions in PBSCa. Drained BHK cell monolayers in 50 mm Petri dishes were inoculated with 0.1 ml of virus dilution. Virus was absorbed for 40 min at the temperature of subsequent incubation (31° or 37°) and then 4 ml EHu5 was added. After incubation for 2-3 days the monolayers were fixed and stained with Giemsa stain for 15 min at room temperature, and plaques counted using a dissecting microscope.

Plaque purification of HSV

The medium (EHu5) was removed from Petri dishes containing well-separated individual plaques and the monolayers were washed twice with PBSCa. Infected cells forming a single plaque were taken up into a finely drawn Pasteur pipette and transferred to 0.5 ml PBSCa in a sterile vial. Removal of the plaque from the dish was carried out under the dark field illumination of a Wild M7A stereoscopic dissecting microscope. The vial was sonicated in an ultrasonic bath and released virus inoculated in tenfold dilutions onto BHK monolayers in Petri dishes, and plaques allowed to develop under 4 ml EHu5. Virus was plaque purified twice more in the same way, and the progeny from the final plaque were used to infect a single 50 mm Petri dish monolayer. Virus from the infected monolayer incubated under 4 ml EC5 was titrated under appropriate conditions and in some cases used to infect 80 oz roller bottles for production of working stocks of virus.

Generation of HSV-1/HSV-2 intertypic recombinant viruses

The marker rescue method of Stow and Wilkie (1976) was used, involving precipitation of the infecting DNA as a calcium phosphate complex and osmotic shocking of the cells by treatment with 25% dimethylsulphoxide (DMSO) to stimulate uptake of the DNA.

Intact DNA from a temperature-sensitive mutant of HSV was mixed at 0.4 μg/ml with 10 μg/ml calf thymus carrier DNA and wild type DNA restriction fragments in Hepes-buffered saline (HeBS: 8.0 g/l NaCl, 0.37 g/l KCl, 0.1 g/l Na_2HPO_4, 1.0 g/l D-glucose, 5.0 g/l N-2-hydroxyethylpiperazine

N'-2-ethanesulphonic acid, pH adjusted to 7.05 with NaOH). The amount of fragment present per ml was that generated from 1 μg intact wild type DNA. 2 M $CaCl_2$ was added to a final concentration of 0.13 M, and 0.5 ml samples added to almost confluent drained BHK cell monolayers. The dishes were incubated at 38.5° from the time of infection, 4 ml EC5 being added after 45 min. At 4 hr p.i. the cells were washed with Eagle's medium and exposed to 1 ml 25% vol/vol DMSO in HeBS for 5 min at room temperature. The cells were carefully washed twice with Eagle's medium and incubated at 38.5° in 4 ml EHu5 for 3 days. Then the monolayers were washed twice with 4 ml PBSCa, 1 ml PBSCa added, and plaques picked.

Preparation of HSV DNA

Confluent monolayers of BHK cells in 80 oz roller bottles were infected as described and incubated at 31°. The infected cells were detached by agitation after 2-3 days and pelleted by centrifugation at 2000 r.p.m. for 10 min. The medium was retained in the case of HSV-1. The cells were resuspended in RSB (0.01 M Tris-HCl pH 7.4, 0.01 M KCl, 0.0015 M $MgCl_2$) containing 0.5% vol/vol Nonidet P40 (NP40) and kept on ice for 10 min. Nuclei were pelleted by centrifugation at 2000 r.p.m. for 5 min and the cytoplasmic supernatant retained. The nuclei were re-extracted with 0.5% NP40 in RSB and the second cytoplasmic supernatant retained. Virus was pelleted by centrifugation of the combined supernatants at 12000 r.p.m. for 3 hr in an MSE 6 x 250 ml rotor.

The pellet was resuspended in NTE, sonicated in an ultrasonic bath, 20% wt/vol sodium dodecyl sulphate (SDS)

added to a final concentration of 0.5%, and extracted with two volumes of NTE-equilibrated redistilled phenol by gentle agitation on a rock-and-roller. The upper aqueous phase was removed and re-extracted with two further volumes of NTE-equilibrated phenol after centrifugation at 2000 r.p.m. for 10 min. After a second centrifugation step the upper phase was extracted for 10 min with isoamyl alcohol in chloroform (1:24 vol/vol) and again centrifuged. The upper aqueous phase containing HSV DNA was dialysed overnight at 4° against 0.1 x SSC, and then stored at -20°.

In some instances HSV DNA was purified further by isopyknic banding on caesium chloride gradients. Solid CsCl was added to the DNA solution to give a refractive index of 1.401-1.402. Centrifugation was for 3 days at 36000 r.p.m. in a Beckman Ti50 rotor at 15°, or for 24 hr at 40000 r.p.m. in a Sorvall TV865B vertical rotor at 15°. The gradients were fractionated by piercing the tube and collecting drops. 5 µl samples of each fraction were assayed by electrophoresis on a 1% agarose gel containing 0.5 µg/ml ethidium bromide, and nucleic acids visualised under long wavelength UV radiation (365 nm). Fractions containing HSV DNA, which is clearly distinguished from degraded cellular DNA and RNA, were combined, dialysed overnight against 0.1 x SSC at 4°, and stored at -20°.

HSV DNA suitable for nick translation was prepared by the method of Wilkie and Cortini (1976). Linear CsCl gradients of refractive indices 1.38 (bottom) to 1.36 (top), containing 20 µg/ml ethidium bromide, were prepared. 0.2-0.3 ml of phenol-extracted or isopyknic banded HSV-1 DNA at 100-500 µg/ml was layered onto each gradient and the

tubes centrifuged at 40000 r.p.m. for 110 min in a Beckman SW50.1 rotor. The band of purified virus DNA was visualised using a long wavelength (365 nm) UV Mineralight handlamp, and removed via an 18-gauge syringe needle. The DNA solution was saturated with CsCl and extracted twice with aqueous propan-2-ol saturated with CsCl to remove ethidium bromide. The lower aqueous phase was dialysed against NTE and precipitated with two volumes of ethanol at -20°. The DNA pellet was redissolved in a small volume of 0.1 x SSC and stored at -20°.

Preparation of PRV DNA

BHK cell monolayers in 80 oz roller bottles were infected with PRV at a m.o.i. of 1 in 300 and incubated at 37° until complete c.p.e. was observed. Virus was harvested by the method of Ben-Porat et al. (1974). The infected cell medium was centrifuged at 5000 r.p.m. for 10 min in a Sorvall GSA rotor, and virus in the supernatant was sedimented onto a 30% wt/vol sucrose "cushion" by centrifugation at 13500 r.p.m. for 1 hr in a Sorvall AH627 rotor. The virus was layered onto a linear sucrose gradient (15-30% wt/vol sucrose in TBSA: 0.01 M Tris-HCl pH 7.5 containing 8 g/l NaCl, 0.2 g/l KCl, 0.1 g/l $MgCl_2.6H_2O$, 0.1 g/l $CaCl_2.2H_2O$ and 10 g/l bovine serum albumin) and centrifuged in a Sorvall AH627 rotor at 10000 r.p.m. for 140 min. The visible virus band was removed by piercing the tube with an 18-gauge syringe needle, diluted sixfold with TBSA and centrifuged at 13500 r.p.m. for 1 hr in a Sorvall AH650 rotor. The virus pellet was resuspended in 5-10 ml NTE by sonication, and DNA prepared by phenol extraction as described for HSV.

Preparation of bacteriophage lambda DNA

Escherichia coli $met^-r^-m^-(cI_{857}S7)$ contains a lambda prophage which is heat inducible and lysis deficient. A starter culture in TP broth was grown at 31° and inoculated into autoclaved H-broth (5 g/l Difco bactopeptone, 1 g/l glucose, 5 g/l NaCl, 8 g/l Difco nutrient broth, pH 7.2) at a dilution of 1:50. The bacteria were grown at 31° in a Gallenkamp orbital incubator. At a cell density of 2-4 x 10^8 per ml the culture temperature was increased to 42° by the addition of boiling H-broth, and further incubation was at 42° for 10 min. The culture temperature was then reduced to 37° for 1-2 hr. The bacteria were centrifuged at 8000 r.p.m. for 2 min in an MSE 6 x 250 ml rotor and resuspended in 20-50 ml T2 buffer (0.05 M Na_2HPO_4, 0.022 M KH_2PO_4, 0.085 M NaCl, 0.001 M $MgSO_4$, 0.0001 M $CaCl_2$, 0.001% wt/vol gelatin). Chloroform was added to a final concentration of 1% vol/vol to lyse the cells, then DNase to 2 mg/ml. To each 10 ml of suspension 0.71 g polyethylene glycol 6000, 0.192 g NaCl and 0.023 g dextran sulphate were added with stirring, and this was left overnight at 4° to allow the bacteriophage to precipitate. The majority of the upper phase was removed and the remainder centrifuged at 2000 r.p.m. for 5 min. The material at the interface was removed and resuspended in about 2 ml of 1% dextran sulphate, adding 0.15 ml 3 M KCl per ml of suspension. After 2 hr at 4° the suspension was centrifuged at 2000 r.p.m. for 5 min, and the supernatant containing the virus removed. The virus was purified by centrifugation on a discontinuous caesium chloride gradient (specific densities of 1.7, 1.5 and 1.3 g/ml of CsCl in 0.005 M Tris-HCl pH 7.2, 0.005 M $MgSO_4$) at 35000 r.p.m. for 1 hr in a Beckman SW50.1 rotor. The band

of virus, in the phase of specific density 1.5, was dialysed against 0.5 x T2 buffer. Bacteriophage DNA was prepared by phenol extraction as described for HSV.

Estimation of DNA concentrations

Approximate estimations of DNA concentrations were obtained by visual comparison of samples with known amounts of DNA on ethidium-stained agarose gels under UV illumination (365 nm). DNA concentrations of RNA-free samples were more accurately estimated by optical density measurement at 260 nm, assuming that an optical density of 1.0 corresponds to a DNA concentration of 40 μg/ml. DNA concentrations of samples also containing RNA were estimated by the method of Hill and Whatley (1975). A solution of mithramycin at 200 μg/ml was made in 0.3 M $MgCl_2$. 15 μl was mixed with 0.3 ml DNA solution and fluorescence measured at 550 nm upon excitation at 440 nm. DNA of known concentration, either lambda or calf thymus DNA, was used to construct a standard curve in the range 0-10 μg/ml.

Purification of restriction endonucleases

Several of the restriction endonucleases used in the initial stages of this work were prepared from bacterial cell pastes (Table B1.1). Brain-heart infusion (BHI) at 37 g/l was used for bulk preparations, which were seeded at a dilution of 1:50 or 1:100 and grown in a Gallenkamp orbital incubator at 200-300 r.p.m. Bacteria were pelleted at early stationary phase by centrifugation in a 6 x 250 ml MSE rotor at 8000 r.p.m. for 2 min. They were resuspended in a small volume of BHI and frozen at -20°.

10-20 g of cell paste was homogenised in a Servall Omnimixer blade homogeniser with an equal volume of acid-

washed glass beads (150-200 μm). Homogenisation was performed on ice for 6 x 2 min periods at 10000 r.p.m. with 2 min intervals, and the extent of homogenisation was checked by microscopic examination. The glass beads were pelleted by centrifugation at 1000 r.p.m. for 5 min, and the supernatant centrifuged at 40000 r.p.m. for 1 hr in a Beckman SW40Ti rotor. The supernatant was made 1 M in NaCl, 10% vol/vol in glycerol and immediately subjected to column chromatography.

The following column chromatographic procedures were employed.

a) Molecular sieve. Proteins were separated according to molecular weight by passing the cell extract through a 2.5 x 100 cm column of Ultrogel AcA34 at 4°. The eluting buffer (CB) was 0.02 M Tris-HCl pH 7.5, 0.001 M EDTA, 0.01 M 2-mercaptoethanol, 10% vol/vol glycerol, and an LKB fraction collector was used with a flow rate of 20-50 ml/hr. 5 μl samples of alternate 6 ml fractions were assayed for nuclease activity as described below, and fractions containing restriction endonuclease activity were pooled and dialysed against CB.

b) Ion exchange. Diaminoethyl cellulose (DE52) or Biorex 70 columns of 5-20 ml bed volume were poured in 20 ml syringe barrels and equilibrated with CB. Dialysed Ultrogel fractions were applied, washed with CB, and eluted with KCl gradients in CB. Gradients of 0-0.6 M KCl were routinely used. Again, 5 μl aliquots of alternate 3 ml fractions were assayed for nuclease activity, and fractions containing restriction endonuclease activity were dialysed against CB. Both types of ion exchange column were used for most endonucleases (Table B1.2). The enzymes were concentrated by application

to small Biorex columns (2-5 ml), elution with 10 ml of 1 M KCl in CB, and dialysis against 50% vol/vol glycerol in CB. 1% wt/vol sterile bovine serum albumin was added to the enzyme to a final concentration of 50 μg/ml, and storage was at -20°.

Restriction endonuclease digestion

All restriction digests were carried out in 0.006 M Tris-HCl pH 7.5, 0.006 M $MgCl_2$, 0.006 M 2-mercaptoethanol, 0.02 M KCl, and 0.02 or 0.1% wt/vol sterile bovine serum albumin. Restriction sites are given in Table B1.4.

In the preparation of restriction endonucleases, 5 μl aliquots of alternate fractions were incubated at 37° for 3 hr with 0.5 μg lambda DNA in 50 μl restriction buffer. Purified endonucleases were titrated against 1 μg lambda DNA in 50 μl for 3 hr at 37°, in order to determine the amount of enzyme required for complete digestion of the DNA. DNA, either unlabelled or nick translated, was incubated at 10-100 μg/ml for 1-3 hr at 37° with the required amount of enzyme. TaqI, BstNI and BclI digestions were done at 65°. 0.15 μg lambda DNA was added to restriction digests containing small amounts of in vivo ^{32}P-labelled HSV DNA or restriction fragments isolated from nick translated HSV DNA.

Restriction digests were terminated by the addition of 10 μl of dye-Ficoll, which contained 10% wt/vol Ficoll 400, 0.1 M EDTA and 0.1% wt/vol bromophenol blue in 5 x E buffer (see below).

Agarose gel electrophoresis

Agarose gel concentrations between 0.4 and 2.0% wt/vol were employed, in E buffer (0.036 M Trizma, 0.036 M NaH_2PO_4,

0.001 M EDTA, pH 7.8) containing 0.5 μg/ml ethidium bromide. The agarose was dissolved by boiling in a Tappan microwave oven and cooled to about 50° before pouring. Horizontal gels were routinely used, and DNA samples with added dye-Ficoll were subjected to electrophoresis at 2 V/cm for 16-20 hr at room temperature in E buffer containing 0.5 μg/ml ethidium bromide. Gels were photographed under long wavelength (365 nm) UV irradiation using Polaroid type 105 or type 107C film. Gels for autoradiography were dried on plain glass plates in an oven at 60°.

Polyacrylamide gel electrophoresis

A stock solution of 29% wt/vol acrylamide and 1% wt/vol NN'-methylenebisacrylamide in water was diluted to the required concentration with the addition of 10 x TBE to a final concentration of 1 x TBE (0.089 M Trizma, 0.089 M boric acid, 0.0025 M EDTA) and 10% wt/vol ammonium persulphate to 0.067%. 60 ml of this solution was degassed using a vacuum pump and 30 μl N,N,N',N'-tetramethylethylenediamine (TEMED) added, and the gel poured (265 mm x 165 mm x 1.5 mm). Electrophoresis was carried out in 1 x TBE at 3 V/cm for 10-20 hr at room temperature, and the gels were dried on Whatman 3MM paper under vacuum in a Biorad heated gel drier.

General labelling of DNA with ^{32}P

Nick translation of DNA *in vitro* was done essentially according to the method of Rigby *et al.* (1977). 180 pmoles of each of α-^{32}P dATP, dGTP, dCTP and dTTP (350 Ci/mmole) were added to a tube and dried in an Edwards lyophiliser. 1 μg DNA in 100 μl nick translation buffer (0.05 M Tris-HCl pH 7.8, 0.005 M $MgCl_2$, 50 μg/ml bovine serum albumin, 0.005 M

2-mercaptoethanol) was added to the tube on ice. 2.5 units DNA polymerase I were added and tube incubated at 14-15° for 90 min. DNA possessing fewer nicks than HSV DNA, such as that from bacteriophage lambda and ØX174, or plasmid DNA, was incubated with the addition of DNase to 10^{-3} or 10^{-4} mg/ml. Isotope incorporation was monitored by spotting 2 µl samples onto filter discs and precipitating the DNA with 5% wt/vol trichloracetic acid for 15 min on ice. After washing in ethanol then acetone the discs were dried and counted for ^{32}P in scintillant. This method produced nick translated DNA of specific activity 1-2 x 10^8 c.p.m./µg, maximal incorporation (30-70%) being reached at 90 min. The nick translation reaction was terminated either by adding EDTA to a final concentration of 0.01 M followed by heating at 65° for 10 min, or by phenol extraction. Labelled DNA was separated from unincorporated deoxynucleoside triphosphates by elution through a 15 cm column of Sephadex G50 in NTE.

HSV DNA was ^{32}P-labelled *in vivo* as described by Preston *et al.* (1978). 50 mm Petri dishes were seeded with 2 x 10^6 cells/plate in EC1-Pi and incubated at 37° overnight. Virus was added in 0.2 ml EC1-Pi at a m.o.i. of 5 p.f.u./cell, incubated at 31° for 1 hr, the monolayers washed twice with EC1-Pi, and 2 ml EC1-Pi added. After a further 2-3 hr at 31°, 0.25-0.5 mCi of carrier-free ^{32}P-orthophosphate was added in 0.5 ml EC1-Pi, and the plates were incubated at 31° for 2-3 days. The infected cells were removed with a rubber policeman or bent Pasteur pipette and centrifuged at 2000 r.p.m. for 5 min. Each cell pellet was extracted once with 5 ml 0.5% NP40 in RSB. The cytoplasmic supernatant was combined with the medium and centrifuged at 35000 r.p.m. for 1 hr at 4° in an Oakridge tube in a Beckman Ti50 rotor.

The virus pellet was resuspended by sonication in 0.5 ml NTE, then 50 µl 25% wt/vol sodium dodecyl sulphate and 0.5 ml NTE-saturated phenol were added to the virus suspension in a 1.5 ml Eppendorf capped plastic tube. After gentle agitation the tube was centrifuged for 5 min at 2000 r.p.m., and the upper phase removed and extracted with 0.5 ml isoamyl alcohol in chloroform (1:24). Boiled RNase A and T1 were added to the removed upper phase to final concentrations of 25 µg/ml and 50 U/ml respectively, and the DNA dialysed overnight against 0.1 x SSC at room temperature. 10 µl samples were scintillation counted in 2 ml water in order to equalise counts before restriction and gel analysis.

Isolation of restriction fragments

The method of Wilkie and Cortini (1976) was used to isolate restriction fragments from agarose gels. Unlabelled fragments were located by staining either the whole horizontal gel or a strip from each edge with 0.5 µg/ml ethidium bromide in E buffer, and nick translated fragments were excised from agarose tube gels (1.5 x 18 cm) which had been electrophoresed in the presence of 0.5 µg/ml ethidium bromide. Agarose fragments were dissolved in 2-10 ml 5 M sodium perchlorate at 60° and applied to hydroxylapatite columns of bed volumes 0.2-1.0 ml in syringe barrels. Application and elution of the DNA were done at 60°. The columns were washed with 5 x 1 ml 5 M sodium perchlorate and then with 2 x 1 ml 0.14 M sodium phosphate pH 6.8. Double-stranded DNA was eluted with 4 x 0.5 ml 0.4 M sodium phosphate pH 6.8, and dialysed overnight against 0.1 x SSC. Radioactive samples were scintillation counted for Cerenkov radiation from ^{32}P.

Transfer of DNA fragments to nitrocellulose

The blotting technique of Southern (1975) involves the immobilisation of single stranded DNA on a nitrocellulose filter. Restricted HSV DNA was electrophoretically separated on an agarose gel (between 0.4 and 1.0% wt/vol) in a single slot spanning the gel. The gel was soaked for 1 hr in gel soak I (0.2 M NaOH, 1 M NaCl) and then for 1 hr in gel soak II (1 M Tris-HCl pH 8, 1 M NaCl). The gel was transferred to two sheets of Whatman 3MM paper on a glass plate supported in a tray of 2 l of 6 x SSC. A sheet of moistened nitrocellulose then a sheet of 3MM paper were applied to the gel surface, followed by a weighted stack of cut paper towels. Transfer of the single stranded DNA to the nitrocellulose was allowed to occur overnight at room temperature. The nitrocellulose sheet was washed in 3 x SSC, dried in air, then baked in a vacuum oven at 70° for 2 hr. It was then used either for strip or cross-blot hybridisation.

Blot hybridisation

Hybridisation at 75° in aqueous solution (Wilkie, 1976) or at 45° in 50% vol/vol formamide (McConaughy et al., 1969) was used. Nick translated probe DNA was denatured by heating at 100° for 5 min in 0.1 M NaOH, and then neutralised on ice with 1 M HCl after the addition of 1 M Tris-HCl pH 7.5 to a concentration of 0.05 M. 10^6-10^7 c.p.m. of ^{32}P-DNA was routinely used per hybridisation. 5 mm strips were cut from a nitrocellulose blot and incubated in a shaking water bath at 75° for 1 hr in 15 ml plastic screw-top tubes containing 5 ml of 3 x SSC and Denhardt's solution (Denhardt, 1966; 0.02% wt/vol Ficoll 400, 0.02% wt/vol polyvinylpyrrolidone, 0.02% wt/vol bovine serum albumin). Hybridisations carried out in 50% formamide in 3 x SSC and Denhardt's solution were

incubated at 45°. The tubes were drained and the denatured probe added after being made up to 5 ml in 3 x SSC and Denhardt's solution, or 50% formamide in 3 x SSC and Denhardt's solution. Hybridisation was allowed to occur for 2-4 days at 75° or 45° in a shaking water bath, and then the strips were washed with 2 x SSC at 60° for at least six hours with several changes of solution. The strips were dried in air, applied to a glass plate with double-sided Sellotape, baked at 70° for 30 min, and autoradiographed.

The method of cross-blot hybridisation, developed by Hutchison (Sato et al., 1977), involves the simultaneous transfer of denatured nick translated DNA fragments from an agarose gel to a nitrocellulose sheet containing immobilised unlabelled DNA fragments. Hybridisation occurs in situ, and homology between fragments is detected as spots on an autoradiograph. A square nitrocellulose blot was prepared as described and soaked for 1 hr at 75° in 4 x SSC and 0.1% wt/vol sodium dodecyl sulphate. 1-2 μg nick translated HSV DNA was restricted and electrophoretically separated on an agarose gel of equivalent dimensions to the first gel, and then autoradiographed. The gel was treated with gel soak I then II, and DNA fragments transferred to the nitrocellulose sheet turned through 90° so that the DNA bands on the sheet and on the gel were perpendicular. Transfer was carried out overnight in 4 x SSC and 0.1% sodium dodecyl sulphate at 75° in an oven. All components of the system except the gel were heated to 75° before commencing transfer. The nitrocellulose sheet was then washed extensively with 3 x SSC at 60°. In order to orientate the unlabelled dimension, a strip was removed from the sheet before cross-blotting and hybridised

with 10^6 c.p.m. of unrestricted nick translated HSV DNA. The strip was washed with the sheet and reassembled alongside it. The assembly was autoradiographed after drying and baking. The labelled dimension was orientated by comparing the autoradiograph of the cross-blot with that of the wet gel containing the nick translated fragments.

Autoradiography

Gels were autoradiographed either at room temperature with Kodak Kodirex film or with Kodak X-Omat H film, or at -70° with preflashed Kodak X-Omat film and a Dupont Cronex phosphotungstate screen.

Restriction fragment nomenclature

Gel electrophoresis of restricted DNA gives a characteristic number of bands which are separated according to molecular weight. Bands are given letters alphabetically or numbers sequentially from slower to faster migrating bands (larger to smaller fragments). This also applies to bands which are less than molar, such as those generated from the terminal and joint fragments of HSV DNA. The only exceptions to this are bands resulting from sequence additions to other fragments. For example, the joint fragments HSV-1 BamHI k and HSV-2 BamHI g consist of HSV-1 BamHI k and HSV-2 BamHI g, respectively, with an additional a sequence (see, for example, Figure C1.3). A band which is more than molar, and yet represents only one sequence because the constituent fragments originate from entirely within a repeat sequence, is given only one designation (e.g. HSV-2 BamHI p, g', m' in Figure C1.20). Fragments which comigrate in the gel system used are designated from left to right on the genome map (e.g. HSV-2 BamHI hij in Figure C1.20).

Calculation of relative molarities of restriction fragments

Autoradiographs were scanned using a Joyce-Loebl autodensitometer, and peaks were cut out and weighed. Molar ratios of individual fragments were calculated from the following formula.

$$\text{Molar ratio} = \frac{\text{wt of peak}}{\text{total wt of peaks}} \times \frac{\text{m.wt. of genome}}{\text{m.wt. of fragment}}$$

Rationale for mapping restriction sites

The purpose of restriction mapping is to relate the restriction profile to the physical location of restriction fragments within the genome. In this study the following methods were used.

1. Recleavage experiments. Individual ^{32}P-labelled restriction fragments were isolated and recleaved with a second enzyme, and the products subjected to electrophoresis alongside the digest of the whole genome with the second enzyme. This method shows which fragments produced by the second enzyme are within the recleaved fragment (products corresponding to bands in the restriction profile of the genome with the second enzyme), and which of the product fragments are at the ends of the recleaved fragment (bands not corresponding). Large parts of the physical map for the second enzyme may then be derived, especially when the map for the first enzyme is already known. This method may be reversed by recleaving fragments produced by the second endonuclease with the first.

2. Double digest experiments. ^{32}P-labelled DNA was cleaved simultaneously with two endonucleases. If a band is present in the single digest profile but not in the double digest profile, this indicates that the fragment contains a restriction site for the second enzyme. The apparent presence of a band in the double digest does not necessarily show that the fragment was uncleaved, as a fragment of larger molecular weight may have been cleaved to give a product which fortuitously comigrated with the band under consideration.

3. Hybridisation experiments. ^{32}P-labelled isolated fragments were hybridised to a nitrocellulose blot of separated restriction fragments produced by another endonuclease. Alternatively the cross-blot method was used, but the data obtained were essentially the same. These methods, taken in conjunction with recleavage experiments, show which fragments are within the complementary fragment and which extend past its ends.

Contamination of restriction fragments isolated from gels

Digested nick translated DNA produced a background of contaminating sequences in preparative gels, especially in the high molecular weight region. This is a feature of the nick translation reaction, but was sometimes due to contamination of the restriction endonuclease with non-specific nucleases. Bands in close proximity to others tended to be contaminated with those specific sequences. Therefore, isolated fragments were electrophoretically separated both uncleaved, and cleaved with an endonuclease of known physical map, in order to ensure identity and estimate purity.

Faint bands on autoradiographs were presumed to result either from general sequence contamination or from proximal band contamination (e.g. in Figure C1.8, KpnI k was not cleaved by HindIII, but it was contaminated both generally, and specifically by KpnI l). In nearly all recleavage experiments some of the parent fragment was refractory to digestion, despite complete digestion of carrier lambda DNA, and the absence of bands resulting from partial digestion of the fragment. The reason for this observation is unknown.

Sequence contamination was also detected in hybridisation experiments involving isolated nick translated fragments.

Generation of bacterial clones bearing recombinant plasmids with HSV DNA inserts

HSV DNA fragments were ligated into the vector pAT153 (Twigg and Sherratt, 1980), which is a deletion mutant of pBR322 unable to undergo conjugal transfer and which carries genes conferring resistance to tetracycline and ampicillin. Recombinant plasmids were transfected into the host Escherichia coli K12 strain HB101 (Boyer and Roulland-Dussoix, 1969). The host was grown in L-broth (0.17 M NaCl, 10 g/l Difco bactotryptone, 5 g/l yeast extract) at 37° in a Gallenkamp orbital incubator. Transformed bacteria bearing pAT153 were grown in L-broth containing 100 μg/ml ampicillin. Stocks were prepared by pelleting bacteria from a 20 ml overnight culture by centrifugation at 8000 r.p.m. for 2 min, and resuspending in 10 ml 40% vol/vol glycerol in 1% wt/vol Difco bactopeptone before storing at -20° in 1 ml aliquots.

HSV-2 HindIII fragments were ligated into pAT153 essentially by the method of Tanaka and Weisblum (1975). 3 μg HSV-2 strain HG52 DNA and .1 μg pAT 153 DNA were

separately digested with HindIII and then heated at 70° for 10 min to inactivate the endonuclease. The two reactions were mixed and appropriate solutions added to bring the final conditions to 0.02 M Tris-HCl pH 7.5, 0.01 M $MgCl_2$, 0.0005 M ATP, 0.01 M dithiothreitol, and 10 U/ml T4 DNA ligase in a final volume of 50 µl. The mixture was incubated at 4° for 16 hr.

The joint fragments HSV-1 BamHI k (strains 17 and USA-8) and HSV-2 BamHI g (strain HG52), and also HSV-1 BamHI y (strain 17), were isolated by hydroxylapatite chromatography (HSV-1 BamHI y was isolated by M-J. Murchie) after electrophoresis of 100 µg digested DNA on agarose gels in the absence of ethidium bromide. The fragments were ligated in 20 µl reaction volumes as described above, using between 0.1 and 0.5 µg BamHI-cleaved pAT153 DNA and the amount of HSV fragment possessing three to five times as many BamHI termini as the pAT153 DNA.

Ligated mixtures were transfected into the host bacterium essentially as described by Cohen et al. (1972), after checking ligation by gel electrophoresis alongside unligated and recleaved reaction mixtures. All procedures involving the use of living bacteria subsequent to ligation of DNA were performed under Category II conditions, having obtained the approval of the Genetic Manipulation Advisory Group. E. coli K12 strain HB101 cells were grown to an optical density of 0.6 at 630 nm, harvested by centrifugation at 8000 r.p.m. for 2 min, washed by resuspending in 400 ml 0.01 M NaCl and again centrifuging. The pellet was resuspended in 400 ml 0.03 M $CaCl_2$, kept on ice for 20 min, and harvested as before. The bacteria were resuspended in 9.4 ml

of 15% vol/vol glycerol in 0.03 M $CaCl_2$, and 0.2 ml aliquots stored at -70°. Under Category II containment conditions aliquots were mixed in 15 ml capped plastic tubes with ligated DNA samples, kept on ice for 30 min, then incubated at 37° for 5 min. 3 ml L-broth was added and the tubes aerated by bubbling at 37° for 90 min. 0.1 ml aliquots were spread on 90 mm L-broth agar plates containing 100 μg/ml ampicillin, and incubation was at 37° overnight. Appropriate controls were included in the transfections: undigested pAT153 DNA, cleaved pAT153 DNA, and cleaved pAT153 DNA which had been ligated.

The hybridisation technique of Grunstein and Hogness (1975) was used to identify colonies bearing recombinant plasmids. Sterile gridded nitrocellulose filters were boiled thrice in water, autoclaved and baked. Bacterial colonies were transferred using sterile cocktail sticks onto the grids on L-broth agar plates containing 100 μg/ml ampicillin, and also to a microtitre plate containing 0.2 ml/well L-broth plus ampicillin. Grids were incubated at 37° overnight and the microtitre plates were kept at room temperature. The nitrocellulose filters were transferred with colonies uppermost in turn at room temperature to filter papers soaked with 0.1 M HCl (1 min), 0.5 M NaOH (15 min), 1 M Tris-HCl pH 7.5 (5 min), and 1.2 M NaCl (15 min), respectively. The filters were dried in air and baked at 70° *in vacuo* for 2 hr. Preincubation and hybridisation of the filters were carried out in 50% formamide at 45° as described for blot hybridisation. Nick translated HSV-2 DNA was used as probe for HSV-2 *Hind*III clones, and the individually isolated restriction fragments for HSV-1 *Bam*HI K and y and HSV-2 *Bam*HI g. Colonies bearing plasmids containing HSV DNA inserts were identified by

autoradiography. Stocks were prepared from the microtitre plate cultures after a further step of colony-purification. The identity of plasmid DNA was ascertained by hybridisation of nick translated plasmid DNA to blot strips of HSV restriction digests, and by restriction mapping of the plasmid in comparison with HSV genome restriction maps.

Between 1 and 10% of ampicillin-resistant colonies were positive for HSV DNA inserts. 37 HSV-2 HindIII clones were characterised, 8 HSV-1 strain 17 BamHI k clones, 3 HSV-1 strain USA-8 BamHI k clones, 9 HSV-2 BamHI g clones, and 1 HSV-1 strain 17 BamHI y clone. Table B1.3 shows the equivalent standard nomenclature for trivial names of the clones used in this work.

Plasmid DNA was isolated essentially as described by Clewell and Helinski (1970), after lysis of bacteria with a neutral detergent (Komano and Sinsheimer, 1968). 1-2 l of L-broth containing 100 µg/ml ampicillin was inoculated with an overnight culture of 10-20 ml bacteria, and grown overnight at 37° in a Gallenkamp orbital incubator. Chloroform was added to 1% vol/vol and the bacteria harvested after 20 min by centrifugation at 8000 r.p.m. for 2 min. The pellet was resuspended in 25% wt/vol sucrose in 0.05 M Tris-HCl pH 8, 10 ml of 5 mg/ml lysozyme was added, and the mixture was kept on ice for 30 min. Incubation on ice was continued for 30 min after the addition of 10 ml 0.2 M EDTA, and then 133 µl NP40 was added. The tube was mixed gently, kept on ice for 10 min, and centrifuged at 20000 r.p.m. for 90 min in a Sorvall SS34 rotor. The supernatant was extracted twice by vigorous shaking with NTE-equilibrated phenol, extracted once with chloroform, and 3 M sodium acetate pH 7.5 was added

to a final concentration of 0.3 M. Nucleic acids were precipitated by the addition of half a volume of propan-2-ol, and pelleted by centrifugation at 2000 r.p.m. for 30 min after 1 hr at room temperature. The pellet was resuspended in 20 ml water, and 25.5 g solid caesium chloride and 1.7 ml ethidium bromide at 10 mg/ml added, and water added to a volume of 34 ml. This was divided between two 17 ml tubes and centrifuged for 20 hr at 15° in a Sorvall TV865B vertical rotor. The resulting band of plasmid DNA in each tube was visualised by long wave UV irradiation (365 nm) and removed by piercing the tube with a syringe needle. The volume of the DNA solution was increased to 17 ml by the addition of 0.75 g/ml caesium chloride containing 0.5 mg/ml ethidium bromide. Plasmid DNA was banded a second time by centrifugation as described above, removed from the tube via a syringe needle, saturated with CsCl, and extracted twice with aqueous propan-2-ol saturated with CsCl to remove ethidium bromide. Purified plasmid DNA was dialysed overnight against NTE, ethanol precipitated, and redissolved in 1-2 ml 0.1 x SSC. One litre of bacterial culture generally yielded 0.5-1 mg of plasmid DNA.

Labelling of DNA termini with ^{32}P

Restriction fragments from cloned DNA were labelled at their 5' termini essentially according to the method of Maxam and Gilbert (1980). 1-4 μg of a DNA fragment isolated from an agarose gel was digested in 50 μl of restriction buffer with the restriction endonuclease cleaving at the sites to be labelled (see Table B1.4). 50 μl of 0.05 M Tris-HCl pH 8 and 20 U bacterial alkaline phosphatase were added and incubated at 65° for 1 hr. The DNA was

extracted with 100 µl of NTE-saturated phenol, ethanol precipitated, washed with 95% vol/vol ethanol, and lyophilised. The DNA was dissolved in 20 µl of 0.05 M Tris-HCl pH 7.6, 0.01 M $MgCl_2$, 0.005 M dithiothreitol, 0.0001 M spermidine, 0.0001 M EDTA. In the case of a restriction site not giving a protruding 5' terminus, the reaction mix was heated at 65° for 2 min and quenched on ice. 5 µl γ-^{32}P-ATP (5000 Ci/mmole) and 20 U T4 polynucleotide kinase were added and the reaction incubated at 37° for 30 min. The reaction mixture was increased in volume to 50 µl with NTE, phenol extracted, ethanol precipitated, washed with 95% ethanol, and lyophilised. Usually the DNA was then restricted in 20 µl with a second restriction endonuclease to give uniquely labelled fragments, 20 µl dyes in glycerol (0.025% wt/vol bromophenol blue, 0.025% wt/vol xylene cyanol FF in 10% vol/vol glycerol) added, and the fragments separated by electrophoresis on a 4% polyacrylamide gel in 0.55 x TBE at 20 V/cm.

In one experiment (see Figure C3.16), TaqI sites were labelled at 3' termini. 2 µg DNA cleaved with TaqI was incubated at 15° for 1 hr in 50 µl of 0.05 M Tris-HCl pH 7.8, 0.005 M $MgCl_2$, 0.001 M dithiothreitol containing 5 µl α-^{32}P-dCTP and 5 U of the Klenow fragment of E. coli DNA polymerase I. The 3' labelled TaqI fragments were separated by electrophoresis on an 8% polyacrylamide 9.3 M urea sequencing gel (described below). The required fragment fortuitously migrated as two separate strands which were isolated directly from the gel.

Polyacrylamide gels containing terminally-labelled restriction fragments were autoradiographed wet with radioactive ink markers, and bands excised and transferred to 1.5 ml Eppendorf tubes. The polyacrylamide slices were

crushed with siliconised glass rods, 0.6 ml of 0.1% wt/vol sodium dodecyl sulphate in NTE added to each tube and incubated overnight at 37°. The tubes were centrifuged in an Eppendorf centrifuge for 5 min, the supernatant removed and the pellet re-extracted with 0.6 ml 0.1% sodium dodecyl sulphate in NTE. The two supernatants were combined in a 15 ml capped plastic tube, and ethanol precipitated in the presence of 30 µg yeast RNA. After centrifugation at 2000 r.p.m. for 30 min the radioactive pellets were redissolved in 0.2 ml 0.1 M sodium acetate pH 7.5, and 0.1 ml 1% wt/vol cetyltrimethylammonium bromide was added to each. The 1.5 ml Eppendorf tubes were kept on ice for 15 min and then centrifuged at 15000 r.p.m. for 15 min at 4° in an MSE centrifuge. The pellets were washed twice with 0.1 M sodium acetate pH 7.5 in 70% ethanol, once with 70% ethanol, once with 95% ethanol, and then lyophilised. The DNA was dissolved in water prior to the chemical degradation reactions for nucleotide sequencing. In some experiments isolated fragments were labelled at both termini and so they were digested with a second endonuclease after the ethanol precipitation step, and uniquely labelled fragments were isolated after electrophoresis on a second 4% polyacrylamide gel. In all the steps described above the whereabouts of the labelled DNA was followed using a radiation monitor.

Determination of the nucleotide sequence of DNA

The chemical degradation method was used (Maxam and Gilbert, 1980). Uniquely 5'-labelled DNA was dissolved in 25 µl water and aliquots put into five 1.5 ml Eppendorf tubes on ice. Reagents were then added as described below in order to modify guanine (G), guanine and adenine (G+A), adenine and to a lesser extent cytosine (A>C), cytosine and thymine (C+T), or cytosine (C) residues. After neutralising the reagents, the DNA was treated in each case with piperidine in order to cleave the DNA strand at the positions of modified residues. The treated DNA samples were then electrophoretically separated. For the chemical degradation reactions all reagents were ice-cold, and manipulations were carried out speedily and, where applicable, on ice. The whereabouts of the radioactive DNA was followed at all stages with a hand radiation monitor. Reaction times were established empirically.

1. G residues. 200 µl DMS buffer (0.05 M sodium cacodylate pH 8.0, 0.01 M $MgCl_2$, 0.001 M EDTA) and 1 µl sonicated calf thymus DNA at 1 mg/ml were added to 5 µl end-labelled DNA. 1 µl dimethylsulphate (DMS) was added in a fume hood to the tube, which was mixed and then incubated at 25° for 2-10 min. The tube was transferred to ice and 50 µl DMS stop added (1.5 M sodium acetate pH 7.0, 1 M 2-mercaptoethanol, 100 µg/ml tRNA) and rapidly mixed. 750 µl ethanol was added and mixed, and the tube put in an ethanol-solid CO_2 bath for 5 min (first ethanol precipitation). The tube was centrifuged for 5 min at 4° in an Eppendorf 5412 centrifuge, and then transferred to ice. The supernatant was removed with a Pasteur pipette and the pellet dissolved in 250 µl 0.3 M sodium acetate pH 6.5 containing 40 µg/ml yeast RNA.

750 µl ethanol was added, and the tube mixed and put for 5 min in an ethanol-solid CO_2 bath (second ethanol precipitation). The supernatant was removed after 5 min centrifugation, and 750 µl ethanol carefully added to the pellet. The tube was centrifuged for 2 min at 4° and the supernatant discarded. The pellet was dried in a lyophiliser. 100 µl 1 M piperidine was added and the tube heated at 90° for 30 min. The solution was frozen on solid CO_2 and lyophilised until no ice remained. 10 µl water was added to the dried pellet, mixed, frozen and lyophilised, and then this step was repeated. The final pellet was dissolved in 5-20 µl formamide-dyes (80% vol/vol formamide, 0.55 x TBE, 0.1% wt/vol xylene cyanol FF, 0.1% wt/vol bromophenol blue).

2. G+A residues. 17 µl water, 1 µl sonicated calf thymus DNA at 1 mg/ml, and 2 µl 1 M pyridinium formate pH 2 were added to 3 µl end-labelled DNA. The tube was incubated at 25° for 30-120 min, and then frozen and lyophilised. The pellet was dissolved in 20 µl water, frozen then lyophilised. Treatment with piperidine and subsequent steps were undertaken as for the G reaction.

3. A>C residues. 100 µl 1.2 M NaOH, 0.001 M EDTA and 1 µl sonicated calf thymus DNA at 1 mg/ml were added to 5 µl end-labelled DNA, and incubated at 90° for 2-10 min. 150 µl 1 M acetic acid, 1 µl yeast RNA at 10 mg/ml, and 750 µl ethanol were added. After the normal ethanol precipitation procedure, treatment with piperidine and subsequent steps were carried out as for the G reaction.

4. C+T residues. 15 µl water, 1 µl sonicated calf thymus DNA at 1 mg/ml, and 30 µl hydrazine were added with mixing to 5 µl end-labelled DNA, and incubated at 25° for 2-10 min.

200 µl hydrazine stop (0.3 M sodium acetate pH 6.5, 0.0001 M EDTA, 50 µg/ml tRNA) was then added and the tube mixed. 750 µl ethanol was added and the first ethanol precipitation carried out. The second ethanol precipitation and subsequent steps were done as for the G reaction.

5. C residues. The steps for the C+T reaction were carried out except that the 15 µl water in the hydrazine reaction was replaced by 15 µl 5 M NaCl.

Aliquots of 3-5 µl of reacted labelled DNA dissolved in formamide-dyes were heated at 100° for 2 min and quenched on ice, before loading onto sequencing gels. For gel preparation, stock solutions of deionised 20% wt/vol acrylamide (29:1 wt:wt acrylamide:NN'-methylenebisacrylamide) in 8 M urea, 10 x TBE, 10 M urea, and 10% wt/vol ammonium persulphate were used. Solutions of the appropriate acrylamide concentration were prepared by diluting the 20% acrylamide solution with 10 M urea. 10 x TBE and 10% ammonium persulphate were added to final concentrations of 0.55 x TBE and 0.067%, respectively, and the polymerising catalyst TEMED added to 0.0005% vol/vol. Sequencing gels of dimensions 450 x 230 x 0.5 mm were poured, using 60 ml solution for each gel. Three concentrations of gel were used: 6% polyacrylamide, 9.3 M urea; 8% polyacrylamide, 9.3 M urea; 19% polyacrylamide, 8 M urea. Pre-electrophoresis of gels was carried out for 1-1.5 hr at 40 W (1.5-2 kV) before loading the samples. Gels were subjected to electrophoresis at 40 W such that 19% polyacrylamide resolved 1-30 n (bromophenol blue marker 15 cm down gel), 8% polyacrylamide resolved 20-80 n (xylene cyanol marker 15 cm down gel) and 80-120 n (xylene cyanol marker to bottom of gel),

and 6% polyacrylamide resolved 120-150 n (xylene cyanol to bottom of gel) and 150 n and larger (second xylene cyanol marker applied when the first had reached the bottom of the gel, then allowed to migrate 15 cm down gel).

After electrophoresis one glass plate was removed. The gel was wrapped in a plain Sterilin bag and autoradiographed for 1-30 days at -70° using preflashed Kodak X-Omat H film and a Dupont Cronex phosphotungstate screen.

Table B1.1

Source bacteria for restriction endonucleases used in this study, with growth temperatures for batch liquid cultures.
Those bacteria not assigned a growth temperature were supplied as frozen cell pastes by the Microbiological Research Establishment, Porton Down, Wiltshire, and by Dr J. Arrand.

Table B1.1

restriction endonuclease	source bacterium	growth temperature ^{o}C
Xba I	Xanthomonas badrii	31
Hind III	Haemophilus influenzae R_d (exo^- mutant)	--
EcoR I	Escherichia coli RY13	--
Bgl II	Bacillus globigii	31
Hpa I	Haemophilus parainfluenzae	--
Kpn I	Klebsiella pneumoniae OK8	37
BamH I	Bacillus amyloliquefaciens H	--
Pvu II	Proteus vulgaris	37

Table B1.2

Elution of restriction endonucleases from ion exchange columns.
The molarity of KCl in the first column fraction containing restriction activity was calculated from the linear KCl gradient used for elution.

Table B1.2

restriction endonuclease	approx. eluting molarities (KCl)	
	DE52	Biorex
Xba I	0.2	--
Hind III	0	0.3
EcoR I	0.13	0.45
Bgl II	0.18	0.3
Hpa I	0.15	0.2
Kpn I	0.1	0.15
BamH I	0.1	0.3
Pvu II	0.15	0.4

Table B1.3

Nomenclature of recombinant plasmids bearing HSV-1 (pGX) or HSV-2 (pGZ) DNA inserts. Trivial names are used in the text.

The insert in pGZ12 consists of HSV-2 HindIII k and about 1 x 10^6 daltons of adjacent sequence from HSV-2 HindIII l (see Figure C2.4). It may have originated from a defective DNA molecule.

Table B1.3

cloned fragment	trivial name	standard name
HSV-2 HindIII a	a	pGZ26
HSV-2 HindIII b	b	pGZ11
HSV-2 HindIII e	e	pGZ25
HSV-2 HindIII h	h	pGZ10
HSV-2 HindIII l	l	pGZ23
HSV-2 HindIII n	n	pGZ32
HSV-2 HindIII o	o	pGZ34
HSV-2 HindIII l2	l2	pGZ12
HSV-2 BamHI g	g	pGZ6
HSV-2 BamHI $\bar{g}$	$\bar{g}$	pGZ1
HSV-1 BamHI y	y	pGX1
HSV-1 BamHI k	k1	pGX2
HSV-1 BamHI k	k2	pGX7
HSV-1 BamHI k	k3	pGX5
HSV-1 BamHI $\bar{k}$	$\bar{k}$1	pGX4
HSV-1 BamHI $\bar{k}$	$\bar{k}$2	pGX8
HSV-1 BamHI k (strain USA-8)	-	pGX10

Table B1.4

Sites for restriction endonucleases used in this work (from Roberts, 1981). Cleavage positions are shown by apostrophes.

Table B1.4

AluI	AG'CT	HindIII	A'AGCTT
AvaI	C'PyCGPuG	HinfI	G'ANTC
AvaII	G'GA/TCC	HpaI	GTT'AAC
BamHI	G'GATCC	KpnI	GGTAC'C
BclI	T'GATCA	MboII	GAAGANNNNNNNN'
BglI	GCCNNNN'NGGC	MnlI	CCTC (5-10 n. 3')
BglII	A'GATCT	PvuII	CAG'CTG
BstNI	CC'A/TCC	SacI	GAGCT'C
ClaI	AT'CGAT	Sau3AI	'GATC
DdeI	C'TNAG	SstI	GAGCT'C (as SacI)
EcoRI	G'AATTC	SstII	CCGC'GG
HaeII	PuGCGC'Py	SmaI	CCC'GGG
HaeIII	GG'CC	TaqI	T'CGA
HhaI	GCG'C	XbaI	T'CTAGA
HincII	GTPy'PuAC	XhoI	C'TCGAG

PART C

RESULTS

AND

DISCUSSION

SECTION 1

MAPPING OF RESTRICTION ENDONUCLEASE SITES ON HSV-1 AND HSV-2 DNA

RESULTS

Introduction

Restriction maps for the genomes of HSV-1 strain 17 and HSV-2 strain HG52 which were available at the commencement of this work were for XbaI, HindIII, EcoRI, BglII and HpaI, and also KpnI for strain HG52 (Wilkie, 1976; Cortini and Wilkie, 1978). These endonucleases produce relatively few restriction fragments from HSV DNA, and so the object of the work described here was to map HSV-1 and HSV-2 DNA for endonucleases which produce many fragments, in order to be able to define more closely those structural and functional aspects of the genome under investigation.

Molecular weight calibration

The standards used to determine the molecular weights of restriction fragments were the EcoRI digest of lambda DNA (Thomas and Davis, 1975), the HindII digest of ØX174 DNA (Maniatis et al., 1975), and the HindII digest of lambda DNA (Maniatis et al., 1975). These digests were used to calibrate molecular weights in the ranges of 1-10 x 10^6 0.1-1 x 10^6, and 0.07-0.1 x 10^6, respectively.

Figure C1.1 shows the gel used to calibrate the larger fragments of the KpnI, BamHI, XhoI, and HpaI digests of HSV-1 DNA. The larger fragments of HSV-1 PvuII were calibrated using HSV-1 HpaI fragment molecular weights (Wilkie, 1976). HSV-2 BglII fragment molecular weights (Cortini and Wilkie, 1978) were used to calibrate larger HSV-2 BamHI fragments.

Figure C1.2 shows the autoradiograph used to calibrate the smaller fragments of HSV-1 BamHI, HSV-1 XhoI, and HSV-2 BamHI. Comparison of HSV-1 XhoI with HSV-1 PvuII allowed

molecular weights of the smaller PvuII fragments to be determined. HSV-1 KpnI b' and c' were calibrated against the HSV-1 BamHI digest, and HSV-1 BamHI k'-n' against lambda HindII fragments on a polyacrylamide gel.

The distance migrated by standard bands was plotted against the logarithm of the molecular weight, and this curve was used to deduce molecular weights of restriction fragments (Tables C1.1-C1.5). It was assumed that the standard curve was valid for all restriction fragments; however, the nucleotide composition of DNA might affect its electrophoretic mobility and hence the apparent molecular weights of some fragments.

Relative molarities of restriction fragments

Figure C1.3 shows restriction profiles of *in vivo* ^{32}P-labelled HSV-1 and HSV-2 DNA. Autodensitometry of these, and other, autoradiographs was carried out to determine the relative molarities of bands in the profiles (Tables C1.1-C1.5). Potential sources of error in such estimations include inaccurate molecular weights, degradation of DNA, a non-linear response of film to radiation exposure, and fragment heterogeneity (for instance, a proportion of the HSV-1 BamHI joint fragment k contains two a sequences instead of one, and therefore comigrated with BamHI j). Nevertheless, in general the measured relative molarities are in agreement with those predicted from the physical maps (below). Each of the restriction endonucleases for which HSV-1 or HSV-2 DNA was mapped in this work has a site in one or both of the sets of inverted repetitions and therefore produces no quarter-molar fragments, since only endonucleases with no sites in repetitive sequences show this characteristic.

HSV-1 KpnI physical map: recleavage data

Figures C1.4-C1.5 show the products of KpnI cleavage of isolated HSV-1 restriction fragments of known map positions. Figure C1.6 shows a summary of these data. In the following argument molecular weights are given in millions.

1. The S segment.

Both HindIII g and BglII l gave KpnI k as an S terminal fragment, and EcoRI h products show KpnI h to map in U_S. Cleavage of EcoRI h and k indicates that S contains three KpnI fragments. The third KpnI fragment is larger than HindIII m (4.6), since this fragment was not cleaved by KpnI, and the products of HindIII n suggest that the molecular weight of the third fragment is 5.5 (4.6+0.9). Similarly, cleavage of EcoRI h and k implies a size of 5.4 (3.5+1.9) for the third fragment. This size corresponds to that of KpnI j, which indeed was a product of the cleavage of XbaI a, XbaI bc, HpaI cde, and BglII gh. Both KpnI j and k were half molar in the HSV-1 KpnI digest (Table C1.1). Therefore the order of KpnI fragments in S is j h k.

This conclusion was used to deduce which products of HpaI gh were produced from HpaI g and which from h, and the products are shown separately in Figure C1.6. Similar reasoning was applied with HpaI cde, BglII gh, and XbaI bc to determine the cleavage products of individual fragments.

2. TR_L/IR_L.

Fragments containing the L terminus gave KpnI r as a common product. Therefore KpnI r maps at the L terminus. This allows the products of HindIII h and i to be deduced from HindIII hi.

3. Joint fragments.

The sizes of L and S terminal fragments indicate that the two half molar joint fragments have molecular weights of 7.9 (5.5+2.4) and 7.2 (4.8+2.4). HpaI a gave KpnI a (8.0), and KpnI e (7.0) was a product of relevant cleavages (HpaI cde, BglII e, XbaI a, XbaI bc). The joint fragments are therefore KpnI a and e.

4. Junctions of TR_L/IR_L with U_L.

There is only one KpnI site in TR_L/IR_L, since only one KpnI fragment was common to both L termini (KpnI r). Therefore fragments adjacent to this site must extend into U_L, and hence must be greater in size than 3.6 (6.0-2.4). The only fragment from BglII f and XbaI d which meets this requirement is KpnI g. Similarly, the results from BglII j and k give a size of 8.1 (7.0+1.1) for the other junction fragment, and this corresponds to KpnI b. Therefore the fragments so far deduced are, as spaced along the genome,

r b .... g r / j h k.

5. U_L from left to right.

Recleavage of HindIII h and BglII k shows that KpnI y is adjacent to KpnI b. Cleavage of BglII o and k, bearing in mind that HpaI k and u were not cleaved by KpnI, indicates the size of the next fragment is 6.9 (4.6+2.3). This corresponds to KpnI f, which was produced from Xba I c. Comparison of BglII p and HpaI j cleavage shows that the next two fragments are KpnI q then w. In like manner the products of XbaI c, BglII m and HpaI i demonstrate that the next three fragments are KpnI i, b' then m. Therefore the order so far is

r b y f q w i b' m .... g r / j h k.

The next region contains KpnI n, p, t, v, x and a', according to cleavage of XbaI f. The fragment at the left hand end of this group is shown by cleavage of HpaI b to be KpnI t. Products of BglII i indicate that KpnI n and p are the next two fragments, but these cannot be ordered with respect to each other by recleavage methods because none of the other endonucleases cleaves within them. The next fragment is larger in size that 1.0, and cleavage of EcoRI m therefore shows the order of KpnI v, x, a' is v a' x. So the order of fragments within XbaI f is KpnI t (np) v a' x.

The next fragment is larger in molecular weight than 6.0, as shown by comparison of the products of HpaI h and EcoRI l. KpnI c, a product of BglII d, is the only fragment meeting this requirement. Cleavage of XbaI e, HindIII k, EcoRI a, BglII d and HpaI f show that the next three fragments are KpnI l c' s, and comparison of the BglII g, HpaI e and EcoRI i results demonstrate that these are followed by KpnI d z u. The final fragment, from cleavage of BglII f and XbaI d, is KpnI o.

In conclusion, the order of KpnI fragments within the HSV-1 genome is

r b y f q w i b' m t (np) v a' x c l c' s d z u o g r / j h k,

with KpnI a and e resulting from fusion at the joint of KpnI j and r, and k and r, respectively. This conclusion accounts for all the bands in the HSV-1 KpnI restriction profile in their respective molarities. No fragment smaller than KpnI c' has been detected.

Figure C1.6, showing a summary of cleavage data, gives the products of individual fragments within comigrating bands as already described, including HpaI opqr.

HSV-1 KpnI physical map: double digest data

Figure C1.7 shows the results of single and double digestion of HSV-1 DNA. Figures C1.8 -C1.9 show the results of cleaving isolated KpnI fragments with other endonucleases. These data are summarised in Table C1.6. The results are consistent with the physical map deduced from recleavage data.

HSV-1 KpnI physical map: hybridisation data

Hybridisation data are provided by the cross-blot shown in Figure C1.10. Each spot on the autoradiograph indicates a region of sequence homology between the two fragments concerned. For example, KpnI wx hybridised to HpaI b and j, and recleavage data show that KpnI w maps in HpaI j and KpnI x in HpaI b. The data are summarised in Figure C1.11, using the physical map already deduced, with which they are consistent. Homology between the L and S termini is explained by the presence at both termini of the terminal repetition, or a sequence, which is also present at the joint of the L and S segments.

HSV-1 KpnI physical map: relative order of fragments n and p

None of the previous methods allowed the ordering of KpnI n and p, as no relevant restriction site is present within the region containing them. Work described in Section 2 shows that the genomes of HSV-1 and HSV-2 are generally colinear, and studies of intertypic recombinants is in accord with this hypothesis (e.g. Preston et al., 1978). Therefore a restriction site within HSV-2 sequences homologous to HSV-1 KpnI n and p could be used to orientate these two fragments. The restriction site used was the BglII site between HSV-2 BglII j and o (Cortini and Wilkie, 1978).

In Figure C1.12 the result is shown of hybridising KpnI no and p to nitrocellulose blot strips containing HSV-1 HpaI or HSV-2 BglII restriction fragments. KpnI no hybridised to HSV-1 HpaI b, e and g. Presumably the former was owing to hybridisation of KpnI n and the latter two to KpnI o. Hybridisation to HpaI i was probably due to contamination of KpnI no by KpnI m. KpnI p hybridised to HpaI b only, showing that this fragment was relatively pure. With HSV-2 BglII blot strips KpnI no hybridised to BglII i and j but not o. Hybridisation to BglII i was presumably due to KpnI o and to BglII j by KpnI n, as they are differentiated by physical map position. KpnI p hybridised to BglII j and o. These results suggest that KpnI n and p are in the order n p in the HSV-1 genome.

HSV-1 KpnI physical map

The physical map consistent with all the data is shown in Figure C1.18.

Physical maps for other restriction endonucleases

The derivation of the HSV-1 KpnI physical map has been described, and maps of HSV-1 DNA for BamHI, PvuII and XhoI, and of HSV-2 DNA for BamHI were constructed in similar manner. Autoradiographs of recleavage, double digestion and hybridisation experiments have not been included, but instead are summarised in diagrammatic (Figures C1.13-C1.17) or tabular form (Tables C1.7-C1.12). Physical maps deduced from recleavage data were confirmed by double digestion and hybridisation experiments, except for HSV-1 PvuII, for which hybridisation experiments were not carried out. All detectable HSV-1 KpnI and BamHI fragments were mapped, but in the other digests an undetermined number of very small

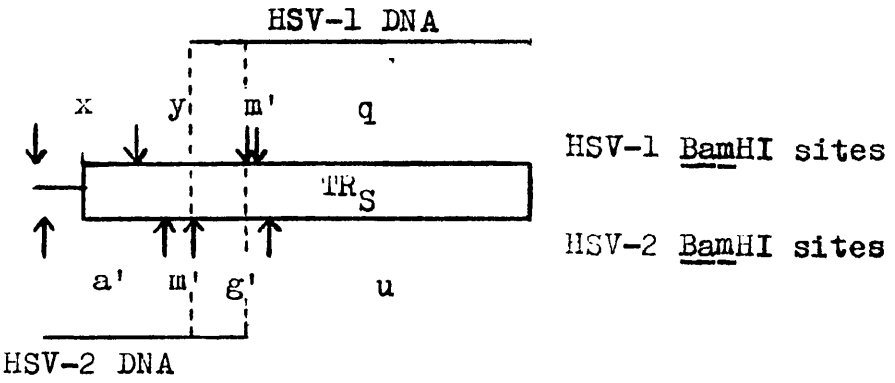
fragments were not resolved and therefore remained unmapped. Final restriction maps are shown in Figures C1.18 and C1.19, and aligned HSV-1 and HSV-2 maps in Figure C1.20.

The methods described above failed to unambiguously map a few fragments which were subsequently assigned as follows.

1. Recleavage experiments showed that HSV-1 BamHI l' and n' are located adjacent to BamHI z. The intertypic recombinant RE4 (Figure C4.26) possesses a crossover in HSV-1 BamHI z, and produced BamHI n' but not l', and therefore the order HSV-1 BamHI n' z l' is the most likely.

2. HSV-2 BamHI i and i' were ordered by inference from data concerning the intertypic recombinant RE4 (Figure C4.26). This recombinant possesses an insertion of the HSV-2 sequence spanning the HSV-2 BamHI i-r region in another region of the genome, as described in Section 4. The inserted piece of DNA does not include HSV-2 BamHI i', and therefore it is likely that i and r are contiguous.

3. Intertypic recombinants with a crossover in HSV-1 BamHI y as shown below lacked HSV-2 BamHI g', but produced a characteristically smaller fragment and HSV-2 BamHI m' (e.g. RE4 subclones 1, 5, 7, 15, 16, 17 in Figure C4.22). These results suggest that g' is adjacent to u in the HSV-2 BamHI map.



DISCUSSION

The general arrangement of the HSV genome has been adequately demonstrated and therefore was assumed in deducing the restriction maps presented here. The presence of TR_L/IR_L and TR_S/IR_S was confirmed. BamHI is the only restriction endonuclease, for which the entire HSV-2 genome has been mapped, which cleaves within both sets of repeats, thus demonstrating their presence.

The subsequently published KpnI and BamHI maps of HSV-1 strains F and Justin (Locker and Frenkel, 1979b) differ from those of HSV-1 strain 17 only in the presence or absence of a few restriction sites, but in general all three strains have very similar restriction maps. In contrast, few if any restriction sites are in equivalent positions in HSV-1 and HSV-2 DNA, even in the case of BamHI which has many more sites in HSV DNA than previously mapped endonucleases. This is true even of the more homologous regions between the two genomes (see Section 2 of Results). Such a large difference is of advantage in determining the genome structures of HSV-1/HSV-2 intertypic recombinants by restriction endonuclease analysis. It cannot be assumed that any restriction sites in the one genome are in equivalent positions in the other, and therefore restriction maps of comparable regions of the two genomes must be determined separately in each case.

Several lines of evidence suggest that the HSV-1 and HSV-2 genomes are colinear, as discussed in Section 2. This was assumed to apply to the region of U_L containing HSV-1 KpnI n and p in determining their relative order by hybridisation to HSV-2 restriction fragments (Figure C1.12). It is possible that colinearity does not apply

in this region, although the simplest interpretation of the recombinant genome analyses described by Chartrand et al. (1979 and 1980) is that colinearity does apply.

Restriction fragments originating from the joint and L terminus of both HSV-1 and HSV-2 DNA demonstrated an additional discrete but faint band migrating at a position 300-400 bp larger than the major species (e.g. HSV-1 BamHI $\bar{k}$ above BamHI k in Figure C1.3, and a faint band above HSV-1 HpaI m in Figure C1.7 track 11). In some autoradiographs very faint bands representing further discrete additions were observed. Fragments from the S terminus were present at this resolution as a single size class. These observations confirm those of Wagner and Summers (1978) for HSV-1 and Wilkie et al. (1977a)for HSV-2. Wagner and Summers (1978) concluded that the additional DNA comprises the a sequence and perhaps part of the b sequence. It contains, in fact, precisely the a sequence (Section 3 of Results).

The m.wt. of the HSV genome can be calculated by summation of m.wts.of the individual restriction fragments. The mean m.wt. of HSV-1 DNA, from the four digests dealt with here, is $96.4 \pm 2.5 \times 10^6$. Bearing in mind the presence of an unknown number of small unmapped restriction fragments in the XhoI and PvuII digests which have not been taken into account, the KpnI (96.7×10^6) and BamHI (97.4×10^6) m.wts. give the most reliable estimates. These sizes are in accord with that of 98×10^6 reported by Wilkie (1976). The m.wt. of HSV-2 DNA, from the BamHI digest, is 97.6×10^6, which compares well with the size of 98.5×10^6 reported by Cortini and Wilkie (1978). This figure includes three small fragments which were resolved by polyacrylamide gel electrophoresis but not mapped.

The mean m.wt. of the S segment of the HSV-1 genome is $16.8 \pm 0.5 \times 10^6$, and that for HSV-2 is 18.6×10^6 (compare Cortini and Wilkie, 1978: 17.9×10^6 for HSV-2). On the basis of these results it seems that the two serotypes differ in the m.wt. of S by $1\text{-}2 \times 10^6$ (1500-3000 bp). It is possible that the HSV-1 and HSV-2 L segments are of equal size, and therefore that the HSV-2 genome is larger than that of HSV-1 by $1\text{-}2 \times 10^6$ in m.wt. Morse *et al.* (1977) likewise mentioned that HSV-2 DNA is 3×10^6 larger in m.wt. than HSV-1 DNA, and Reyes *et al.* (1979) indicated that the extra DNA is in S. The precise location of the extra DNA in the HSV-2 S segment cannot be determined from the results presented here, but the alignment of restriction sites concluded from the recombinant analysis summarised in Figure C2.13 suggests that the major proportion is located in HSV-2 BamHI 1 in the middle of U_S. The function of the additional DNA is unknown.

The physical maps also provide information regarding the sizes of inverted repeats, which is summarised in Table C1.13. These results are in agreement with previously published estimates for HSV-1 and HSV-2. The size of HSV-1 TR_S (i.e. ca) has since been more accurately estimated as 6720 bp (m.wt. 4.37×10^6), from detailed restriction mapping and nucleotide sequencing experiments (Section 3 of Results; M-J. Murchie, personal communication).

Tables C1.1-C1.5

Molecular weights and relative molarities of restriction fragments of HSV-1 DNA produced by KpnI, BamHI, XhoI and PvuII, and of HSV-2 DNA produced by BamHI.

All restriction fragments of a digest could not be resolved on a gel of single concentration, so relative molarities of larger fragments were estimated from lower concentration gels, and those of smaller fragments from higher concentration gels. The results were linked using fragments of intermediate size which were resolved on both gel concentrations.

Table C1.1

HSV-1 Kpn I fragment(s)	mol. wt. x 10^{-6}	relative molarity (± standard deviation)	predicted relative molarity
abcd	8.0	2.9 (0.2)	3.5
efg	7.0	2.5 (0.2)	2.5
h	6.0	1.3 (0.2)	1.0
i	5.8	1.0 (0.2)	1.0
j	5.5	0.6 (0.1)	0.5
k	4.8	0.6 (0.1)	0.5
l	4.4	1.0 (0.1)	1.0
m	3.2	1.0 (0.1)	1.0
no	3.0	2.0 (0.2)	2.0
p	2.8	1.0 (0.1)	1.0
q	2.4	1.1 (0.1)	1.0
r	2.4	1.1 (0.1)	1.0
s	2.2	1.1 (0.1)	1.0
t	2.0	1.1 (0.1)	1.0
u	1.6	1.3 (0.2)	1.0
v	1.2	1.2 (0.1)	1.0
wx	1.1	2.4 (0.4)	2.0
yz	1.0	2.3 (0.3)	2.0
a'	0.8	1.0 (0.2)	1.0
b'	0.5	1.1 (0.2)	1.0
c'	0.42	1.1 (0.2)	1.0

Table C1.2

HSV-1 BamH I fragment(s)	mol. wt. x 10^{-6}	relative molarity (± standard deviation)	predicted relative molarity
a	8.0	0.8 (0.1)	1.0
b	6.2	1.1 (0.2)	1.0
c	6.0	1.0 (0.0)	1.0
d	5.6	1.0 (0.0)	1.0
e	5.4	1.0 (0.1)	1.0
f	5.0	1.1 (0.1)	1.0
g	4.9	1.0 (0.0)	1.0
h	4.8	0.9 (0.1)	1.0
i	4.3	1.1 (0.1)	1.0
j	4.2	1.3 (0.1)	1.0
k	3.9	0.9 (0.2)	1.0
l	3.5	1.1 (0.1)	1.0
m	3.3	1.2 (0.2)	1.0
n	3.2	1.2 (0.2)	1.0
o	2.4	1.1 (0.2)	1.0
pq	2.2	1.8 (0.2)	2.0
r	2.0	1.3 (0.2)	1.0
st	1.8	1.9 (0.1)	2.0
uv	1.7	1.7 (0.1)	2.0
w	1.5	1.0 (0.1)	1.0
xyz	1.3	4.4 (0.2)	4.0
a'	1.1	1.2 (0.1)	1.0
b'	0.90	1.2 (0.2)	1.0
c'	0.63	1.3 (0.2)	1.0
d'	0.58	1.3 (0.3)	1.0
e'	0.55	1.0 (0.1)	1.0
f'g'	0.51	2.8 (0.5)	2.0
h'	0.48	1.3 (0.1)	1.0
i'	0.45	1.2 (0.2)	1.0
j'	0.35	1.2 (0.2)	1.0
k'	0.14	---	1.0
l'	0.13	---	1.0
m'	0.10	---	2.0
n'	0.06	---	1.0

Table C1.3

HSV-1 *Xho* I fragment(s)	mol. wt. x 10^{-6}	relative molarity (±standard deviation)	predicted relative molarity
a	10.0	0.7 (0.1)	1.0
b	9.0	0.9 (0.1)	1.0
c	6.6	0.7 (0.0)	0.5
d	6.2	0.5 (0.0)	0.5
e	5.7	1.3 (0.0)	1.0
f	5.2	1.1 (0.1)	1.0
ghi	4.9	2.5 (0.2)	2.5
j	4.6	0.6 (0.0)	0.5
k	4.0	1.2 (0.1)	1.0
mn	2.5	1.8 (0.2)	2.0
o	2.3	1.2 (0.1)	1.0
pqrst	2.1	4.5 (0.7)	5.0
u	1.8	1.0 (0.1)	1.0
v	1.5	1.0 (0.1)	1.0
w	1.4	1.1 (0.1)	1.0
xyz	I.4	3.0 (0.1)	3.0
a'	1.4	0.9 (0.2)	1.0
b'	1.3	0.9 (0.1)	1.0
c'	1.2	0.9 (0.2)	1.0
d'	1.0	1.1 (0.3)	1.0
e'f'	0.83	2.2 (0.1)	2.0
g'	0.73	1.1 (0.2)	1.0
h'i'	0.62	1.7 (0.1)	2.0
j'	0.54	1.0 (0.1)	1.0
k'	0.43	0.8 (0.0)	1.0
l'm'n'	0.37	2.8 (1.0)	3.0
o'p'	0.30	1.4 (0.2)	2.0

Table C1.4

HSV-1 Pvu II fragment(s)	mol. wt. x 10^{-6}	relative molarity (±standard deviation)	predicted relative molarity
a	17	0.3 (0.1)	0.5
b	14	0.5 (0.1)	0.5
c	12	0.4 (0.1)	0.5
d	9	0.5 (0.1)	0.5
e	5.3	1.2 (0.2)	1.0
f	4.2	0.9 (0.1)	1.0
g	3.8	1.0 (0.1)	1.0
h	3.7	0.8 (0.1)	1.0
i	3.5	1.1 (0.1)	1.0
jklm	3.2	3.2 (0.5)	4.0
n	2.7	1.1 (0.1)	1.0
o	2.2	1.0 (0.1)	1.0
p	2.0	1.0 (0.1)	1.0
qrst	1.9	4.0 (0.6)	4.0
u	1.7	1.3 (0.2)	1.0
v	1.5	1.2 (0.2)	1.0
w	1.5	1.5 (0.2)	1.0
x	1.4	0.9 (0.1)	1.0
y	1.4	0.9 (0.2)	1.0
z	1.3	0.9 (0.1)	1.0
a'	1.25	0.9 (0.1)	1.0
b'c'	1.1	2.1 (0.2)	2.0
d'e'	1.05	1.8 (0.1)	2.0
f'	0.9	1.0 (0.1)	1.0
g'h'	0.83	2.1 (0.1)	2.0
i'	0.80	1.0 (0.0)	1.0
j'k'l'	0.70	2.9 (0.4)	3.0
m'n'o'	0.60	3.3 (0.2)	3.0
p'q'	0.40	---	2.0
r's'	0.38	---	2.0
t'	0.35	---	1.0
u'	0.33	---	1.0
v'	0.30	---	1.0
w'x'	0.28	---	2.0

Table C1.5

HSV-2 BamH I fragment(s)	mol. wt. x 10^{-6}	relative molarity (±standard deviation)	predicted relative molarity
ab	8.0	1.4 (0.3)	2.0
c	5.2	1.2 (0.0)	1.0
d	4.9	1.1 (0.3)	1.0
e	4.7	1.0 (0.3)	1.0
f	4.3	1.2 (0.2)	1.0
g	3.7	1.0 (0.1)	1.0
hij	3.3	3.2 (0.2)	3.0
k	3.1	1.1 (0.2)	1.0
l	2.8	1.3 (0.2)	1.0
m	2.6	1.2 (0.2)	1.0
n	2.4	1.2 (0.2)	1.0
o	2.3	1.3 (0.2)	1.0
pq	2.2	3.5 (0.2)	3.0
r	2.0	1.4 (0.2)	1.0
st	1.9	2.6 (0.4)	2.0
u	1.8	0.9 (0.1)	1.0
vw	1.7	2.0 (0.2)	2.0
x	1.7	1.0 (0.1)	1.0
yz	1.6	1.8 (0.2)	2.0
a'	1.5	0.7 (0.1)	1.0
b'	1.3	1.0 (0.3)	1.0
c'a'	1.2	1.8 (0.2)	2.0
e'	1.15	0.9 (0.1)	1.0
f'	1.05	1.2 (0.1)	1.0
g'	0.96	1.6 (0.1)	2.0
h'	0.80	1.0 (0.1)	1.0
i'	0.70	1.3 (0.3)	1.0
j'	0.58	1.2 (0.6)	1.0
k'l'	0.47	2.1 (0.8)	2.0
m'	0.39	---	2.0

Table C1.6

Digestion of HSV-1 Kpn I fragments by other restriction endonucleases. The summarised data are taken from Figures C1.7-9.
Molecular weights refer to the product fragments of recleavage of purified Kpn I fragments.
Letters are given when a product fragment corresponds to a fragment produced by the restriction of HSV-1 DNA with the recleaving endonuclease alone.

x this fragment is not recleaved

* this fragment is recleaved, but the product sizes are not known

Table C1.6

recleaved band (Kpn I)	recleaving endonuclease (product fragments in megadaltons)				
	Xba I	Hind III	EcoR I	Bgl II	Hpa I
a	x	x	6 5.4 4.0	x	x
b	6.5 4.4	7 5.4	4.0 l 3.0	x	6 6 4.0
c	4.2 3.6	5.0 o	2.6 2.1	x	2.3 2.3
d	3.4 1.6	0.6	o 0.8	7 0.7	o 1.4
e	x	x	x	x	x
f	x	x	x	x	k r s 1.6
g	x	x	6.1 1.1	4.6 2.2	1.5 u v
h	x	3.5 2.6	x	4.8 1.2	4.0 2.0
i	x	3.1 2.7	x	n 1.2 0.7	n 1.3 1.0
j	x	m 0.6	k 2.1	x	x
k	x	x	k 1.1	x	x
l	x	3.9 0.5	x	x	3.5 0.9
m	3.0 0.2	x	n 0.9 0.8	2.5 0.7	x
n	x	x	x	x	x
o	x	x	2.0 1.0	x	2.0 1.0
p	x	x	x	x	x

Table C1.6 (continued)

recleaved band (Kpn I)	recleaving endonuclease (product fragments in megadaltons)				
	Xba I	Hind III	EcoR I	Bgl II	Hpa I
q	x	x	2.1 0.3	*	x
r	x	x	x	x	x
s	x	2.0 0.2	x	*	x
t	x	x	x	x	0.6 t 0.4
u	x	x	x	*	x
v	x	x	x	*	x
wx	x x	x x	x x	x x	x x
yz	x x	x x	x x	x x	x 0.9 0.1
a'	x	x	0.6 0.2	x	x
b'	x	x	x	x	x
c'	x	x	x	x	x

Table C1.7

Digestion of HSV-1 BamH I fragments by other restriction endonucleases. The summarised data are from double digestion and recleavage experiments. Molecular weights refer to the product fragments of recleavage of isolated BamH I fragments. Letters are given when a product fragment corresponds to a fragment produced by the restriction of HSV-1 DNA with the recleaving endonuclease alone.

x this fragment was not cleaved.

Table C1.7

1. HSV-1 BamH I a-j. The following fragments were cleaved.

Xba I: e i EcoR I: a c d h i Hpa I: a b c e h i j

Hind III: a c h Bgl II: a c f

2. HSV-1 BamH I k-i'.

recleaved band (BamH I)	recleaving endonuclease (product fragments in megadaltons)			
	Hind III	EcoR I	Hpa I	Kpn I
k	x	x	x	x
l	x	2.0 1.4	o 0.6 0.5	2.4 1.0
m	x	x	2.4 1.0	x
n	1.8 1.4	x	x	2.2 1.0
o	1.5 1.1	x	x	1.6 1.0

Table C1.7 (continued)

recleaved band (BamH I)	recleaving endonuclease (product fragments in megadaltons)			
	Hind III	EcoR I	Hpa I	Kpn I
pqr	xxx	x 1.5 n 1.1 0.4 0.3	xxx	xx a' 0.8 0.8
s	x	x	x	x
t	x	x	x	1.2 b' (0.1)
uv	xx	xx	x 0.8 0.7	1.1 1.0 0.6 0.5
w	x	1.3 (0.1)	x	1.1 0.2
x	x	x	x	x
y	x	x	x	x
z	x	x	x	x
a'	0.55 0.45	x	x	x
b'	x	0.5 0.4	x	x
c'	x	x	x	0.45 0.2
d'	x	x	0.35 0.25	x

continued overleaf

Table C1.7 (continued)

recleaved band (BamH I)	recleaving endonuclease (product fragments in megadaltons)			
	Hind III	EcoR I	Hpa I	Kpn I
e'	x	x	x	x
f'g'	xx	xx	x 0.27 0.25	xx
h'	0.4 (0.1)	x	x	x
i'	x	x	x	x

Bam c' is not cleaved by Bgl II.

Table C1.8

Summary of HSV-1 Xho I double digestion data. Numbers in parentheses refer to the number of molar fragments which were cleaved within a multiple band.

Table C1.8

second endonuclease	Xho fragments which were cleaved in double digest
Xba I	(a f h d')
Hind III	a e m ghi(0.5) pqrs(2) a' c'
EcoR I	a b c d f ghi(1.5) j mn(1) pqrs(1) u
Bgl II	a e f ghi(1) l mn(1) pqrs(3) u
Hpa I	a b e f ghi(2) k l a' c' d'
Kpn I	a b f k mn(1) o pqrs(3) v w x y z e'
BamH I	a b c d e f g h i j l m n pqrs(3) v w xyz(2)

Table C1.9

Summary of HSV-1 Xho I fragments to which isolated nick translated HSV-1 BamH I fragments hybridised. Hybridisation of BamH I k was not as specific as for the other fragments, presumably owing to contamination by other fragments.

Table C1,9

HSV-1 BamH I fragment	homologous fragments in HSV-1 Xho I digest
k	(c d ghi j pqrs)
l	b
m	l
n	xyz c e i
o	a xyz
pqr	c d f ghi j pqrs xyz e'
s	c d pqrs
t	f pqrs
uv	f pqrs j'
w	xyz ghi

Table C1.10

Summary of HSV-1 Pvu II double digestion data. Numbers in parentheses refer to the number of fragments within a multiple band which were cleaved.

Table C1.10

second endonuclease	Pvu II bands which were cleaved in the double digest
Xba I	c d f g j'k'l'(1)
Hind III	c d g rst(1) x b'c'(1) d'e'(1)
EcoR I	a b c d e f p q w y j'k'l'(1)
Bgl II (bands larger than u)	a b e g h q rst(1)
Hpa I	a b c d e f g h i n b'c'(1)

Table C1.11

Digestion of HSV-2 BamH I fragments by other restriction endonucleases. Molecular weights refer to the product fragments of recleavage of isolated BamH I fragments. Letters are given when a product fragment corresponds to a fragment produced by the restriction of HSV-2 DNA with the recleaving endonuclease alone.

x this fragment was not recleaved.

Table C1.11

recleaved fragment (BamH I)	recleaving endonuclease (product molecular weight in millions)				
	Xba I	Hind III	EcoR I	Hpa I	Kpn I
a	x	x	5 2.5 p	x	i m s 3.0 0.6
b	x	7 1.1	x	x	0.5
c	x	2.6 2.5	x	3.2 1.8	x
d	x	x	3.5 1.4	x	p 1.5 1.0
e	x	x	x	3.2 1.2	x
f	x	2.0 o 1.0	x	2.1 1.1	x
g	x	x	x	x	3.0 0.9
hij	xxx	xxx	xxx	xxx	x 2.4 1.9 1.4 0.6
k	x	x	1.7 1.3	x	x
l	1.5 1.2	x	1.4 1.3	x	x
m	x	1.4 1.1	x	x	x

Table C1.11 (continued)

recleaved fragment (BamH I)	recleaving endonuclease (product molecular weight in millions)				
	Xba I	Hind III	EcoR I	Hpa I	Kpn I
n	x	x	2.2 (0.2)	1.3 0.8	1.3 0.9
o	1.8 0.6	x	x	x	x
pq	xx	xx	xx	xx	xx
r	x	n 0.4	x	x	1.5 0.5
st	xx	xx	xx	xx	x 1.5 (0.4)
u	x	x	x	x	0.9 0.9
vw	xx	xx	xx	xx	xx
x	x	x	x	x	1.5 1.2
yz	xx	xx	xx	xx	xx

Further notes. Bam k was recleaved by Xba in the double digest, but this was not resolved upon recleavage of the isolated fragnent. In the double digests, Bam b' was cleaved by Xba, Bam e' by Hind, and Bam c'd' (one fragment) and h' and k' by Eco. Bam a and b, and c and d were distinguished on the double digest gel, but not as isolated fragments.

Table C1.12

Summary of HSV-2 BamHI fragments to which isolated nick translated HSV-2 HindIII/EcoRI fragments hybridised. The HSV-2 HindIII/EcoRI map is from Wilkie et al. (1979b).

Table C1.12

HSV-2 Hind/Eco fragment	homologous fragments in HSV-2 BamH I digest
1-6	ab c e f g hij m o p st u vw yz a' e' h'
7-8	ab d hij n q
9	ab k w
10-11	c f g p vw yz
12	d hij i'
13	g p u vw yz a' e'
14-15	c g p u vw x yz a'
16	l' c'd'
17	l e' f'
18	f k
19	st
20	r
21	b'
22	ab
23	f

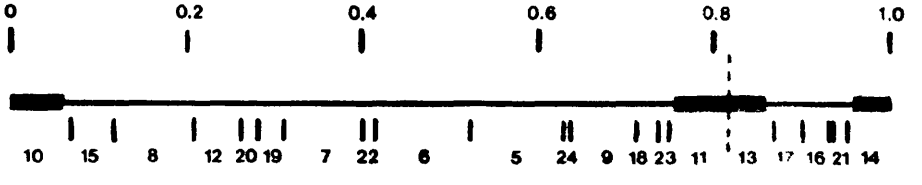


Table C1.13

Estimates of upper and lower limits for molecular weights of the repeat sequences of HSV-1 and HSV-2. Molecular weights are given in millions and the relevant fragments are quoted.

Table C1.13

		short repeat sequence	long repeat sequence	IR_S+IR_L
HSV-1	upper	4.6 (Xho j)	6.1 (Xho pk)	10.7
	lower	3.5 (Eco k)	3.7 (Hpa m)	7.2
HSV-2	upper	4.65 (Bam ug'm'a')	6.2 (Hpa g)	10.9
	lower	3.15 (Bam ug'm')	3.9 (Bam vp)	7.1

Figure C1.1

Molecular weight calibration of HSV-1 DNA restriction fragments (1-10 x 10^6 daltons).

1. Lambda DNA cleaved with Hind III.
2. Lambda DNA cleaved with EcoR I.
3. HSV-1 DNA cleaved with Xho I.
4. HSV-1 DNA cleaved with BamH I.
5. HSV-1 DNA cleaved with Kpn I.
6. HSV-1 DNA cleaved with Hpa I.

The 0.5% agarose gel containing ethidium bromide was photographed under UV illumination. Molecular weights (x 10^{-6}) of lambda DNA fragments are indicated (Thomas and Davis, 1975; Murray and Murray, 1975).

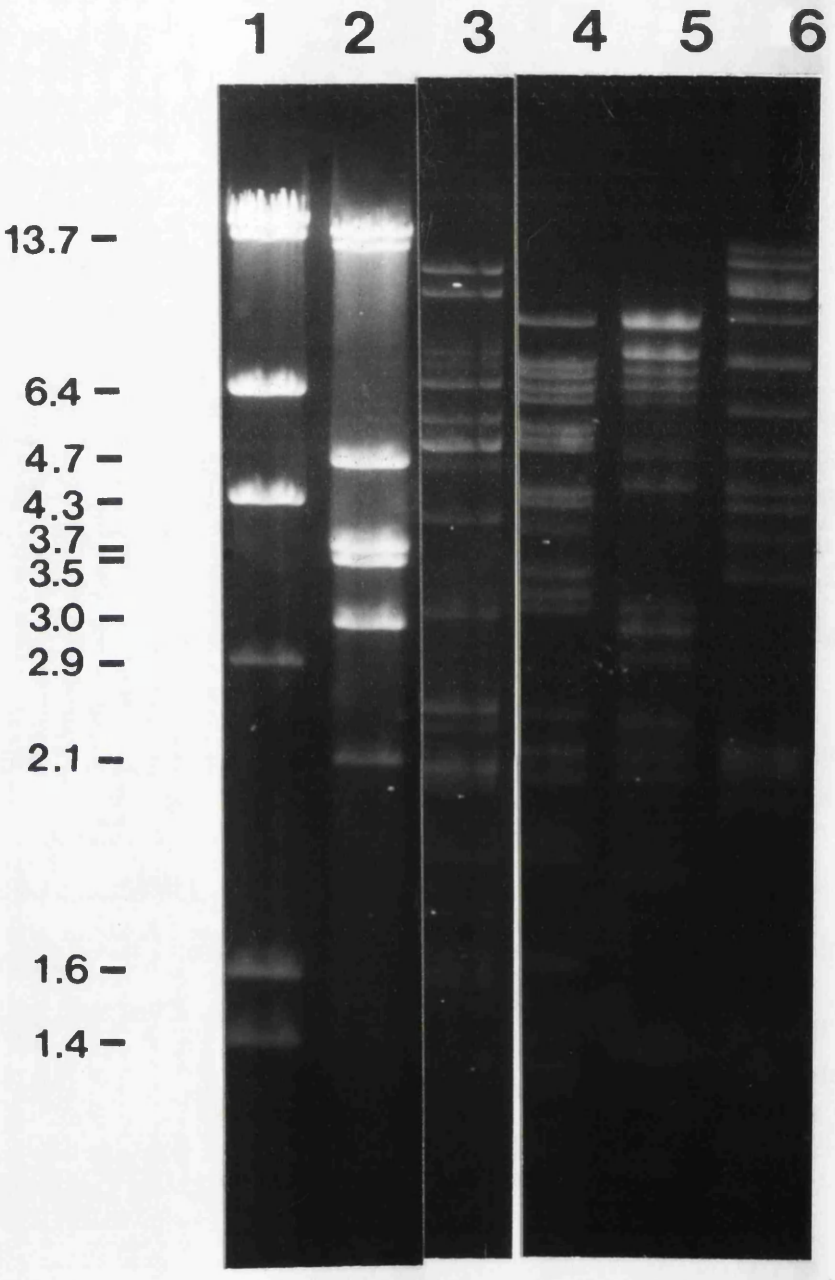

1
2
3
4
5
6
13.7
6.4
4.7
4.3
3.7
3.5
3.0
2.9
2.1
1.6
1.4

Figure C1.2

Molecular weight calibration of HSV-1 and HSV-2 DNA restriction fragments (0.1-1.0 x 10^6 daltons).

M. ØX174 DNA cleaved with HindII.

1. HSV-1 DNA cleaved with BamHI.

2. HSV-1 DNA cleaved with XhoI.

3. HSV-2 DNA cleaved with BamHI.

ØX174 DNA was ^{32}P-labelled by nick translation and HSV-1 and HSV-2 DNA were ^{32}P-labelled *in vivo*. Electrophoresis was carried out on a 1.7% agarose gel which was dried then autoradiographed. Molecular weights (x 10^{-6}) of ØX174 DNA fragments are indicated.

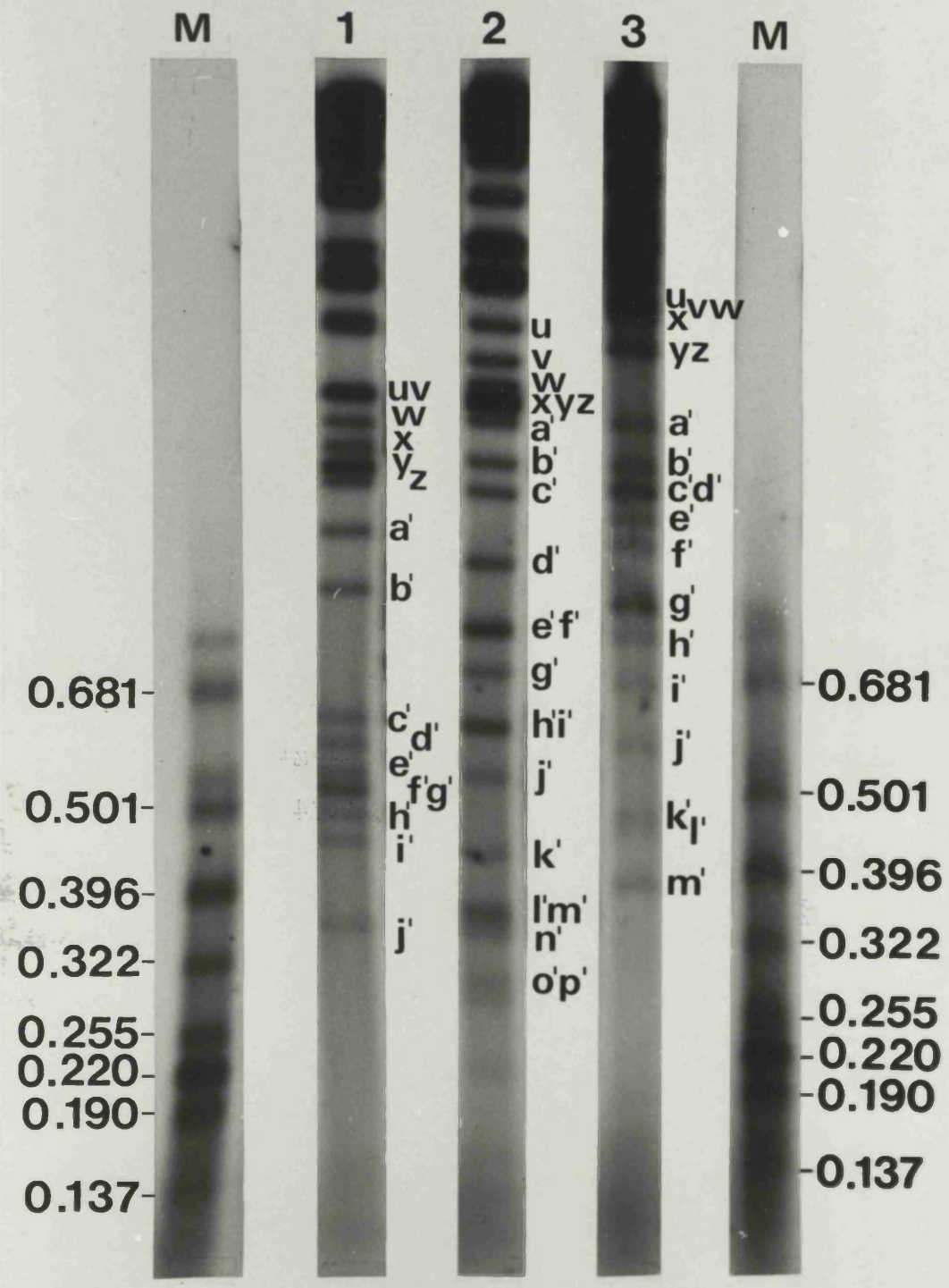

M
1
2
3
M
0.681
0.501
0.396
0.322
0.255
0.220
0.190
0.137
uv
w
x
yz
a'
b'
c'
d'
e'
f'g'
h'
i'
j'
u
v
w
x
yz
a'
b'
c'
d'
e'f'
g'
h'i'
j'
k'
l'm'
n'
o'p'
uvw
x
yz
a'
b'
c'd'
e'
f'
g'
h'
i'
j'
k'l'
m'
0.681
0.501
0.396
0.322
0.255
0.220
0.190
0.137

Figure C1.3

Restriction profiles of HSV-1 (upper) and HSV-2 DNA (lower) which were ^{32}P-labelled *in vivo*. Electrophoresis was carried out on 0.5% (upper portions) and 1.2% agarose (lower portions). Bands are indicated only for those digests for which restriction maps are already known, or were deduced in this work.

Xba I
Cla I
Bcl I
Hind III
EcoR I
Bgl II
Hpa I
Kpn I
BamH I
Xho I
Pvu II

Xba I
Cla I
Bcl I
Hind III
EcoR I
Bgl II
Hpa I
Kpn I
BamH I
Xho I
Pvu II

Figure C1.4

Results of recleavage of isolated HSV-1 XbaI, HindIII and EcoRI restriction fragments by KpnI. Nick translated fragments were cleaved and the products electrophoretically separated on 0.5% (upper portions) and 1.5% agarose gels (lower portions), which were then dried and autoradiographed. The identity of the parent fragment is given at the top of each track, and positions of HSV-1 KpnI fragments are indicated in the dimension of electrophoresis. Cleavage products are marked to the right of each track.

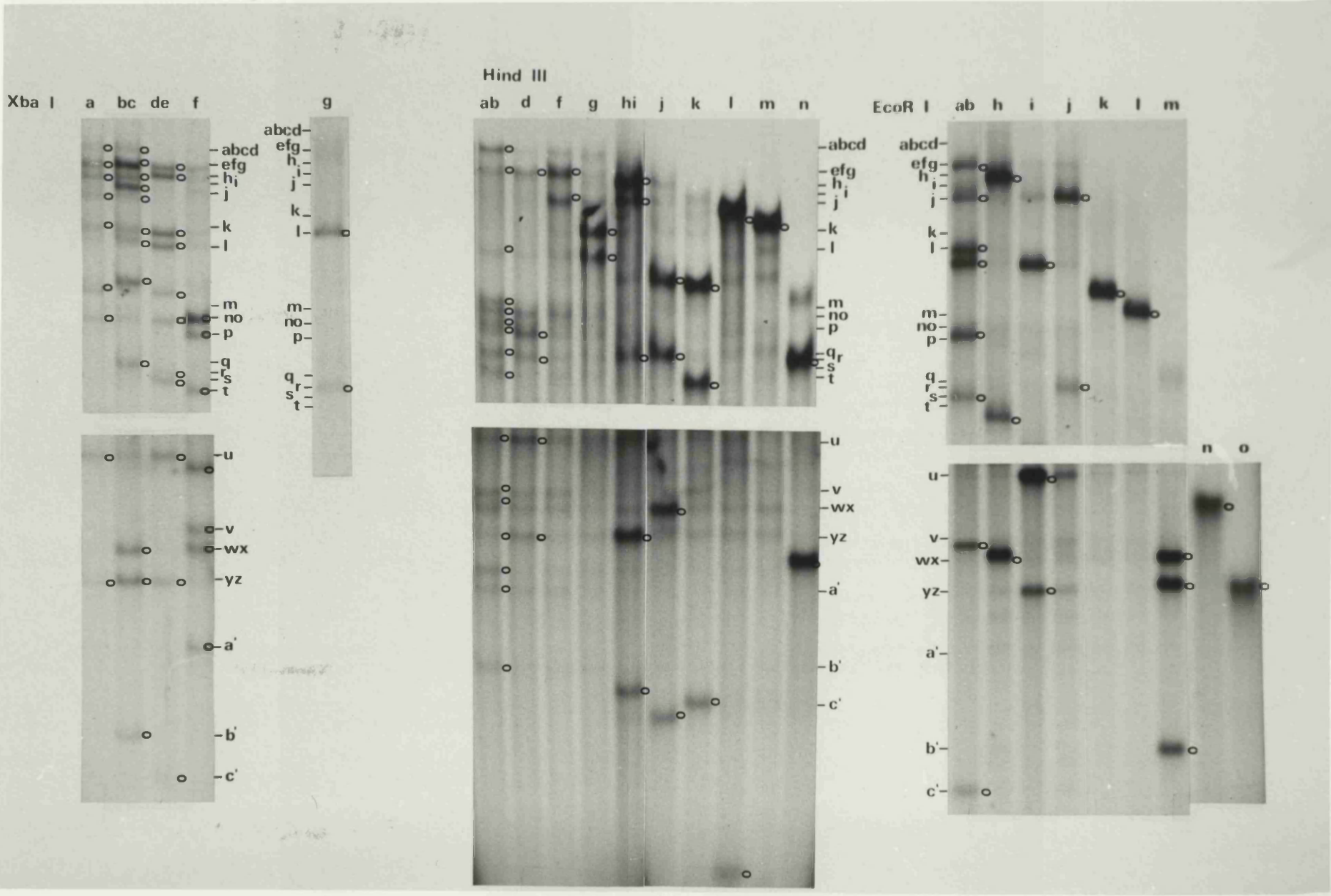

Xba I
a
bc
de
f
g
abcd
efg
h
i
j
k
l
m
no
p
q
r
s
t
u
v
wx
yz
a'
b'
c'
Hind III
ab
d
f
g
hi
j
k
l
m
n
EcoR I
ab
h
i
j
k
l
m
n
o

Figure Cl.5

Results of recleavage of isolated HSV-1 BglII and HpaI restriction fragments by KpnI.
Details are given in the Legend to Figure Cl.4.

Figure C1.6

Combined recleavage data of the HSV-1 genome for Kpn I. The scale is of molecular weight (x 10^{-6}). HSV-1 Kpn I fragments produced by recleavage with Kpn I are listed under each parent fragment, as well as the molecular weights (x 10^{-6}) of recleavage fragments not corresponding to HSV-1 Kpn I fragments. The symbol □ indicates fragments not cleaved by Kpn I. Recleavage products of joint fragments are shown on the right. The Kpn I map deduced from these data is shown at the bottom.

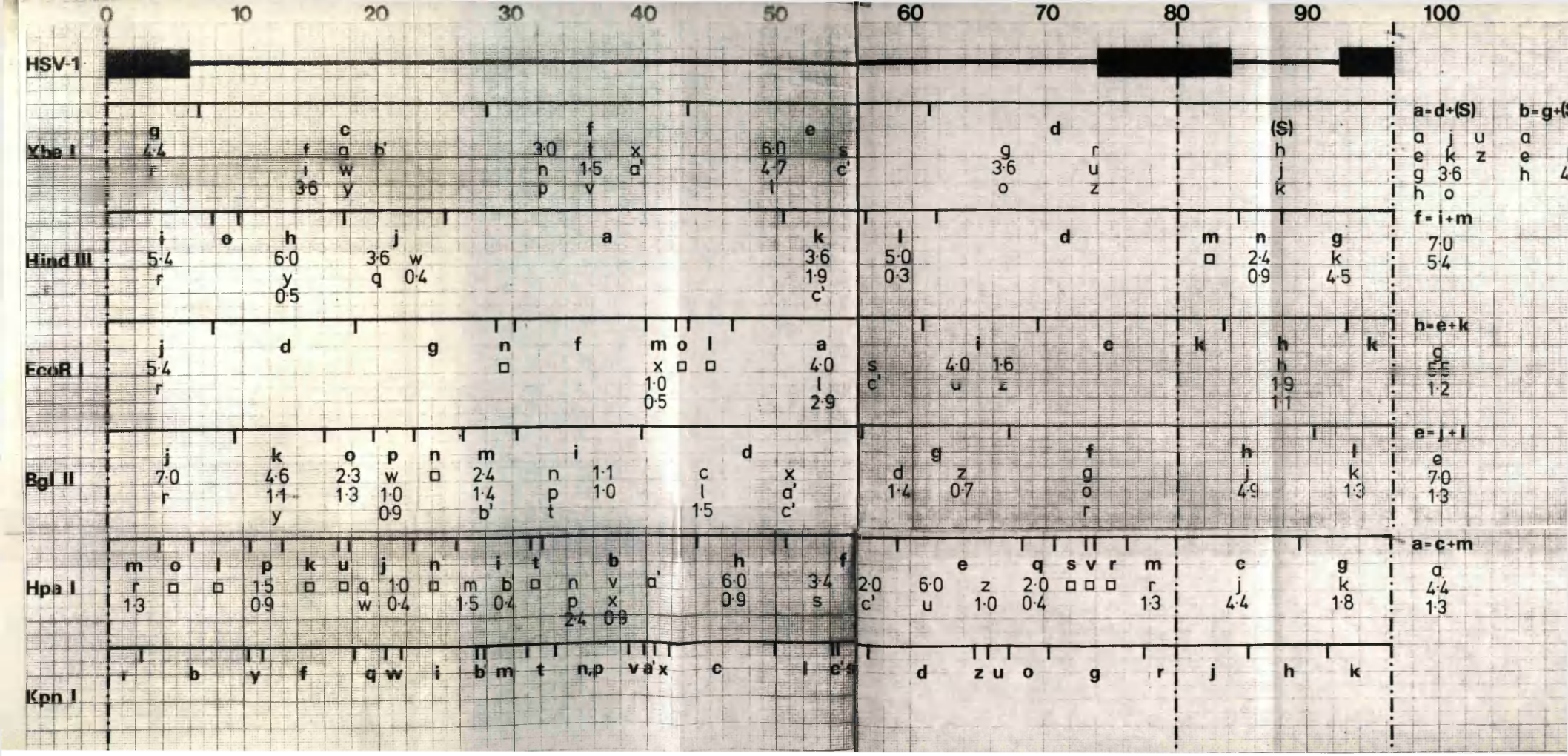

0
10
20
30
40
50
60
70
80
90
100
HSV-1
Xba I
Hind III
EcoR I
Bgl II
Hpa I
Kpn I
a-d+(S)
b=g+(S
f=i+m
b=e+k
e=j+l
a=c+m

Figure C1.7

Double digests of *in vivo* ^{32}P-labelled HSV-1 DNA.

1. *Xba* I.
2. *Xba* I/*Kpn* I.
3. *Kpn* I.
4. *Hind* III/*Kpn* I.
5. *Hind* III.
6. *EcoR* I.
7. *EcoR* I/*Kpn* I.
8. *Kpn* I.
9. *Bgl* II/*Kpn* I.
10. *Bgl* II.
11. *Hpa* I.
12. *Hpa* I/*Kpn* I.
13. *Kpn* I.

Electrophoresis was carried out on a 0.6% agarose gel which was then dried and autoradiographed. HSV-1 *Kpn* I restriction fragments are indicated.

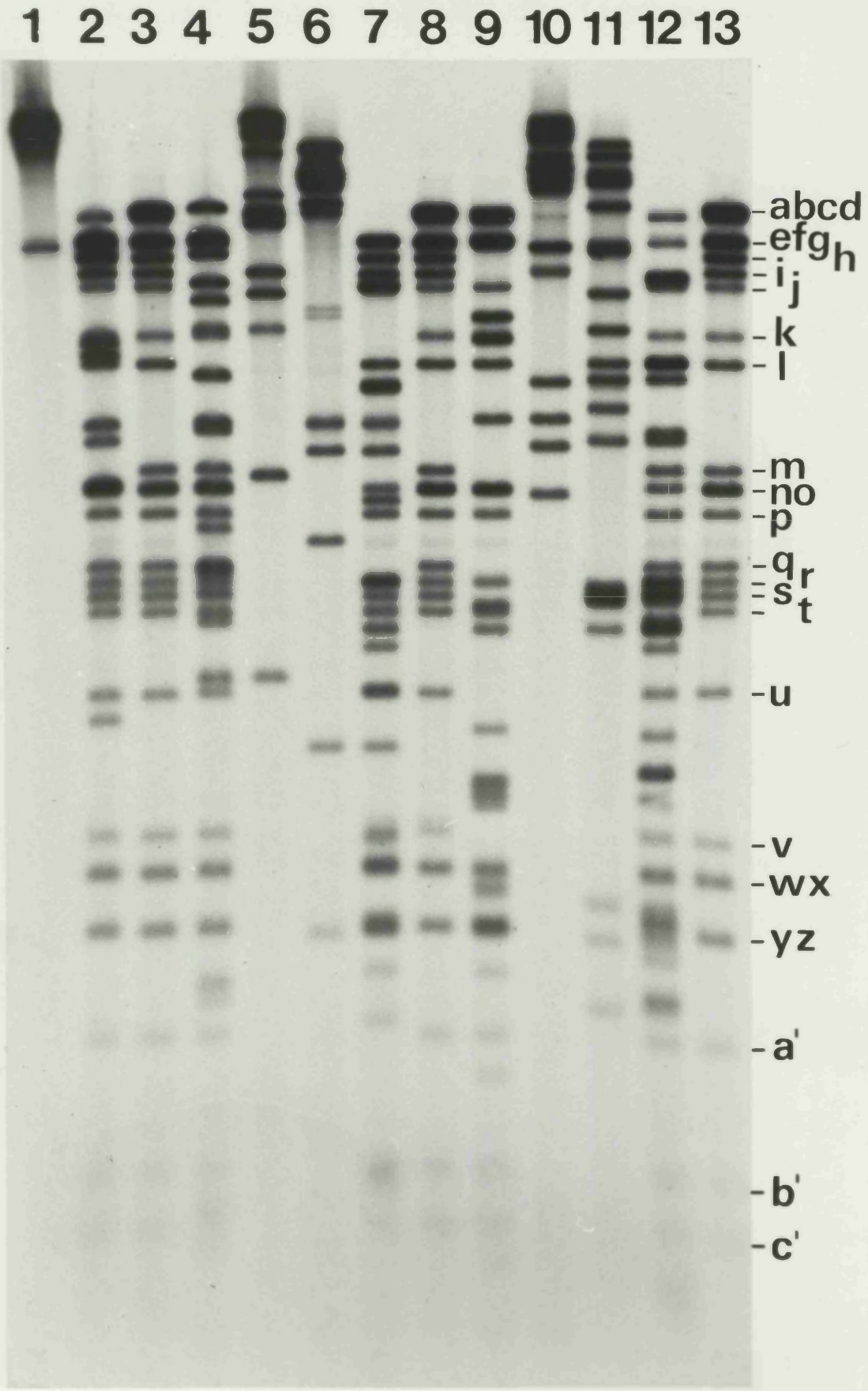

1 2 3 4 5 6 7 8 9 10 11 12 13
abcd
efg h
i j
k
l
m
no
p
q r
s t
u
v
wx
yz
a'
b'
c'

Figure Cl.8

Cleavage of HSV-1 Kpn I restriction fragments with various restriction endonucleases. Nick translated fragments were cleaved and the products electrophoretically separated on 1% agarose gels, which were then dried and autoradiographed. The Kpn I fragments cleaved are indicated at the top of the tracks, with the recleaving endonuclease above each panel. The marker digest of the recleaving endonuclease is indicated in the dimension of electrophoresis. Not all recleavages are shown, but all the data has been included in Table Cl.6.

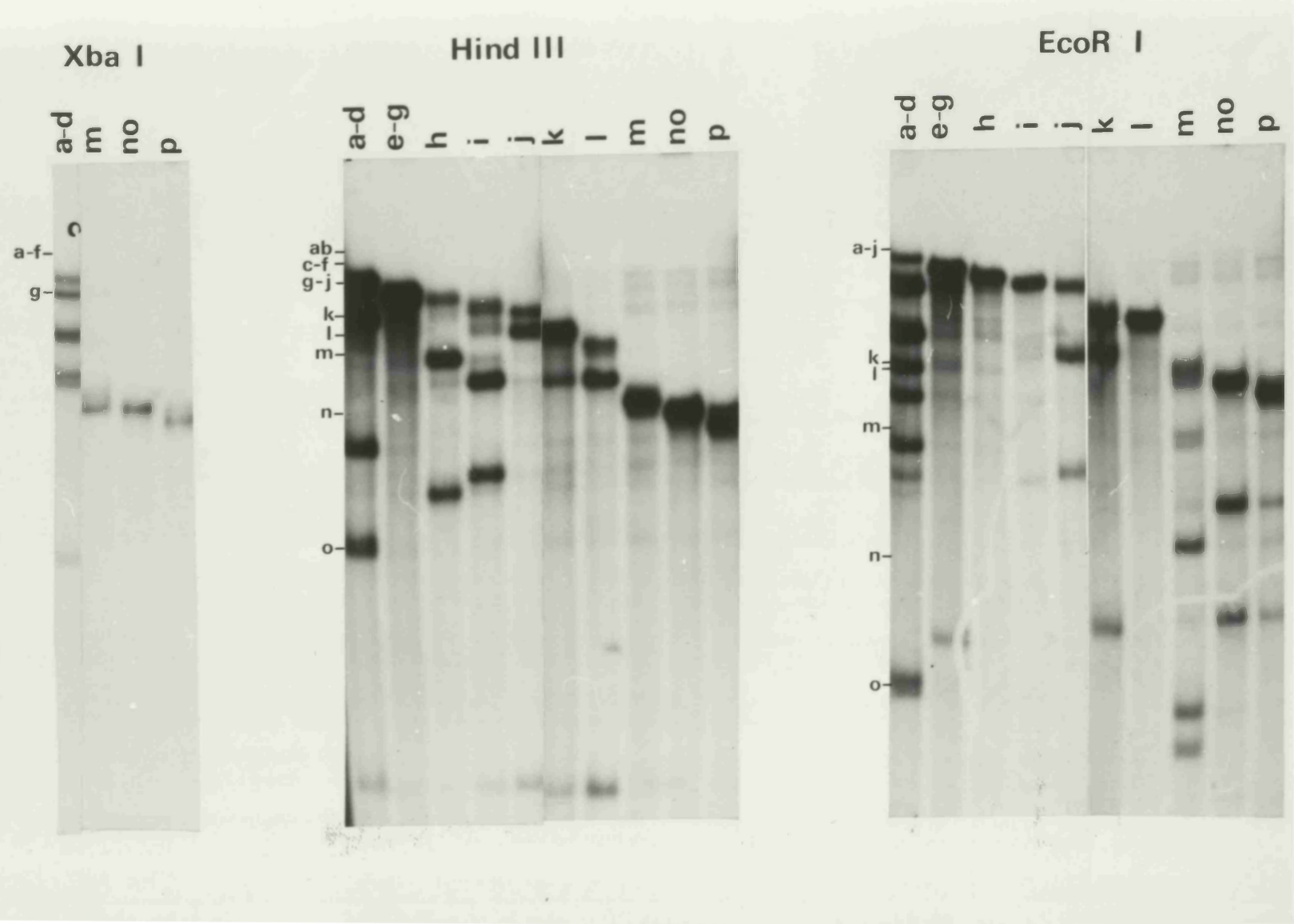
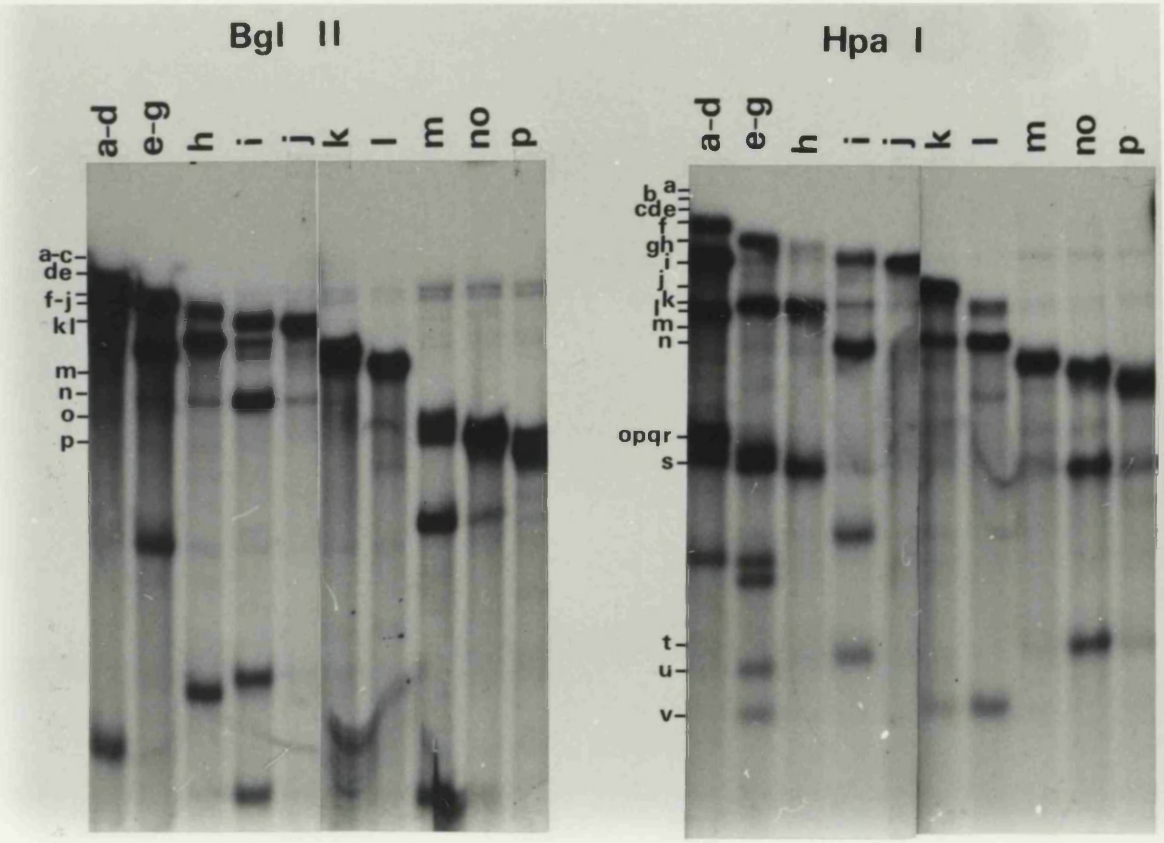

Xba I
a-d
m
no
p
a-f
g
Hind III
a-d
e-g
h
i
j
k
l
m
no
p
ab
c-f
g-j
k
l
m
n
o
EcoR I
a-d
e-g
h
i
j
k
l
m
no
p
a-j
k
l
m
n
o

Figure C1.9

Cleavage of HSV-1 Kpn I restriction fragments with various restriction endonucleases. Details are given in the Legend to Figure C1.8.


Bgl II
a-d
e-g
h
i
j
k
l
m
no
p
a-c
de
f-j
kl
m
n
o
p
Hpa I
a-d
e-g
h
i
j
k
l
m
no
p
a
b
cd
e
f
gh
i
j
k
l
m
n
opqr
s
t
u
v

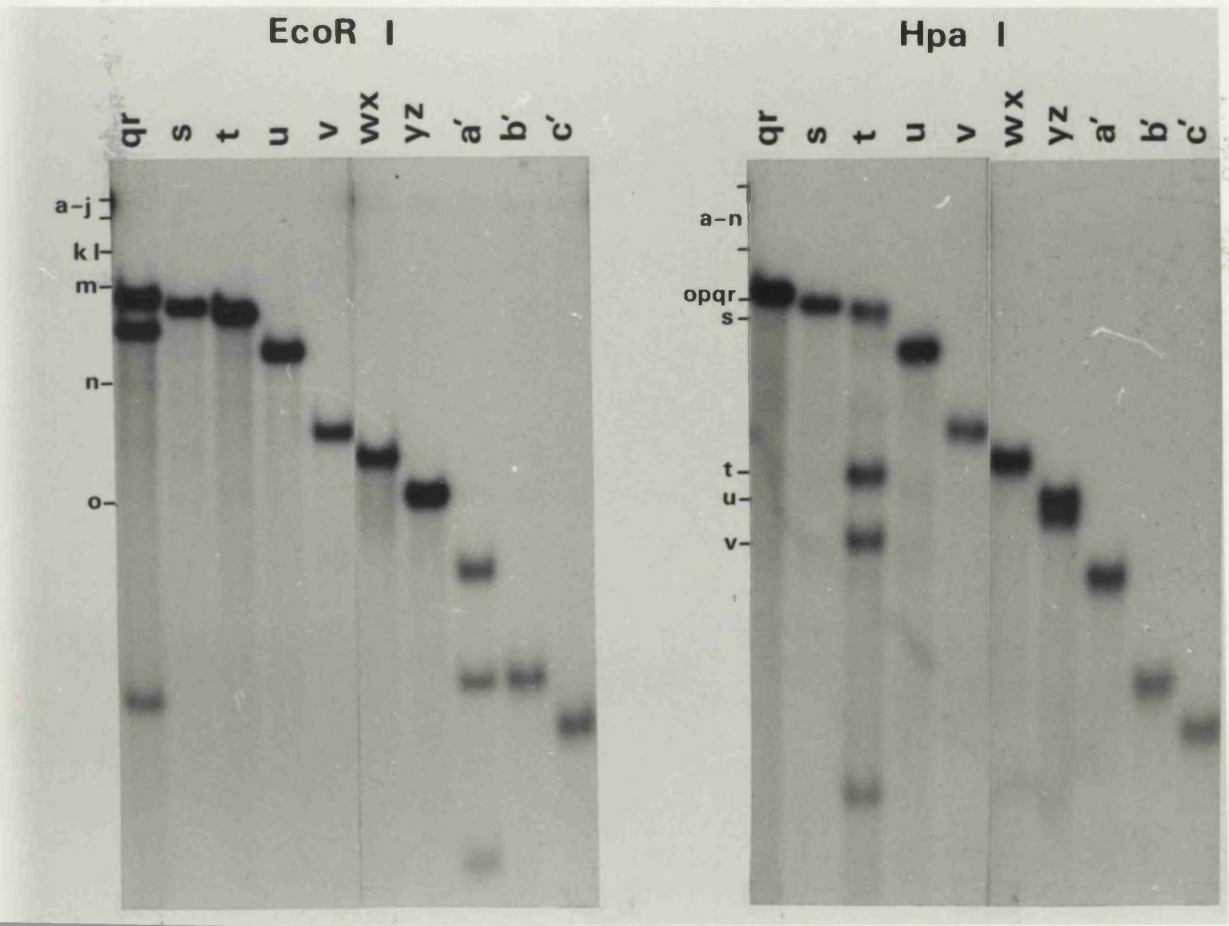

EcoR I
qr
s
t
u
v
wx
yz
a'
b'
c'
a-j
kl
m
n
o
Hpa I
qr
s
t
u
v
wx
yz
a'
b'
c'
a-n
opqr
s
t
u
v

Figure C1.10

Cross-blot of HSV-1 HpaI against nick translated HSV-1 KpnI. The unlabelled dimension is indicated by the marker strip to the right, and the labelled dimension across the top. Faint spots not corresponding to KpnI bands are due to some fragments resulting from slight underdigestion of the DNA.

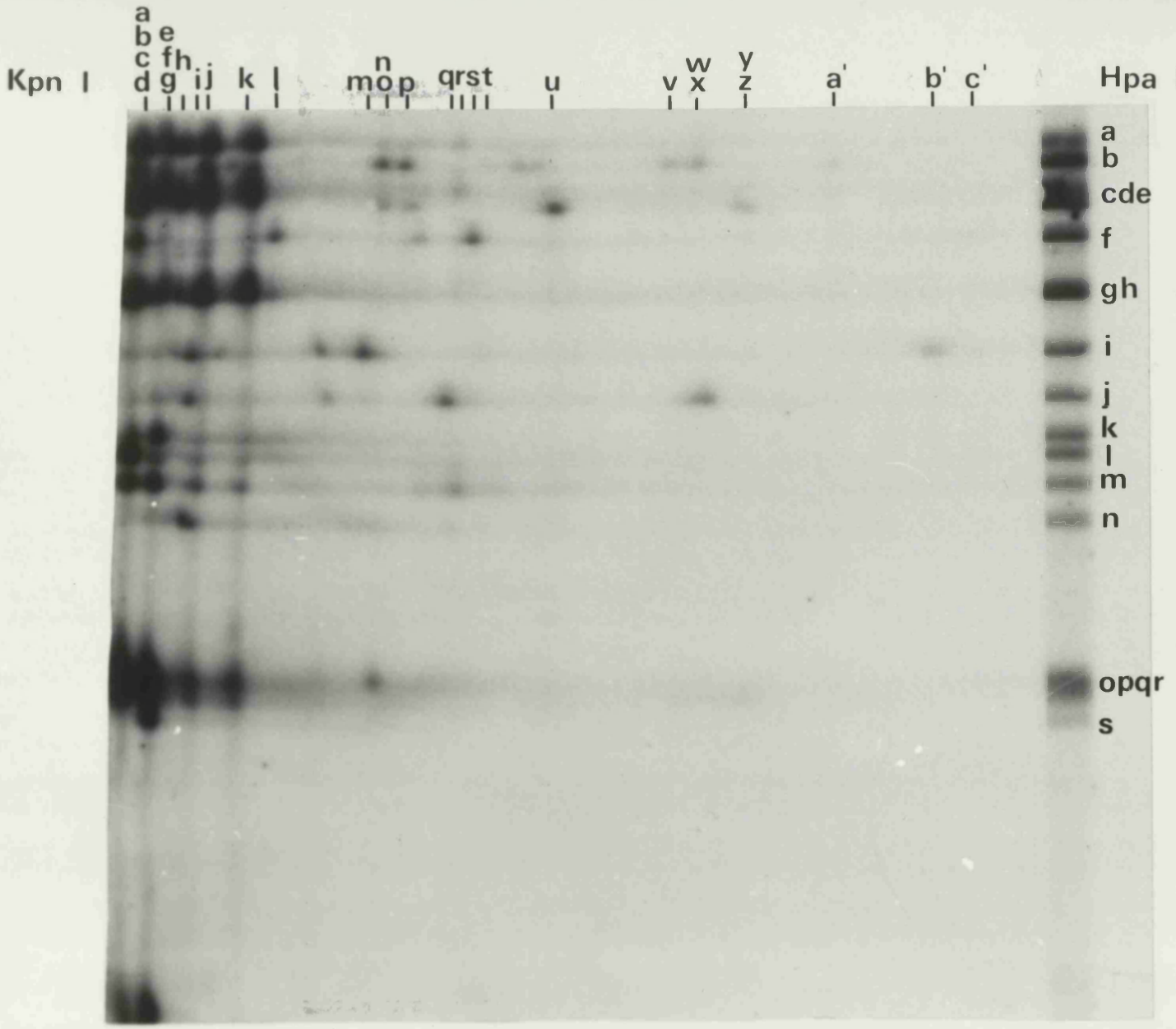

Kpn I
a
b
c
d
e
f
g
h
i
j
k
l
m
n
o
p
q
r
s
t
u
v
w
x
y
z
a'
b'
c'
Hpa I
a
b
cde
f
gh
i
j
k
l
m
n
opqr
s

Figure C1.11

Summary of HSV-1 KpnI hybridisation data, using the physical map deduced from recleavage experiments. Beneath each fragment is written the fragment of the other digest with which it has homology, as deduced from Figure C1.10. The scale is molecular weight ($\times 10^{-6}$).

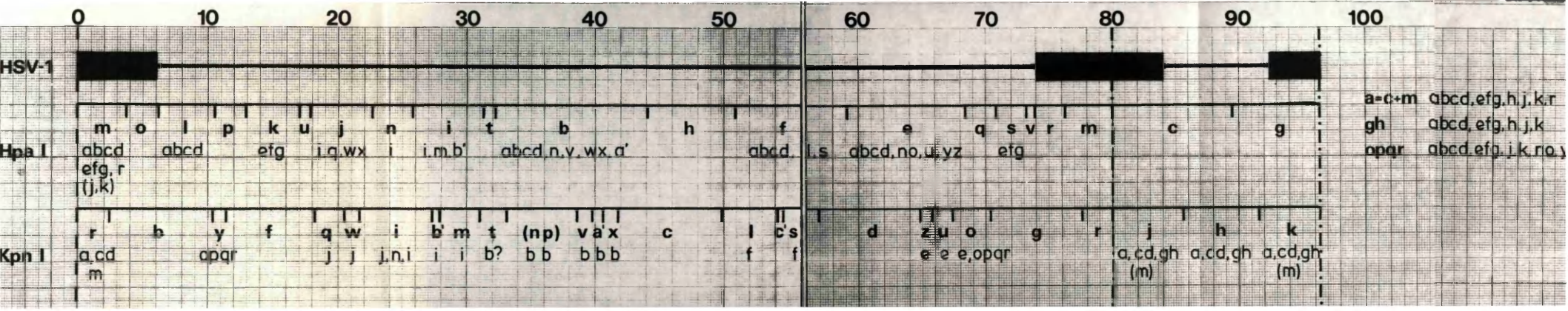

0
10
20
30
40
50
60
70
80
90
100
HSV-1
m o l p k u j n i t b h f
Hpa I
abcd abcd efg i,q,w,x i i,m,b' abcd,n,v,wx,a' abcd
efg, r
(j,k)
e q s v r m c g
l,s abcd,no,u,yz efg
r b y f q w i b' m t (np) v a' x c l c's
Kpn I
a,cd m
opqr
j j j,n,i i i b? b b b b b
d z u o g r j h k
e e e,opqr
a,cd,gh (m)
a,cd,gh
a,cd,gh (m)
a=c+m abcd,efg,h,j,k,r
gh abcd,efg,h,j,k
opqr abcd,efg,j,k,r,o,y

Figure C1.12

Determination of the relative order of HSV-1 Kpn I n and p. The restriction digest present on the nitrocellulose strips is noted at the top, and the fragments of the digest to the left of each panel. Bands to which hybridisation of the nick translated DNA probe occurred are shown to the right of each panel.
The probes were as follows.

1. HSV-1 (left panel) or HSV-2 DNA (right panel).
2. HSV-1 Kpn I no.
3. HSV-1 Kpn I p.

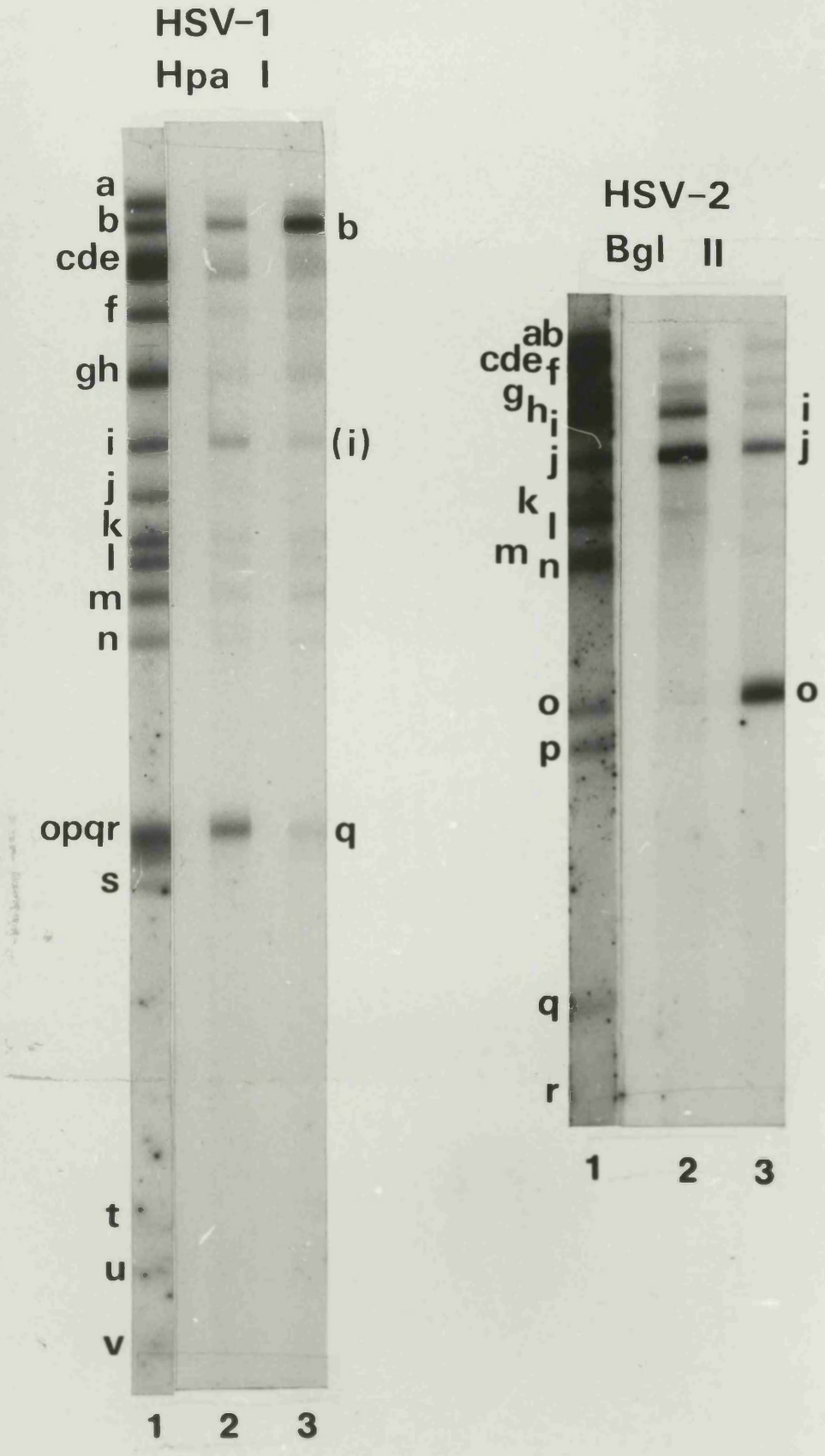

HSV-1
Hpa I
a
b
cde
f
gh
i
j
k
l
m
n
opqr
s
t
u
v
b
(i)
q
1
2
3
HSV-2
Bgl II
ab
cde
f
g
h
i
j
k
l
m
n
o
p
q
r
i
j
o
1
2
3

Figure C1.13

Combined recleavage data of the HSV-1 genome for BamH I. The scale is of molecular weight (x 10^{-6}). HSV-1 BamH I fragments produced by recleavage with BamH I are listed under each parent fragment, as well as the molecular weights (x 10^{-6}) of recleavage fragments not corresponding to HSV-1 BamH I fragments. The symbol □ indicates fragments not cleaved by BamH I. Recleavage products of joint fragments are shown on the right. The BamH I map deduced from these data is shown at the bottom.

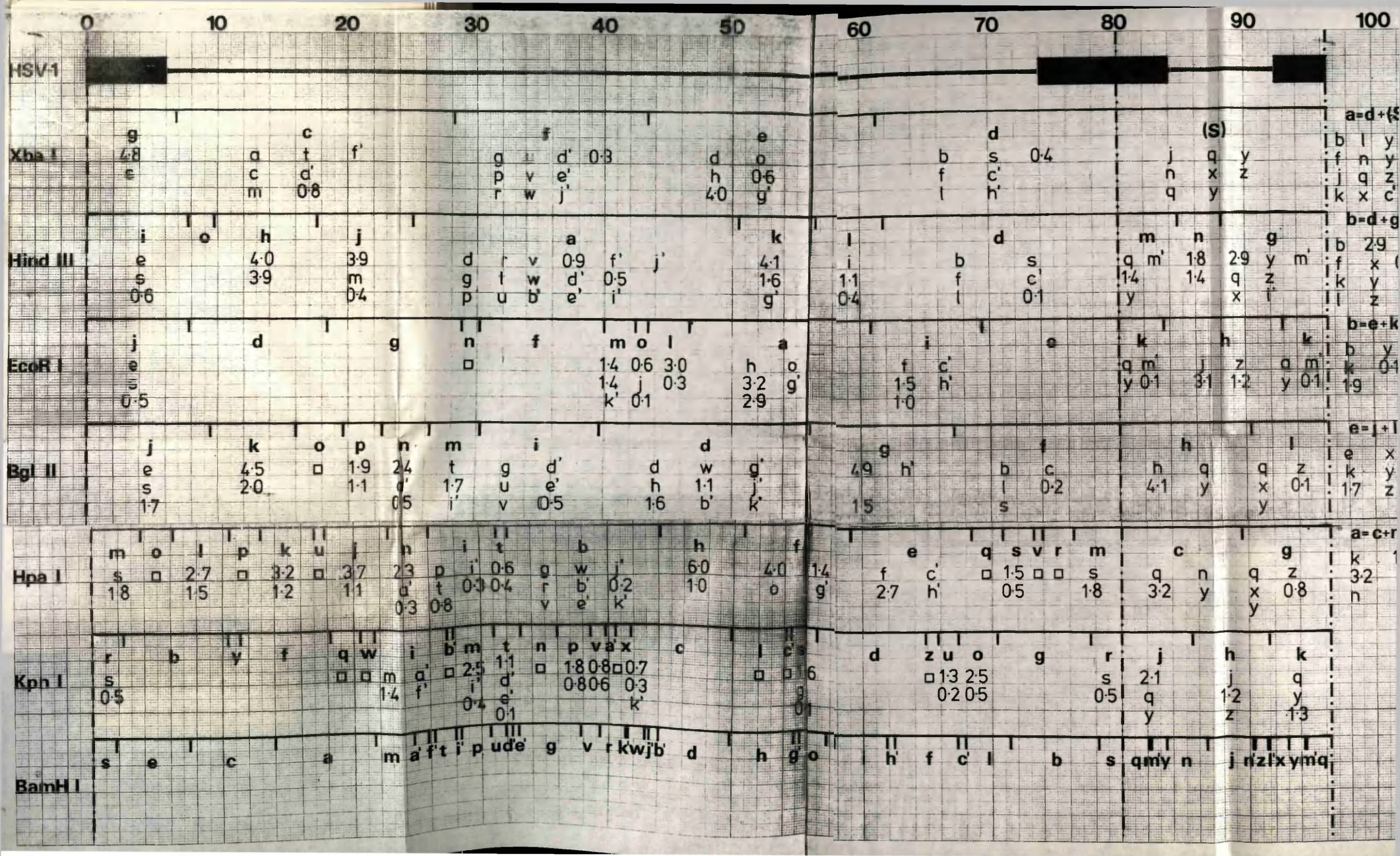

0
10
20
30
40
50
60
70
80
90
100
HSV-1
Xba I
Hind III
EcoR I
Bgl II
Hpa I
Kpn I
BamH I

Figure C1.14

Summary of HSV-1 BamH I hybridisation data, using the physical map deduced from recleavage experiments. Beneath each fragment is written the fragment of the other digest with which it has homology. For the BamH I map, those fragments underlined refer to homology with Hpa I fragments, and the others refer to the Kpn I map.

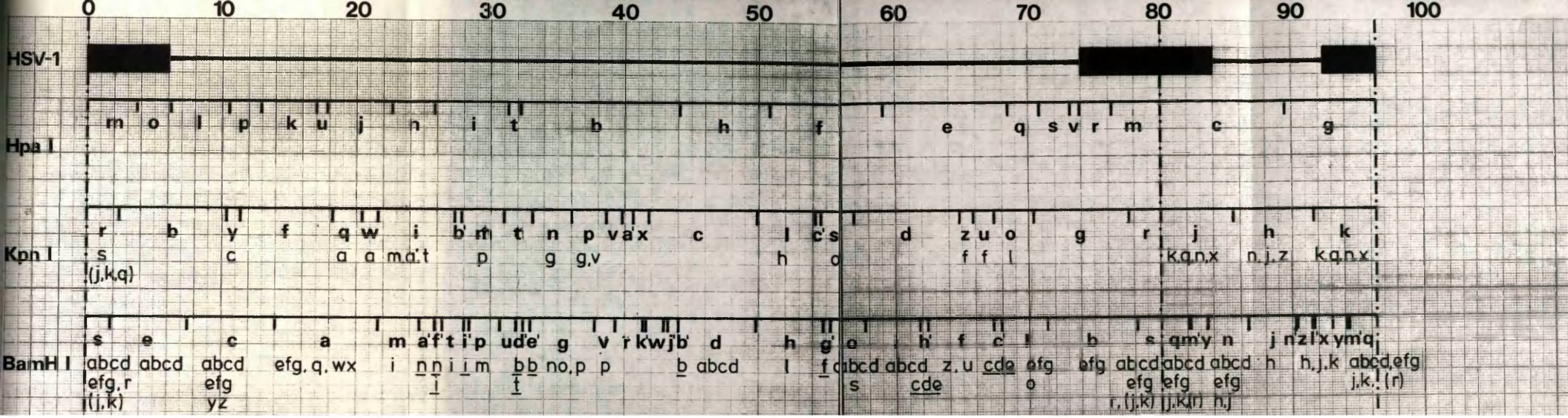

0
10
20
30
40
50
60
70
80
90
100
HSV-1
Hpa I
m o l p k u j n i t b h f e q s v r m c g
Kpn I
r b y f q w i b' m t n p v a' x c l c' s d z u o g r j h k
s c a g g,v h o f f l k,q,n,x n,j,z k,q,n,x
(j,k,q)
BamH I
s e c a m a' f' t i' p u d' e' g v r' k' w' j' b' d h g'
abcd abcd abcd efg, q, wx i n n̲ i i̲ m b b̲ no, p p b̲ abcd l f̲ a
efg, r efg i̲ t̲
(j,k) yz
o h' f c' i b s qm' y n j n' z l' x y m' q
abcd abcd z, u cde efg efg abcd abcd abcd h h, j, k abcd, efg
s cde o efg efg efg j, k, l (r)
r, (j,k) (j,k) (r) h, j

Figure C1.15

Combined recleavage data of the HSV-1 genome for Xho I. The scale is of molecular weight (x 10^{-6}). HSV-1 Xho I fragments produced by recleavage with Xho I are listed under each parent fragment, as well as the molecular weights (x 10^{-6}) of recleavage fragments not corresponding to HSV-1 Xho I fragments. The symbol □ indicates fragments not cleaved by Xho I. Recleavage products of joint fragments are shown on the right. The Xho I map deduced from these data is shown at the bottom.

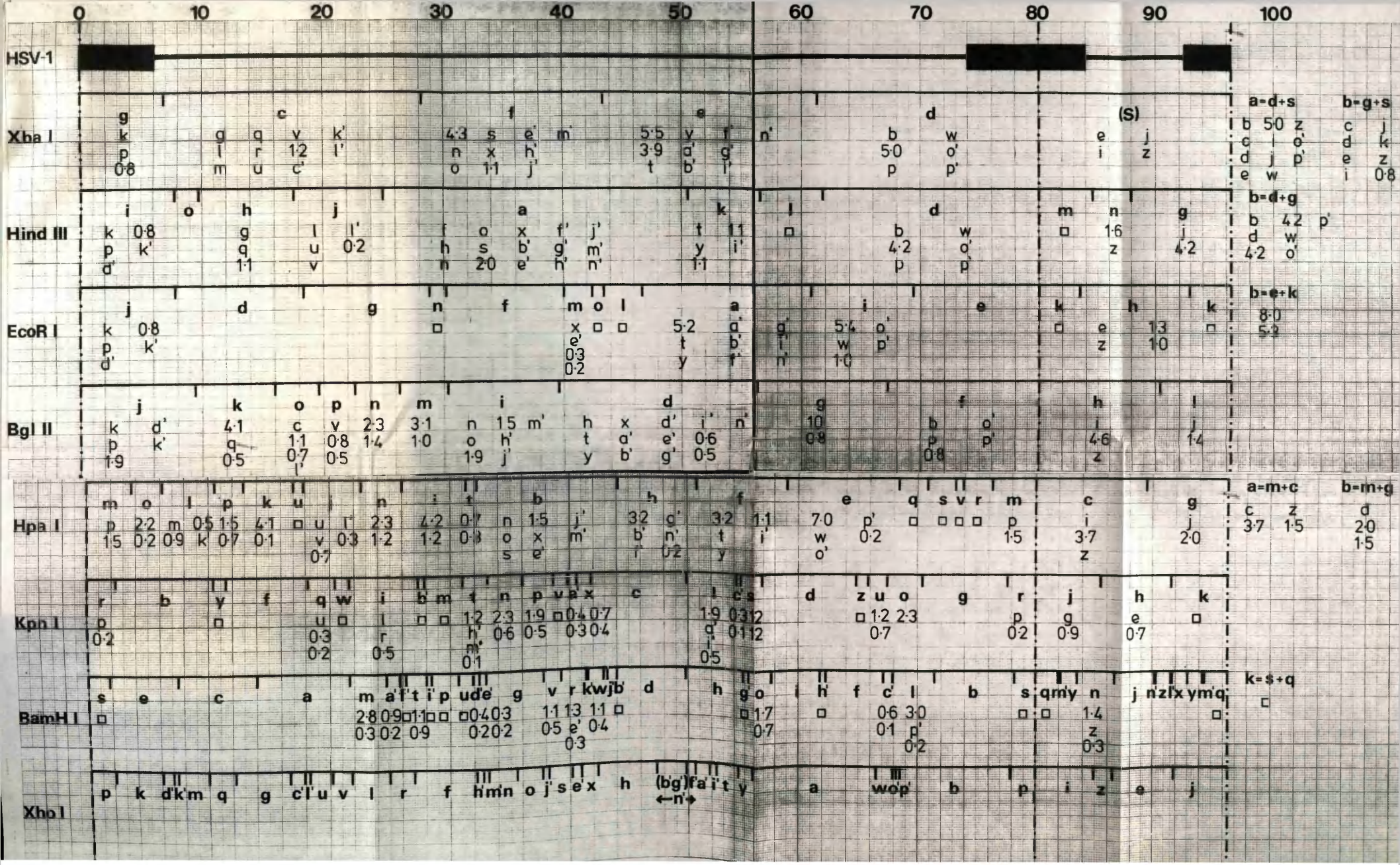

0
10
20
30
40
50
60
70
80
90
100
HSV-1
Xba I
Hind III
EcoR I
Bgl II
Hpa I
Kpn I
BamH I
Xho I
(S)
a=d+s
b=g+s
b=d+g
b=e+k
a=m+c
b=m+g
k=$+q

Figure C1.16

Combined recleavage data of the HSV-1 genome for Pvu II. The scale is of molecular weight (x 10^{-6}). HSV-1 Pvu II fragments produced by recleavage with Pvu II are listed under each parent fragment, as well as the molecular weights (x 10^{-6}) of recleavage fragments not corresponding to HSV-1 Pvu II fragments. The symbol □ indicates fragments not cleaved by Pvu II. Recleavage products of joint fragments are shown on the right. The Pvu II map deduced from these data is shown at the bottom.

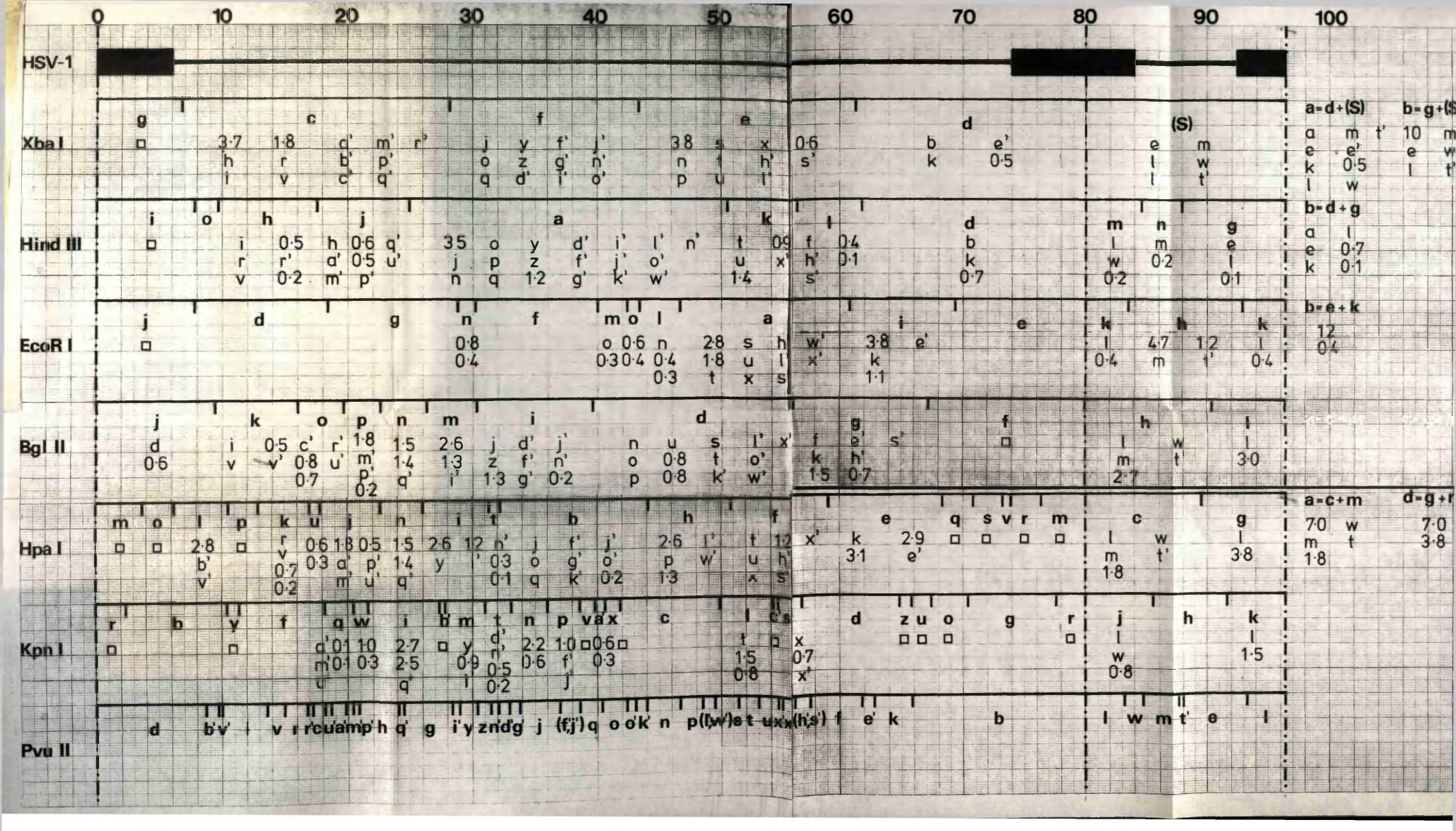
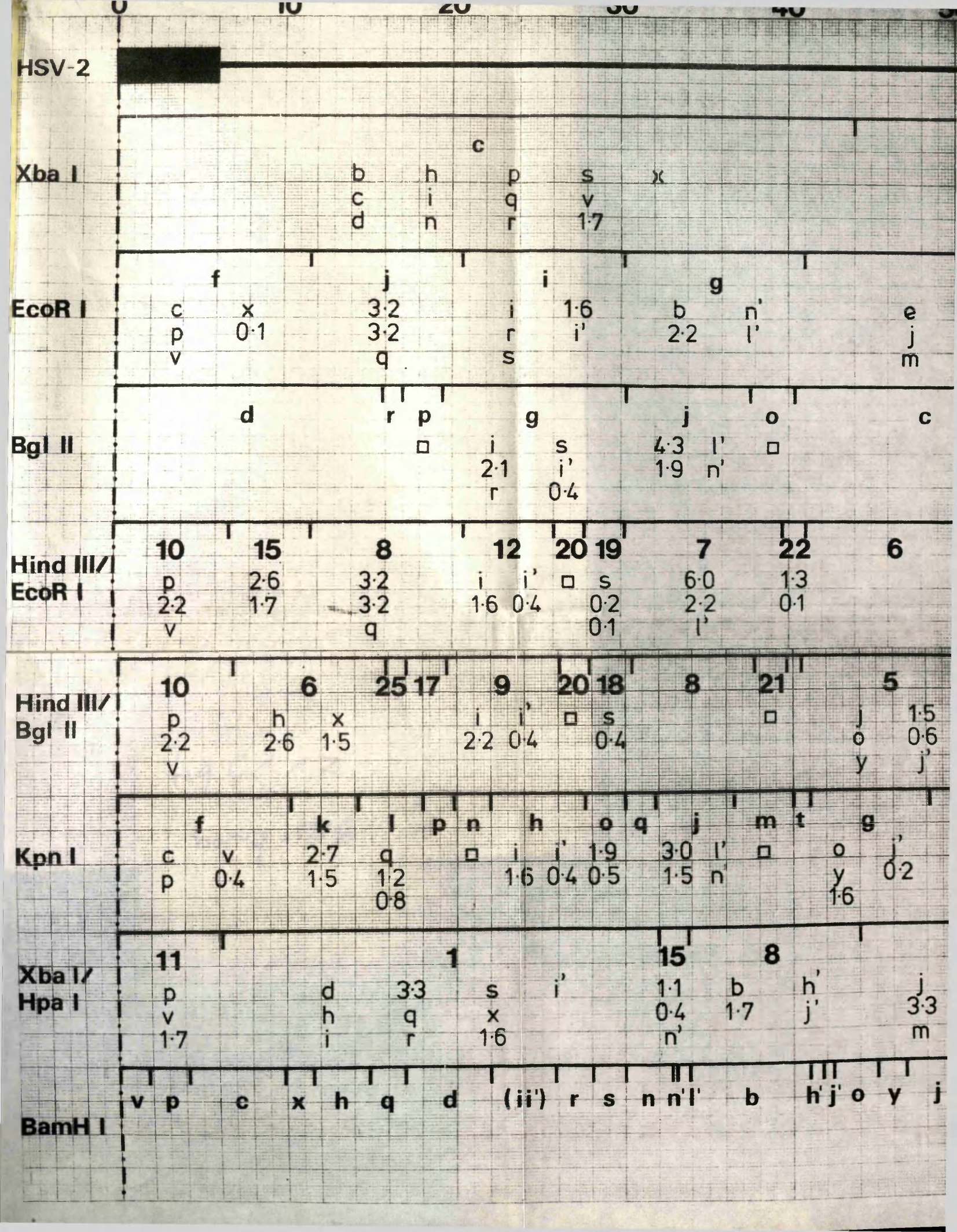

0
10
20
30
40
50
60
70
80
90
100
HSV-1
Xba I
Hind III
EcoR I
Bgl II
Hpa I
Kpn I
Pvu II
a=d+(S)
b=g+(S)
b=d+g
b=e+k
a=c+m
d=g+r

Figure C1.17

Combined recleavage data of the HSV-2 genome for BamH I. The scale is of molecular weight (x 10^{-6}). HSV-2 BamH I fragments produced by recleavage with BamH I are listed under each parent fragment, as well as the molecular weights (x 10^{-6}) of recleavage fragments not corresponding to HSV-2 BamH I fragments. The symbol □ indicates fragments not cleaved by BamH I. Recleavage products of joint fragments are shown on the right. The BamH I map deduced from these data is shown at the bottom.


0
10
20
30
40
HSV-2
Xba I
c
b h p s x
c i q v
d n r 1·7
EcoR I
f j i g
c x 3·2 i 1·6 b n' e
p 0·1 3·2 r i' 2·2 l' j
v q s m
Bgl II
d r p g j o c
□ i s 4·3 l' □
2·1 i' 1·9 n'
r 0·4
Hind III/
EcoR I
10 15 8 12 20 19 7 22 6
p 2·6 3·2 i i' □ s 6·0 1·3
2·2 1·7 3·2 1·6 0·4 0·2 2·2 0·1
v q 0·1 l'
Hind III/
Bgl II
10 6 25 17 9 20 18 8 21 5
p h x i i' □ s □ j 1·5
2·2 2·6 1·5 2·2 0·4 0·4 o 0·6
v y j'
Kpn I
f k l p n h o q j m t g
c v 2·7 q □ i i' 1·9 3·0 l' □ o j'
p 0·4 1·5 1·2 1·6 0·4 0·5 1·5 n' y 0·2
0·8 1·6
Xba I/
Hpa I
11 1 15 8
p d 3·3 s i' 1·1 b h' j
v h q x 0·4 1·7 j' 3·3
1·7 i r 1·6 n' m
BamH I
v p c x h q d (ii') r s n n' l' b h' j' o y j

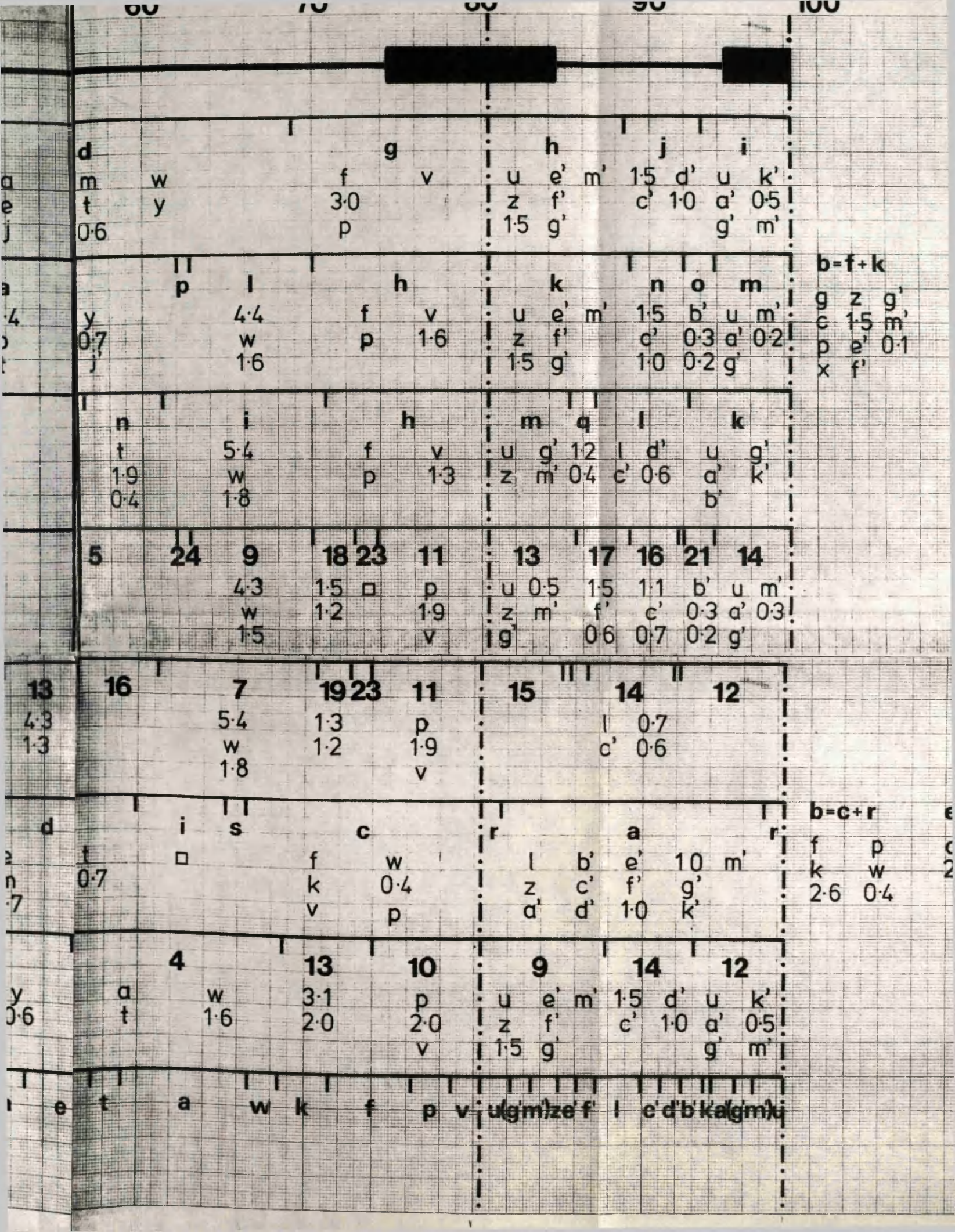

60
70
80
90
100
d
m w
t y
0·6
g
f v
3·0
p
h
u e' m'
z f'
1·5 g'
j i
1·5 d' u k'
c' 1·0 a' 0·5
g' m'
p l
y 4·4
0·7 w
j' 1·6
f v
p 1·6
k
u e' m'
z f'
1·5 g'
n o m
1·5 b' u m'
c' 0·3 a' 0·2
1·0 0·2 g'
b=f+k
g z g'
c 1·5 m'
p e' 0·1
x f'
n i
t 5·4
1·9 w
0·4 1·8
h
f v
p 1·3
m q l k
u g' 1·2 l d' u g'
z m' 0·4 c' 0·6 a' k'
b'
5 24 9
4·3
w
1·5
18 23 11
1·5 □ p
1·2 1·9
v
13 17 16 21 14
u 0·5 1·5 1·1 b' u m'
z m' f' c' 0·3 a' 0·3
g' 0·6 0·7 0·2 g'
13 16 7
4·3 5·4
1·3 w
1·8
19 23 11
1·3 p
1·2 1·9
v
15 14 12
l 0·7
c' 0·6
d i s
t □
0·7
c
f w
k 0·4
v p
r a r
l b' e' 1·0 m'
z c' f' g'
a' d' 1·0 k'
b=c+r
f p
k w
2·6 0·4
4 13 10
a w
t 1·6
3·1
2·0
p
2·0
v
9 14 12
u e' m' 1·5 d' u k'
z f' c' 1·0 a' 0·5
1·5 g' g' m'
e t a w k f p v u(g'm')ze'f' l c'd'b'k'a(g'm')u

Figure C1.18

Aligned restriction maps for HSV-1 DNA.
The scale is fractional genome length.
The maps for Kpn I, BamH I, Xho I and Pvu II
were determined as a result of this study,
and the others were the work of Wilkie (1976).

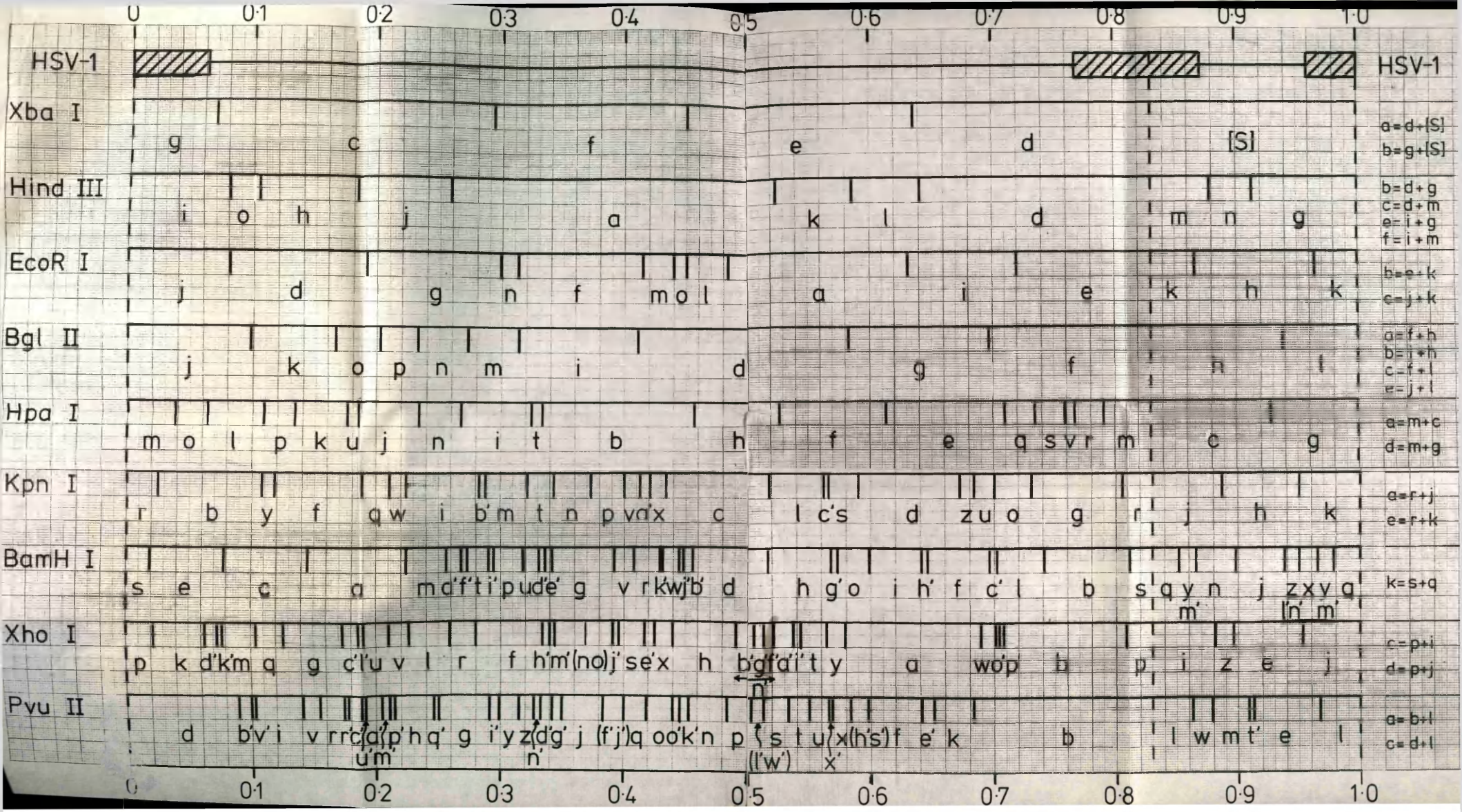

0
0·1
0·2
0·3
0·4
0·5
0·6
0·7
0·8
0·9
1·0
HSV-1
Xba I
Hind III
EcoR I
Bgl II
Hpa I
Kpn I
BamH I
Xho I
Pvu II
a=d+[S]
b=g+[S]
b=d+g
c=d+m
e=i+g
f=i+m
b=e+k
c=j+k
a=f+h
b=j+h
c=f+l
e=j+l
a=m+c
d=m+g
a=r+j
e=r+k
k=s+q
c=p+i
d=p+j
a=b+l
c=d+l

Figure C1.19

Aligned restriction maps for HSV-2 DNA.
The scale is fractional genome length.
The map for BamH I was determined as a result of this study, and the others were the work of Cortini and Wilkie (1978).

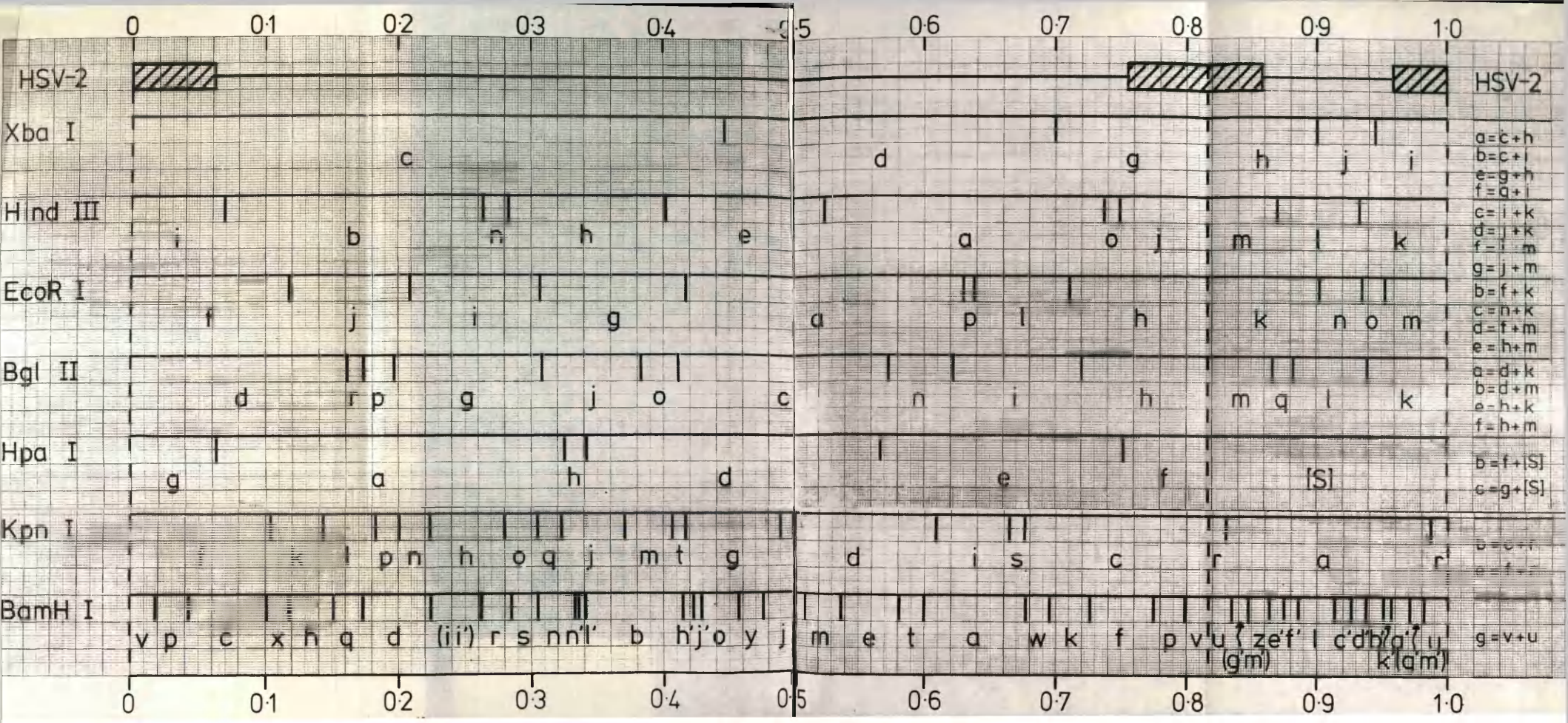
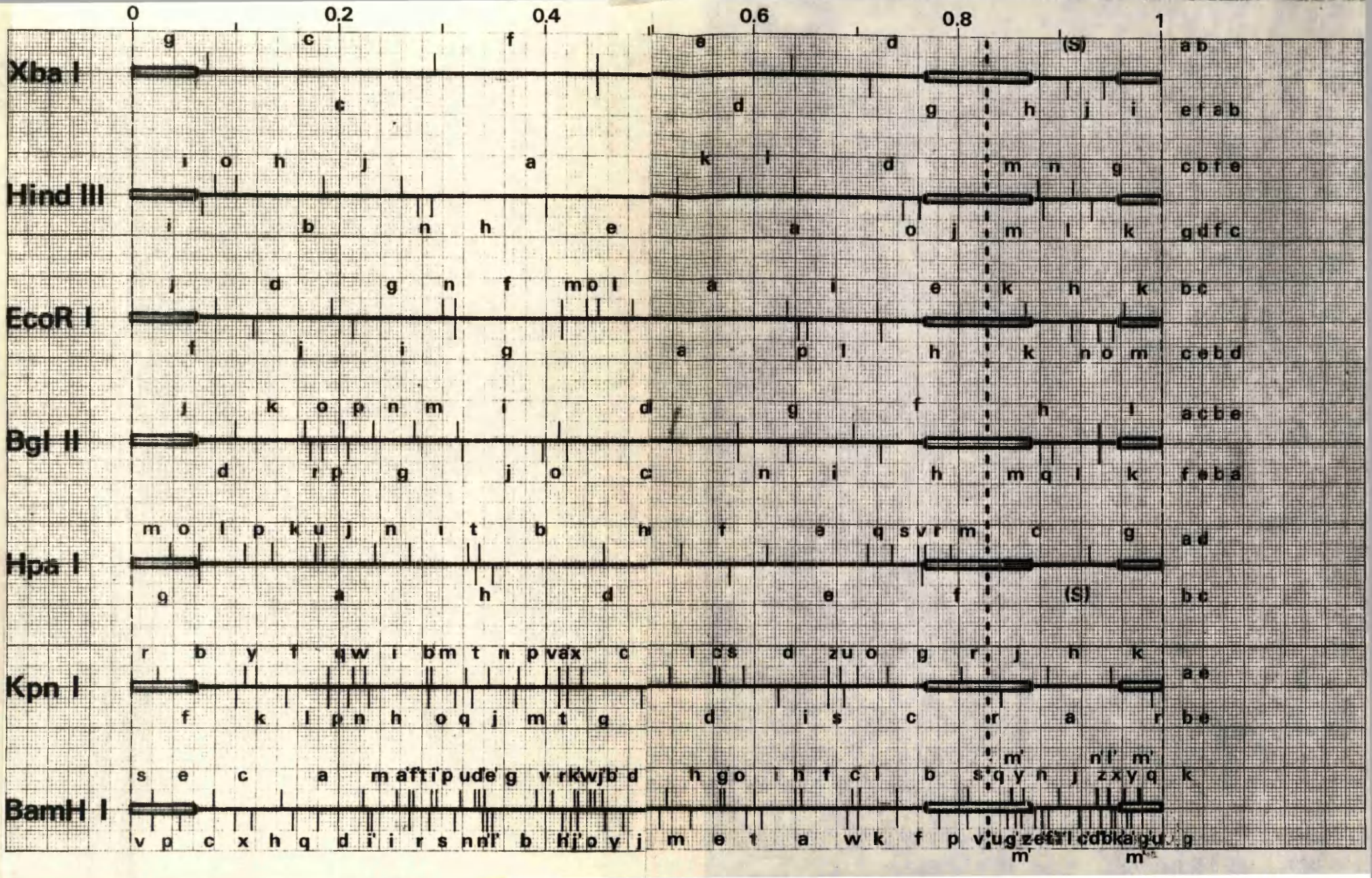

0
0·1
0·2
0·3
0·4
0·5
0·6
0·7
0·8
0·9
1·0
HSV-2
Xba I
Hind III
EcoR I
Bgl II
Hpa I
Kpn I
BamH I
a=c+h
b=c+i
e=g+h
f=g+i
c=i+k
d=j+k
f=l+m
g=j+m
b=f+k
c=n+k
d=f+m
e=h+m
a=d+k
b=d+m
e=h+k
f=h+m
b=f+[S]
c=g+[S]
g=v+u

Figure C1.20

Restriction maps for HSV-1 DNA aligned with those for HSV-2 DNA. Data used to determine the best alignment included restriction fragment sizes, intertypic hybridisation (Section 2 of Results), and analysis of intertypic recombinants (Preston et al., 1978; Stow and Wilkie, 1978; Marsden et al., 1978; Chartrand et al., 1979 and 1980; Section 2 of Results).
Since the HSV-2 genome has an extra 1-2 x 10^6 daltons of DNA in the S segment compared with that of HSV-1, the following fragments have been shortened in the HSV-2 maps: Xba I j, Hind III l, EcoR I n, Bgl II l, Hpa I (S), Kpn I a, and BamH I l. The scale shows fractional genome length, and the nature of joint fragments is shown on the right.


0
0.2
0.4
0.6
0.8
1
Xba I
Hind III
EcoR I
Bgl II
Hpa I
Kpn I
BamH I

SECTION 2

HOMOLOGY BETWEEN HERPESVIRUS GENOMES

RESULTS

Introduction

HSV-1 and HSV-2 share homology in about 50% of their DNA sequences (Kieff et al., 1972), whereas the DNA of pseudorabies virus (PRV) has been reported to be less than 8% homologous to HSV-1 and HSV-2 DNA (Bronson et al., 1972). The purpose of the work described here was firstly to determine the genomic distribution of homologous sequences between HSV-1 and HSV-2, and to ascertain whether the genomes are colinear. Second, experiments were carried out to determine whether regions of greater homology between HSV-1 and HSV-2 DNA are also homologous to PRV DNA and equine herpes virus type 1 (EHV-1) DNA to a greater extent than other regions. Homology between the HSV and human cytomegalovirus (CMV) genomes was also investigated. Third, colinearity of the genomes of HSV and EHV-1 or PRV was considered. Last, a study was made of a large number of HSV-1/HSV-2 intertypic recombinants in order to determine the relationship between crossover location and regions of higher homology.

Distribution of homologous sequences between HSV-1 and HSV-2 DNA

Blot hybridisation provides a good method for semi-quantitative estimation of the proportion of probe which hybridises to each restriction fragment of a genome. Hybridisation in solution is quantitative, but requires each fragment to be isolated and hybridised separately, and therefore is much less convenient for initial determination of the distribution of homology between regions of HSV-1 and HSV-2 DNA.

Figures C2.1-C2.2 show examples of the results of hybridising HSV-1 or HSV-2 probes to nitrocellulose filters containing fragments of HSV-1 or HSV-2 DNA. The probes in

these, and subsequent, experiments were prepared by nick translation in the presence of 10^{-8} g/ml DNase, and under these conditions the mean size of single-stranded DNA fragments is 400 n (Rigby et al., 1977). Each blot strip of a pair contained an equal amount of DNA (0.5-1.0 μg), and resulted from transfer of DNA from the same gel. An equal amount of probe DNA (0.1-0.2 μg) was added to each strip and allowed to anneal to saturation (3-5 days) under stringent hybridisation conditions (3 x SSC and Denhardt's solution in 50% formamide at 45°). The experimental design ensured that potential causes of artefacts, such as inefficient transfer of some restriction fragments in the blotting procedure, were cancelled out.

The extent of intertypic homology can in principle be determined for each restriction fragment by comparison of the band intensities produced by homologous and heterologous probes. In Figure C2.1, for example, HSV-1 BamHI o and r are more intense than pq with HSV-2 probe. A semi-quantitative estimate of hybridisation to each fragment was made by autodensitometric scanning of several autoradiographs. Peaks were cut out and weighed, and the following ratios calculated.

$$R_1 = \frac{\text{wt of peak with heterologous probe}}{\text{wt of peak with homologous probe}}$$

$$R_2 = \frac{\text{total wt of peaks with heterologous probe}}{\text{total wt of peaks with homologous probe}}$$

$$R_3 = R_1/R_2.$$

R_3 is independent of specific activities of the probes and duration of autoradiographic exposure in the linear range of the film, and is an arbitrary value for relative homology.

All the fragments of restriction digests could not be resolved on a single gel, so it was necessary to scan two autoradiographs: one from a gel of lower agarose concentration for bands of larger molecular weights, and one of higher concentration for bands of smaller molecular weight. This approach led to two internally consistent sets of R_3 values in each case. A few bands of intermediate molecular weight were resolved on both gels, so R_3 values for smaller fragments were multiplied by a factor which equalised the two sets of R_3 values for the intermediate bands, thereby linking the two sets of data. R_3 values are, of course, merely an approximate indication of relative homology, the intertypic genome-to-genome homology being unity.

Figure C2.3 shows histographically the results of several experiments, and summarises the regions of greater homology between HSV-1 and HSV-2 DNA. There is one region of greater homology in TR_S/IR_S, and six, or perhaps seven, in U_L. Regions of least homology include TR_L/IR_L, the junctions between U_S and TR_S/IR_S, and the central portion of U_S.

Figure C2.2 shows the effect of temperature of hybridisation upon hybridisation of HSV-2 probe to HSV-1 restriction fragments. The patterns at three different temperatures are similar, except that HSV-2 DNA hybridised more efficiently to the joint and S terminal fragments of HSV-1 DNA (BamHI k and q) at the higher temperatures. The general saturation level of HSV-2 probe to HSV-1 DNA decreased with increasing temperature.

Colinearity of the genomes of HSV-1 and HSV-2

It has been assumed in interpreting the results of homology experiments that the genomes of HSV-1 and HSV-2 are colinear. To test this assumption plasmids containing HSV-2

HindIII fragments were nick translated and hybridised to blot strips containing BamHI fragments of HSV-1 or HSV-2 DNA (Figure C2.4). Fragments complementary to each probe are listed in Table C2.1. No hybridisation of probes to regions of the genome in which they do not map was detected in this experiment. The few faint bands observed when HSV-1 BamHI blot strips were used (Figure C2.4) were probably due to slight under-digestion of the HSV-1 DNA.

These results are consistent with colinearity of the HSV-1 and HSV-2 genomes. There is no evidence from these experiments for homology between one region of a genome and a region of the same or the heterologous genome mapping at a different position.

Non-colinear homology between the genomes of HSV-1 and HSV-2

The results of cross-blot experiments suggested that certain fragments of one genome also hybridised to distal fragments of the same, or the heterologous, genome. In Figure C1.10 there is evidence of homology between HSV-1 KpnI j and k (the S termini) and HSV-1 HpaI m (the L terminus) and opqr. In Figure C2.5, HSV-1 KpnI j and k hybridised to HSV-1 BamHI d, j and s, and perhaps to b, c and e. Homology between L and S termini is explained by the presence to the a sequence at both termini and at the L-S joint. Hybridisation to HSV-1 BamHI j was the result of BamHI k (BamHI k with an additional a sequence) which comigrated with this fragment. Figure C2.5 shows a cross-blot of HSV-1 BamHI against HSV-1 BamHI, and demonstrates some hybridisation of HSV-1 BamHI k and pq to d. Figure C2.6 shows some hybridisation of EcoRI k to l. These results suggest a region in HSV-1 BamHI d and EcoRI l to which the S terminus has some sequence homology. Hybridisation between the S terminus and the

junctions between U_L and TR_L/IR_L was detected in some, but not all, experiments.

Figure C2.6 shows a cross-blot between HSV-1 BamHI and HSV-2 BamHI. The most obvious aberrant homologies are those between HSV-1 BamHI k and pq and HSV-2 BamHI p and yz. Fainter spots include HSV-1 BamHI k and pq with HSV-2 BamHI c, f, l and a', and HSV-2 BamHI a' with HSV-1 BamHI b, d and e. Many of these aberrant homologies involve repetitive sequences or their junctions with unique regions.

Homology between the HSV genome and the genomes of EHV-1 and PRV

Figure C2.7 shows the results of hybridising nick translated HSV-1, HSV-2, EHV-1 or PRV DNA to blot strips containing restriction fragments of HSV-1 or HSV-2 DNA. Tables C2.2-C2.3 summarise the HSV fragments to which EHV-1 and PRV probe hybridised. The regions of homology of either EHV-1 or PRV DNA with the genomes of HSV-1 and HSV-2 are shown in the upper panel of Figure C2.10, and are largely similar in position to the more homologous regions between HSV-1 and HSV-2 DNA.

In order to determine the relative mapping positions of HSV DNA sequences homologous with EHV-1 and PRV DNA, nick translated plasmids containing HSV DNA fragments were hybridised at 40° to blot strips containing EHV-1 or PRV restriction digests. The results are shown in Figures C2.8 C2.9, and are summarised in Tables C2.4-C2.5. The middle and lower panels of Figure C2.10 show the positions in the genomes of EHV-1 and PRV to which each cloned fragment hybridised. In instances where hybridisation occurred to a band containing more than one restriction fragment, results of the other digests were used to determine to which of the fragments hybridisation had occurred. For example, HSV-2

HindIII h hybridised to PRV KpnI jk, but to BamHI k, indicating that hybridisation was to KpnI k rather than to j. The faint bands present on the PRV DNA blot strips (Figure C2.9 such as those just below BamHI ij and KpnI l, which have not been included in Table C2.5, were due to the presence of minute amounts of contaminating pAT153 DNA in the dye-Ficoll which was added to the digested PRV DNA prior to electrophoresis; the probes contained nick translated pAT153 DNA as well as the cloned HSV DNA fragments, and therefore revealed these bands, which were not disclosed in the control strips.

Homology between the HSV and CMV genomes

Similar experiments to those already described were carried out by probing blot strips containing restriction digests of HSV-1 or HSV-2 DNA with human cytomegalovirus (CMV) strain AD169 DNA. The reverse experiment of probing blot strips containing CMV HindIII fragments with HSV-1 or HSV-2 DNA was also performed. No hybridisation was detected under conditions which allowed EHV-1 and PRV homology to HSV DNA to be detected. However, when excess probe (1 μg at a specific activity of 2 x 10^8 c.p.m./μg) was used, and long exposures made of autoradiographs, some hybridisation was detected (Figure C2.11). CMV DNA hybridised to HSV-2 BamHI p, and HSV-2 DNA to CMV HindIII no and hi; these results were reproduced in a second experiment. No homology between HSV-1 and CMV DNA was detected.

Studies of recombination between the HSV-1 and HSV-2 genomes

The technique of marker rescue of HSV mutants (Stow and Wilkie, 1978) has been very successfully used to generate HSV-1/HSV-2 intertypic recombinants (Preston et al., 1978; Stow and Wilkie, 1978; Marsden et al., 1978; Knipe

et al., 1978; Chartrand et al., 1979 and 1980). Structures of the recombinant genomes were routinely deduced by comparing their DNA restriction profiles with those of the parental viruses, and scoring for the presence or absence of restriction sites. The experiments described here were aimed at three aspects of recombination. The first aim was to investigate the possibility that only one of the four isomers of the HSV genome is involved in recombination. The second aim was to determine whether recombination tends to occur in regions of higher intertypic homology. The third aim was to attempt to construct recombinants with complete type-specific heterology between repetitive regions; it was predicted that such recombinants would not invert normally in the segment(s) bounded by heterotypic repetitions. This aspect is dealt with in Section 4.

The mutation in HSV-1 tsD maps in TR_S/IR_S (BamHI q; Preston, in press), and may conveniently be used to produce intertypic recombinants possessing HSV-2 DNA in repetitive regions. Cell monolayers were coinfected with tsD DNA and HSV-2 EcoRI bcde (fragments spanning the joint region) isolated from agarose gels, by the method of Stow and Wilkie (1976). The monolayers were incubated under EHu5 at 38.5°, and six plaques picked from each of fifteen Petri dishes. The ninety plaques were purified by two further rounds of plaque-picking at 38.5°. Virus stocks were grown at 31° on Petri dishes, titrated, and in vivo ^{32}P-DNA was prepared. Genome structures were analysed by restriction endonuclease digestion with XbaI, HindIII, EcoRI, BglII, HpaI, KpnI and BamHI. All crossovers mapped within the rescuing HSV-2 joint fragments, and genome structures of the six isolates from any single dish suggested that in

general they were not related.

The recombination events which occur in an experiment of this type are rather complex owing to the facility with which secondary recombination events occur in repetitive regions. This is discussed in detail in Section 4. In short, a primary crossover in one repetitive region is also present in the other repetition at the stage of genome analysis, due to secondary recombination events. This means that if the primary crossover occurred in a repetitive region of a segment, it is, in the great majority of cases, not possible to state the orientation of that segment during recombination. Nevertheless, recombination events in U_L or U_S are preserved unambiguously regardless of further crossovers in repetitive regions. Therefore, careful analysis of the resulting genomes from marker rescue of tsD allows either the orientation of both L and S at primary recombination to be deduced (crossovers in U_L and U_S), or the orientation of S only (crossovers in TR_L/IR_L and U_S), or the orientation of L only (crossover in U_L and TR_S/IR_S), or neither orientation (crossovers in TR_L/IR_L and TR_S/IR_S, or TR_S/IR_S and U_S, or TR_S/IR_S only).

Table C2.6 shows the numbers of recombinants representing each orientation of the rescued genome. It is clear from these results that tsD was rescued with the genome in both the P and the I_L orientations, and all the observed crossovers could have occurred between tsD and HSV-2 EcoRI b or c. It cannot be concluded, however, that recombination in the I_S or I_{SL} orientations (with HSV-2 EcoRI d and e) did not occur, because the great majority of recombinants possessed symmetrical crossovers in TR_S/IR_S and therefore might equally well have been the result of rescue by HSV-2 EcoRI d or e. The position of tsD in

TR_S/IR_S means that upon rescue of this mutation at least one crossover event in S is obligatory, whereas a concurrent crossover event in L is optional. It may be concluded that at least the P and I_L orientations of the genome participate in recombination.

A second feature of recombination is revealed by these results. About ten times as many recombinants resulted from marker rescue of the I_L orientation compared with the P orientation, although EcoRI b and c differ in size by only about 5%. The distribution of primary crossover positions in U_L are shown in Figure C2.12. Two recombinants possessed symmetrical crossovers in TR_L/IR_L, so it was assumed for the purposes of the diagram that the primary event could have occurred with equal probability in either TR_L or IR_L. Recombination seems to have occurred preferentially in the more homologous regions between the HSV-1 and HSV-2 genomes.

In a second experiment, twenty-eight recombinants were isolated by marker rescue of tsD with HSV-2 HindIII cdefg (cdfg are the joint fragments), of which it was possible to determine the structures of twenty-seven. All crossovers in U_S (7/27) occurred in regions of HSV-1 DNA complementary to HSV-2 HindIII k; other crossovers were within TR_S/IR_S, and in all except one case were symmetrically positioned in both repetitions. Other crossover positions were either in TR_L/IR_L close to the joint, or in TR_S/IR_S: these two possibilities could not be distinguished from each other. Although the rescuing fragments extended into U_L, no crossovers in U_L were detected. Primary crossover positions in S from both

experiments are shown in Figure C2.13. Only HSV-2 EcoRI joint fragments produced crossovers at the left hand side of U_S, and only HSV-2 HindIII joint fragments recombined at the right hand side. The comparison of recombination frequencies on the two sides of U_S was constructed on the assumption that primary recombination within TR_S/IR_S could occur with equal probability in either; therefore in each of the two cases the number of primary recombination events occurring in TR_S/IR_S was divided by two. The results are expressed as crossover frequencies relative to those within HSV-1 BamHI y. Again, recombination events occurred preferentially within the most homologous regions.

DISCUSSION

Features of nucleic acid hybridisation

Hybridisation in solution has been used successfully for many years to determine relationships between nucleic acids, and the features of this assay have been well characterised both theoretically and empirically. Hybridisation rate is maximal at approximately 25° below the hybrid melting temperature (T_m), and varies minimally over a range of 10-15° in that region. T_m is related to ion concentration and GC content (Schildkraut and Lifson, 1965), and is decreased by approximately 1° per 1% base mismatching (Hutton and Wetmur, 1973). The use of single stranded DNA bound to a nitrocellulose filter in the hybridisation reaction is a less well-characterised system, since under certain conditions the rate of reaction is limited by a diffusion factor, and also concatenation of partially reannealed probe to the filter-bound DNA may occur (Flavell et al., 1974a and b). The use of formamide in filter hybridisation still further reduces the possibility of meaningful quantification of results. In solution hybridisation, formamide reduces T_m by 0.6° per 1% formamide, and the hybridisation rate by half, although the rate retains an approximate independence of temperature in the region of T_m-25° (Hutton, 1977). Formamide effectually increases the stability of mismatched hybrids on filters when compared with what are presumably comparable conditions in the absence of formamide (Schmeckpeper and Smith, 1972).

Therefore filter hybridisation in 50% formamide, which was the method used in this study, can provide an indication of relative hybridisation between restriction fragments of heterologous DNAs, but not reliable quantitative estimates. However, quantitative estimations of the degree of homology

between heterologous DNAs derived from hybridisation experiments bear no direct relationship to similarity of nucleotide sequences, and this is of course the more meaningful description of homology. For example, it is not known whether homologous sequences detected in this study are widely distributed within the homologous regions, or whether they consist of local regions of very high homology. These alternatives could be distinguished by estimating the stability of the hybrids. A large difference in the extent of apparent homology between two strains of vesicular stomatitis virus determined by the two methods of hybridisation and nucleotide sequencing was discussed by McGeoch et al. (1980).

In hybridising heterologous DNAs, the T_m of hybrids, and hence their rate of formation and resulting stability, is dependent upon the GC content of homologous regions and the extent of base mismatching. Using the equation of Schildkraut and Lifson (1965) and assuming that 50% formamide reduces T_m by 30° (Hutton, 1977), the DNAs of HSV-1, HSV-2, PRV and EHV-1 under the conditions used have T_m-25° values of 48.9, 49.7, 51.4 and 44.4°, respectively. These are mean values, and T_m will differ for regions of the genome with GC contents different from the mean. In general, however, hybridisation of HSV-1 and HSV-2 DNA at 50° allows up to 10-15% mismatching, 45° allows 15-20%, and 40° allows 20-25%, assuming that the effect of mismatching on T_m is the same in solution hybridisation and under the conditions used here. Hybridisation of HSV-1 and HSV-2 DNA at these three temperatures resulted in similar, but not identical, patterns (Figure C2.2). This is in agreement with Kieff et al. (1972), who concluded that the homologous sequences possess a low degree of mismatching. Hybridisation of EHV-1 DNA to HSV-1 or HSV-2 DNA was much more temperature-

dependent in the 40-50° range (Figure C2.7), presumably as a result of greater mismatching.

The equation of Schildkraut and Lifson (1965) shows that homologous regions may vary in GC content over a 35% range, other factors being equivalent, without greatly affecting the rate of hybridisation. The distribution of the more homologous regions between HSV-1 DNA and the other herpesvirus DNAs does not obviously reflect the distribution of GC-rich regions in the HSV-1 genome located by thermal denaturation experiments (Delius and Clements, 1976). It is therefore probable that the regions of greater homology detected in this study are largely the result of greater similarity of nucleotide sequences rather than merely a reflection of local GC content.

Colinearity of the genomes of HSV-1 and HSV-2

Hybridisation of cloned DNA fragments to nitrocellulose strips containing HSV-1 or HSV-2 restriction fragments showed that, within the resolution attained, the two genomes are colinear (Figure C2.4, Table C2.1). Hybridisation of HSV-1 and HSV-2 BamHI fragments in the cross blot of Figure C2.6 also demonstrated colinearity, with the exception of some non-colinear homology as discussed previously. Esparza et al. (1976) concluded that there is at least some degree of colinearity between HSV-1 and HSV-2 DNA from the observation that heterologous mutant pairs which failed to complement also failed to recombine. Analyses of intertypic recombinants have shown that crossovers occur throughout the genome, reflecting functional colinearity. Specific functions, such as the dPyK gene (Halliburton et al., 1980), the DNA polymerase gene (Chartrand et al., 1979), and the IE genes (Clements et al., 1977; Easton and Clements, 1980) are in equivalent locations in the two genomes. The morphological transforming regions of

HSV-1 and HSV-2 are not equivalently located, but the DNA in each region is colinear with the analogous region in the other genome (Reyes et al., 1979). Ruyechan et al. (1979) concluded that the genes specifying gC are not colinear, although this was not supported by Halliburton (1980). The HSV-1 BamHI/HSV-2 BamHI cross blot of Figure C2.6 did not suggest any non-colinearity in this region (0.53-0.69 fractional genome units).

Conserved regions in the genomes of HSV-1, HSV-2, EHV-1 and PRV

Seven major regions of higher homology between HSV-1 and HSV-2 DNA were demonstrated (I-VII in Figure C2.10), and of these I, III, V, VI and VII were also homologous to PRV and EHV-1 DNA, although at a much lower level. The extent of region II was more limited using EHV-1 DNA as probe, and region IV was detected with PRV as probe only when HSV-1 DNA filters were used. Although the same regions are conserved between the four genomes, the results did not show whether the same DNA sequences are conserved in each case. The observation that nearly all HSV-1 and HSV-2 restriction fragments showed some level of hybridisation to the heterologous probe (Figures C2.1 and C2.2), whereas only certain fragments hybridised to PRV or EHV-1 probes (Figure C2.7), may be attributed to the much lower level of hybridisation in general in the latter case, and hence a lower level of stringency may be required to detect hybridisation to the other fragments. The seven regions of higher homology probably reflect constraints on DNA sequences imposed by conservation of amino acid sequences in the proteins encoded by the DNA. The following paragraphs discuss the identities and functions of putative conserved proteins.

Region I maps within the HSV-2 morphological transforming region (Reyes et al., 1979). One possibility for the conserved

protein is gC, the structural gene for which is located in this region but has not been precisely mapped (Marsden et al., 1978; Ruyechan et al., 1979; Halliburton, 1980). Type-specific antigenic sites have been reported for this glycoprotein (Norrild and Vestergaard, 1979). Perhaps a more likely candidate is V_{mw} IE 136'(143), the gene for which is transcribed in small quantities in the presence of cycloheximide, and in greatly increased amounts after removal of the inhibitor (Clements et al., 1977; J. McLauchlan and J.B. Clements, personal communication). The mRNA has a size of 5.0 kb and has been accurately located on the genome within region I (Anderson et al.,1981; J. McLauchlan and J.B. Clements, personal communication). The function of the protein is unknown, although the HSV-2 function causing inhibition of host macromolecular synthesis maps in the same location (0.56-0.57).

Region II is located in the position of the structural gene coding for the HSV DNA polymerase (Chartrand et al., 1979). Halliburton and Andrew (1976) were able to detect a degree of cross-neutralisation between the DNA polymerases of HSV-1 and HSV-2, but not between these two viruses and PRV.

The data of Conley et al. (1981) suggest that region III contains the gene coding for the major DNA-binding protein (MDBP) of HSV-1. Yeo et al. (1981) showed that antisera to cells infected with HSV-1, HSV-2, BMV, EHV-1 and PRV react heterologously predominantly with MDBP, and they proposed this protein as a putative herpesvirus group specific antigen. Although MDBP is likely to have a central role in DNA replication, its precise function is unknown. The loci for gA and gB also map in this region, and these glycoproteins have been reported to be serologically conserved in HSV-1 and HSV-2 (Norrild et al., 1978; Eberle and Courtney, 1980a). Others

have reported that gA and gB possess type-specific antigenic sites (Powell and Watson, 1975; Courtney and Powell, 1975).

Yeo et al. (1981) also reported limited cross-reactivity between the major capsid proteins (MCP) of the five herpesviruses they considered. Courtney and Powell (1975) showed type-common antigenic sites on the MCP of HSV-1 and HSV-2 using specific antisera, and Cohen et al. (1980b)demonstrated conservation of MCP in HSV-1 and HSV-2 by serological and proteolytic methods. MCP has a m.wt. of 140,000-160,000 in a wide range of herpesviruses (Spear et al., 1978) and the gene has been precisely located for HSV-1 (Costa et al., 1981), corresponding well with the position of region V.

Region VII is certainly located, for HSV-1, within the coding region of the V_{mw} IE 175 gene (M-J. Murchie and F.J. Rixon, personal communications). Detailed homology studies of the joint regions of HSV-1 and HSV-2 DNA, as discussed in Section 3, indicate that homology extends throughout at least the polypeptide coding region of the gene: the arrangement of restriction sites falsely gives the impression that HSV-1/HSV-2 homology in this region is confined to HSV-1 BamHI y, whereas it does extend into BamHI k. An IE polypeptide thought to be analogous to HSV-1 V_{mw} IE 175 has been mapped in a similar position in TR_S/IR_S of PRV (Powell, 1979). V_{mw} IE 175 functions in promoting transcription of early genes. It is noted that, although homology between HSV-1 and HSV-2 DNA in this region of the genome is greater than in adjacent regions, it seems not to be as great as in regions I-VI (Figure C2.3). Courtney and Powell (1975) reported type-specific sites on this polypeptide in HSV-1 and HSV-2, using a specific antiserum to V_{mw} IE 175.

Knowledge of the locations of gene functions on the HSV

genome is not yet sufficiently advanced to allow the identification of the proteins which may be responsible for the greater homology in regions IV and VI. With the exception of the putative analogue of V_{mw} IE 175 in PRV, nothing is directly known of the proteins coded by the homologous regions in the PRV and EHV-1 genomes.

The proteins described above comprise early and late, structural and non-structural members. It is noted that the dPyK gene does not reside within a region of greater homology in HSV-1 and HSV-2. The regions of least homology between HSV-1 and HSV-2 DNA encode HSV-1 V_{mw} IE 12 and 68 at the TR_S/U_S and IR_S/U_S junctions, and V_{mw} IE 110 in TR_L/IR_L. A strict comparison between serological and hybridisation studies in the search for conserved proteins is not straightforward, since gD has been reported to be conserved in HSV-1 and HSV-2, both serologically (Sim and Watson, 1973; Honess and Watson, 1974) and by tryptic peptide mapping (Eisenberg et al., 1980). The gene coding for this glycoprotein resides within U_S (Ruyechan et al., 1979), none of the sequences of which were detected as being particularly homologous in HSV-1 and HSV-2.

Homology between the genomes of HSV and HCMV

A very small amount of homology between HSV-2 DNA and at least two loci in U_L of the CMV genome, and between CMV DNA and TR_L/IR_L of the HSV-2 genome was detected at the temperature of hybridisation used. It is questionable whether such a low level of hybridisation is meaningful at all, since it may result from non-specific hybridisation of DNA of unusual composition (e.g. high GC content). These results are in agreement with those of Huang and Pagano (1974), who failed to detect significant hybridisation of HSV-1 or HSV-2

DNA to CMV DNA. Several other lines of investigation have demonstrated that apart from virion morphology, gross genome structure and some aspects of virus growth, HSV and CMV show little similarity. For example, CMV does not possess a dPyK or thymidine kinase gene (Miller et al., 1977; Jerkofsky et al., 1980), and exhibits an IE transcriptional pattern markedly different from that of HSV (Wathen et al., 1981).

Distribution of regions homologous to HSV DNA in the genomes of EHV-1 and PRV

The results summarised in Figure C2.10 show that the homologous regions in the L segment of EHV-1 DNA are colinear with those in the L segment of HSV DNA in the I_L orientation. It must be stressed that the experiments allowed only homologous regions to be located. Although it is reasonable to propose that other genes are colinear in the two virus genomes, there is no evidence to support this hypothesis. Some homology was detected which does not map colinearly. HSV-2 HindIII e hybridised to some extent to the same region as HSV-2 HindIII h. This might represent a limited region of genetic non-colinearity, or might be an aberrant result in that it does not signify functional colinearity. Apparently aberrant hybridisation of HSV (especially HSV-2) fragments containing the S terminus to the middle of L in EHV-1 is discussed below.

The conclusions for PRV are broadly in agreement with those of Rand and Ben-Porat (1980), who described the pattern of hybridisation between the HSV and PRV genomes using virion DNA, noting that most of the homologous sequences are situated in the L segment. The pattern of homologous regions in the L segment of PRV DNA shown in Figure C2.10 does not fit a colinear arrangement with respect to HSV DNA. Inversion of the region hybridising to HSV-2 HindIII h-e-a would lead to

approximate colinearity between the homologous regions in the L segment of PRV DNA and the I_L orientation of HSV DNA (i.e. analogous to the colinearity of EHV-1 and HSV DNA). Therefore the possibility exists that this region of the PRV genome is inverted with respect to HSV. The actual situation may be, of course, more complex. As with EHV-1 DNA, fragments from HSV TR_S/IR_S hybridised to equivalent positions in PRV DNA, and the orientation of HSV-1 BamHI k and y with respect to the PRV genome is in agreement with the orientation of the PRV gene analogous to HSV-1 V_{mw} IE 175 (Powell, 1979; F.J. Rixon, Personal communication).

Non-colinear homology

Non-colinear homology was detected within the HSV-1 genome and between HSV-1 and HSV-2 DNA using the cross blot technique. Such homology was not detected in experiments where cloned DNA probes and nitrocellulose strips were used. The difference is probably a result of the different techniques. The cross blot method allows the detection of regions of poor homology since at that point in the nitrocellulose filter there is no competition for the probe by filter-bound sequences of greater homology. The significance of non-colinear homology is in doubt. Most instances involve the inverted repeats or their junctions with unique regions, sequences known to possess regions of intra- or inter-strain size variability and, at least in some instances, to contain multiple reiterations of short GC-rich sequences (Lonsdale et al., 1980; Section 3 of Results; M-J. Murchie and F.J. Rixon, personal communications). It is possible, therefore, that non-colinear homology reflects the locations of such sequences within the genome. A similar consideration may be applied to the observed hybridisation of HSV fragments containing the S terminus to regions in the L

segment of both PRV and EHV-1 DNA, and of several HSV fragments to repetitive regions of the PRV and EHV-1 genomes (Tables C2.4-C2.5). The non-colinear homology in these cases was far more prominent than that detected within HSV-1 DNA or between the HSV-1 and HSV-2 genomes, probably as a result of the generally low level of hybridisation of PRV and EHV-1 DNA to HSV DNA.

Genetic relatedness of herpesviruses

The main approach to studies of genetic relatedness among herpesviruses has been an immunological one. A number of techniques are widely used, including virus neutralisation, immune precipitation and immunofluorescence. Antisera may be raised against virions, nucleocapsids, virus-infected cells or purified proteins. The criterion of relatedness is whether an antiserum produced against one virus cross-reacts with another virus in the test used. The pertinent point has been made, however, that antigens with type-specific determinants also possess type-common sites, thus limiting the conclusions which may be drawn by this approach to herpesvirus relatedness (Killington et al., 1978; Yeo et al., 1981). The search for a herpesvirus group specific antigen has nonetheless proceeded from the MCP (Kirkwood et al., 1972; Honess et al., 1974) to the virion glycoproteins (Norrild et al., 1978) and at present centres on the MDBP (Yeo et al., 1981). Killington et al. (1978) have proposed an immunological classification of herpesviruses with two main classes: a neutroseron, containing viruses which cross-neutralise, and a seron, containing viruses which cross-react antigenically but which do not necessarily cross-neutralise. This group has presented evidence, from perhaps the clearest work on the subject so far, that HSV-1, HSV-2 and BMV form a neutroseron,

and that these three viruses plus PRV and EHV-1 form a seron (Killington et al., 1977 and 1978; Yeo et al., 1981). The implication that HSV-2 is more closely related to HSV-1 than is BMV, and that PRV and EHV-1 are more distantly related to HSV-1, has the support of nucleic acid hybridisation studies (Kieff et al., 1972; Bronson et al., 1972; Sterz et al., 1974).

Nucleic acid hybridisation lends itself as an alternative approach to the study of genetic relatedness between herpesviruses. In the past, however, this technique has been unsatisfactory in that only gross genome-to-genome homology was measured. Moreover, most herpesvirus DNAs share less than 5-10% homology, a level which by this method is difficult to distinguish from zero. The availability of cloned DNA fragments and the use of blot hybridisation clearly enhances the usefulness of hybridisation, as demonstrated in this study. Liquid hybridisation experiments would allow better quantification of results, although nucleotide sequencing of analogous genes in different herpesvirus genomes will supply the ultimate estimation of homology.

One application of the study presented here is that the locations of EHV-1 genes can be predicted by virtue of the colinearity between homologous regions in EHV-1 and HSV DNA. Further, an unmapped gene in either EHV-1 or PRV DNA could be located by using as a probe the HSV-1 gene, providing there is sufficient homology between the respective DNA sequences. For example, the MCP gene in EHV-1 and PRV could be located using an HSV-1 MCP gene probe. This would obviate the need to locate this function by marker rescue of a mutation in the gene, which requires a mutant, or by analysis of intertypic recombinants, which requires two serotypes with distinctive restriction and polypeptide profiles which are able to recombine. It may prove possible to locate with HSV-1 probes even apparently

non-homologous genes in PRV or EHV-1 DNA, such as the thymidine kinase gene, by decreasing the stringency of hybridisation.

Homology and recombination in HSV

It was concluded from the structures of HSV-1/HSV-2 intertypic recombinants produced by marker rescue of HSV-1 tsD that recombination tends to occur in regions of greater homology. In attempting to obtain the distribution of crossovers between the two ends of L and S it was assumed, and probably not unreasonably, that recombination within either TR_S or IR_S and either TR_L or IR_L occurs with equal probability with the appropriate segment in either orientation. Even if this were not the case, the conclusion would be unaffected. Morse et al. (1977) suggested from their limited study that crossovers tended to cluster in the region of 0.4 and 0.62-0.69 fractional genome units. An analysis of recombinants from several laboratories reviewed by Halliburton (1980) shows a rather good fit between the distribution of crossovers throughout the genome and the distribution of homology presented here, with the exception of a high frequency of crossovers at 0.62-0.69 observed by Morse et al. (1977a) but not by other groups. No recombinant has yet been described, including those analysed here, which has a crossover in the region of least homology between the two genomes, that is, at the TR_S/U_S and IR_S/U_S junctions.

Does only one HSV genome arrangement participate in recombination?

The results of the work presented here are important with respect to the hypothesis that not all four genome arrangements may participate in the generation of viable intertypic recombinants. Analyses of the numbers of crossovers in intertypic recombinants have suggested that only one or both of the P and I_S arrangements participates in recombination

(Morse et al., 1977 ; Preston et al., 1978; Roizman et al., 1979; Wilkie et al., 1979b). The evidence is that genomes possessing an odd number of crossovers in U_L display the least number of crossovers in the P or I_S arrangements. Indeed, this hypothesis finds support in the observation that the majority of virion DNA of recombinants Bxl(28-1) and RE4 is in the P or I_S, and I_S, arrangements, respectively (Preston et al., 1978; Section 4 of Results). The argument has three weaknesses which were emphasised by its proposers. Firstly, it is based on a small number of recombinants with an odd number of crossovers in U_L: 9/62 recombinants produced from genetic crosses in the studies of Morse et al. (1977), Preston et al. (1978), Marsden et al. (1978) and Halliburton et al. (1980). The assumption that an odd number (n) of recombination events in the genome is more likely to occur than an even number (n+1) must be viewed with caution since the majority of recombinants (44/62) possess even numbers of crossovers in the genome. Secondly, undetected crossovers in the recombinants with odd numbers of crossovers in U_L could reduce the number put forward as evidence. At least two have been shown to have an additional crossover near the L terminus (Section 4 of Results). Thirdly, the way in which recombinants were made may have biased their orientation durimg recombination. Recombination is homology-dependent, and therefore recombinant genome structures are dictated not only by the particular mutants used in a genetic cross and by the sequences available for recombination in the case of marker rescue, but also by the extent and distribution of homology within those sequences.

The second argument in favour of the hypothesis that not all four arrangements take part in recombination stems

from genetic mapping of mutant markers. Clements et al. (1976) have made the point that inversion of L and S would be expected to lead to equal recombination frequencies between any marker in L and any marker in S. That is, it would not be possible to align markers in L with respect to those in S. Brown et al. (1973) obtained a linear genetic map between several markers in U_L and one in TR_S/IR_S. Unfortunately, Stow et al. (1978) were unable to correlate this map with the physically determined locations of the markers, and therefore it was not possible to state which orientation of U_L the genetic map represents. Parris et al. (1980) claimed to be able to correlate the genetically and physically determined mapping positions of several mutations. Their interpretation was that markers in L and S were not equidistant because only one or both of the I_L and I_{SL} arrangements participates in genetic recombination. Clearly this is the opposite conclusion from that attained by argument from recombinant genome structures.

The data presented here show that at least the P and I_L arrangements are able to participate in the generation of viable recombinants, but that available homologous sequences profoundly affect the frequency of recombination in each orientation. Taken together with the above contradictory conclusions, there is at present no good reason for supposing that not all four arrangements are able to recombine. A recent contribution is the hypothesis of Honess et al. (1980), who presented a detailed genetic analysis of several HSV-1 markers, and concluded that recombination in HSV occurs by a circular or concatemeric mechanism. This conclusion is not negated by inclusion in the study of HSV-1 tsc75, which had previously been erroneously mapped in each a sequence (Knipe et al., 1979), but has since been shown to map only in the

c sequences (Preston, in press). Two features of this model are that it better accommodates inclusion of all four arrangements in recombination, and that all recombinants conceptually have an even number of crossovers.

Table C2.1

Hybridisation of nick translated recombinant plasmids containing HSV DNA fragments to nitrocellulose blot strips of HSV-1 and HSV-2 BamH I digests.
The data are taken from Figure C2.4.

Table C2.1

cloned fragment probe	HSV-2 DNA BamH I strips	HSV-1 DNA BamH I strips
HSV-2 Hind III a	ab e f k m st vw	f h i l o c' f'g' h'
HSV-2 Hind III b	c d hij q r x i'	a c e m a' f'g'
HSV-2 Hind III e	ab hij m o yz h' j'	d h r w b' j'
HSV-2 Hind III h	ab n st k'l'	g pq uv d' e' i'
HSV-2 Hind III l	l c'd' e' f'	j n
HSV-2 Hind III n	r	st
HSV-2 Hind III o	f	b
HSV-2 Hind III 12	g l u yz a' b' c'd' g' k'l' m'	j k pq x y z
HSV-1 BamH I k	g u v	k pq st
HSV-1 BamH I y	g'	y
HSV-2 BamH I g	g u v	k pq st

Table C2.2

Hybridisation of nick translated EHV-1 and PRV DNA to nitrocellulose blot strips of the HSV-1 BamH I digest. Data are taken from Figure C2.7. BamH I fragments to which the probes hybridised are indicated, the number of circles displaying an estimation of the amount of hybridisation.

Table C2.2

HSV-1 BamH I fragment	50° EHV-1	45° PRV	45° EHV-1	40° EHV-1
a		oo	oo	ooo
b				
c		oo	oo	ooo
d				
e				
f				
g		ooo		oo
h				
i				
j				
k, $\bar{k}$	ooo	oo	ooo	oo
l				
m		oo		oo
n				
o		oo		oo
p				
q	ooo	oo	ooo	oo
r		ooo		oo
s				
t				
uv		oo		
w				
x				
y	o	oo	oo	oo
z				
a'		ooo		oo
b'		oo		
c'				
d'		o		
e'				
f' g'				
h'				
i'				
j'				

Table C2.3

Hybridisation of nick translated EHV-1 and PRV DNA to nitrocellulose blot strips of the HSV-2 BamH I digest. Data are taken from Figure C2.7. BamH I fragments to which the probes hybridised are indicated, the number of circles displaying an estimation of the amount of hybridisation. The use of the HindIII/EcoRI digest of HSV-2 DNA in another experiment confirmed that hybridisation was to HSV-2 BamHI t and not s.

Table C2.3

HSV-2 BamH I fragment	50° EHV-1	45° PRV	45° EHV-1	40° EHV-1
a				
b		ooo	o	o
c		oo	oo	ooo
d		ooo		o
e		o		
f		o	o	o
g, ḡ	ooo	oo	ooo	oo
ni j		ooo		ooo
k				
l				
m			o	
n				
o		oo		o
p		o		o
q			o	
r				
st		oo		o
u	ooo	ooo	ooo	oo
vw				
x		o		
yz				
a'				
b'				
c' d'				
e'				
f'				
g'		oo	oo	
h'		ooo	oo	
i'				
j'		ooo		
k' l'				
m'		oo		

Table C2.4

Hybridisation of nick translated recombinant plasmids containing HSV DNA fragments to nitrocellulose blot strips of EHV-1 DNA digests.

Data for BglII and BglII/EcoRI digests are taken from Figure C2.8. Fragments in parentheses hybridised more weakly. The temperature of hybridisation was 40° except for EHV-1 BamHI, when it was 45°. In the latter experiment the increased temperature significantly reduced hybridisation when HSV-2 HindIII a, b and h were used as probes.

Table C2.4

cloned fragment probe	EHV DNA EcoRI strips	EHV DNA BglII strips	EHV DNA EcoRI/BglII strips	EHV DNA BamHI strips
HSV-2 HindIII a	m (b) (cd) (ef)	j (a)	m (a) (cd)	m
HSV-2 HindIII b	a ef l	e gh i n (a)	ghi pqr uv	b fg o (c)
HSV-2 HindIII e	a	b o (a)	cd xyz	a
HSV-2 HindIII h	a	b gh p	cd ghi	a i
HSV-2 HindIII l	-	-	-	-
HSV-2 HindIII n	-	-	-	-
HSV-2 HindIII o	-	-	-	-
HSV-1 BamHI k	a b cd ef	a b	a b cd e	e qrs
HSV-1 BamHI y	b cd ef	a	a b cd e	(not done)
HSV-2 BamHI g	a b cd ef	a b	a b cd e	b e qrs
HSV-2 HindIII 12	a b cd ef	a b	a b cd e	b e qrs

Table C2.5

Hybridisation of nick translated recombinant plasmids containing HSV DNA fragments to nitrocellulose blot strips of PRV digests. Data are taken from Figure C2.9. Fragments in parentheses showed less hybridisation.

Table C2.5

cloned fragment probe	PRV DNA BamH I strips	PRV DNA Kpn I strips
HSV-2 Hind III a	b (ij) (o)	a (d)
HSV-2 Hind III b	c d g	c f gh m
HSV-2 Hind III e	a (o)	a
HSV-2 Hind III h	a k	c jk
HSV-2 Hind III l	-	-
HSV-2 Hind III n	-	-
HSV-2 Hind III o	-	-
HSV-2 Hind III 12	ij k o (a) (b) (c) (d) (ef) (g) (h)	e gh jk (a) (b) (c) (d) (i) (l)
HSV-1 BamH I k	ij k o (a) (b) (c) (d) (ef) (g) (h)	e gh jk (a) (b) (c) (d) (i) (l)
HSV-1 BamH I y	ij	e gh
HSV-2 BamH I g	ij k o (a) (b) (c) (d) (ef) (g) (h)	e gh jk (a) (b) (c) (d) (i) (l)

Table C2.6

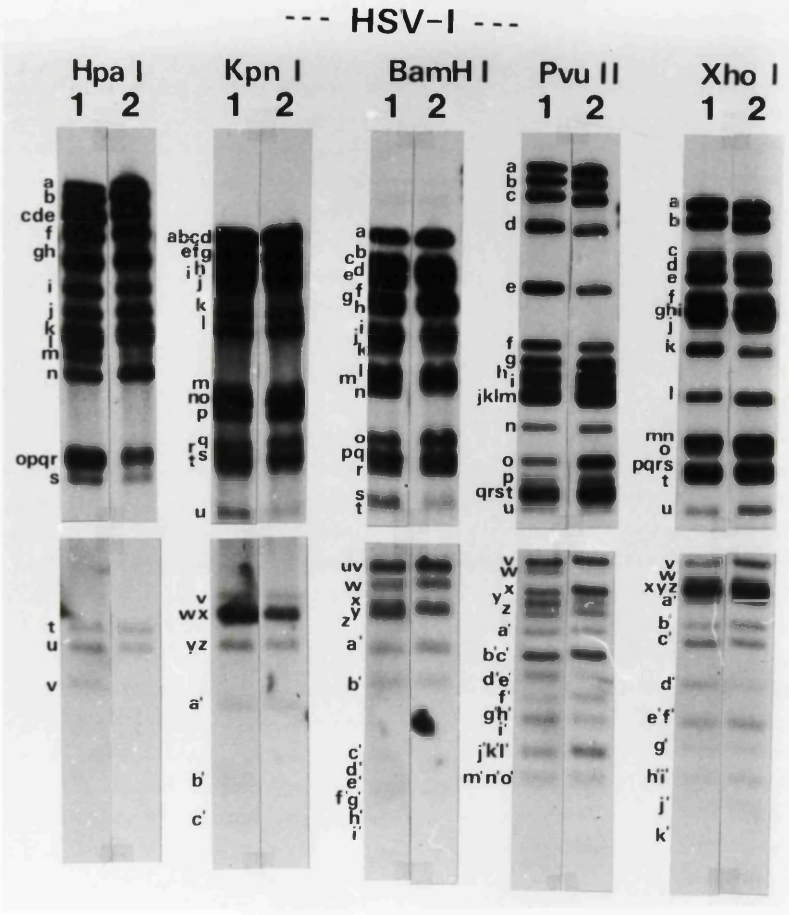
Genome arrangements of recombinants produced by marker rescue of HSV-1 tsD with HSV-2 EcoR I joint fragments. The original arrangements of the tsD genome involved in recombination were deduced from the resulting recombinant structures, according to arguments described in the text, and are listed with the numbers of recombinants reflecting each particular arrangment.

Table C2.6

orientation of genome	rescuing HSV-2 EcoRI fragment	crossovers	number of recombinants
genome in P orientation	c	U_L and U_S	1
genome in I_S orientation	e	U_L and U_S	0
genome in I_L orientation	b	U_L and U_S	13
genome in I_{SL} orientation	d	U_L and U_S	0
L in P orientation, S unspecified	c or e	U_L and TR_S/IR_S	2
L inverted, S unspecified	b or d	U_L and TR_S/IR_S	19
S in P orientation, L unspecified	b or c	TR_L/IR_L and U_S	0
S inverted, L unspecified	d or e	TR_L/IR_L and U_S	0
neither orientation determined	b or c	TR_S/IR_S and U_S	7
	b or c or d or e	TR_L/IR_L and TR_S/IR_S TR_S/IR_S and TR_S/IR_S	45
uninterpreted structures	undetermined	undetermined	3
TOTAL			90

Figure C2.1

HSV-1/HSV-2 intertypic homology. Restriction digests of HSV-1 or HSV-2 DNA, indicated at the top of each pair of tracks, were transferred from the same gel to nitrocellulose. The blot strips were hybridised with nick translated HSV-1 (1) or HSV-2 (2) DNA, and autoradiographed. The five pairs of tracks on the left represent 0.5% (upper panels) and 1% agarose gels (lower panels). The five pairs of tracks on the right represent 1% agarose gels, the lower panels showing a longer exposure of the bottom part of the gel. Tracks of a pair were not autoradiographed for equal durations. The temperature of hybridisation was 45°.


--- HSV-I ---
Hpa I
1 2
Kpn I
1 2
BamH I
1 2
Pvu II
1 2
Xho I
1 2

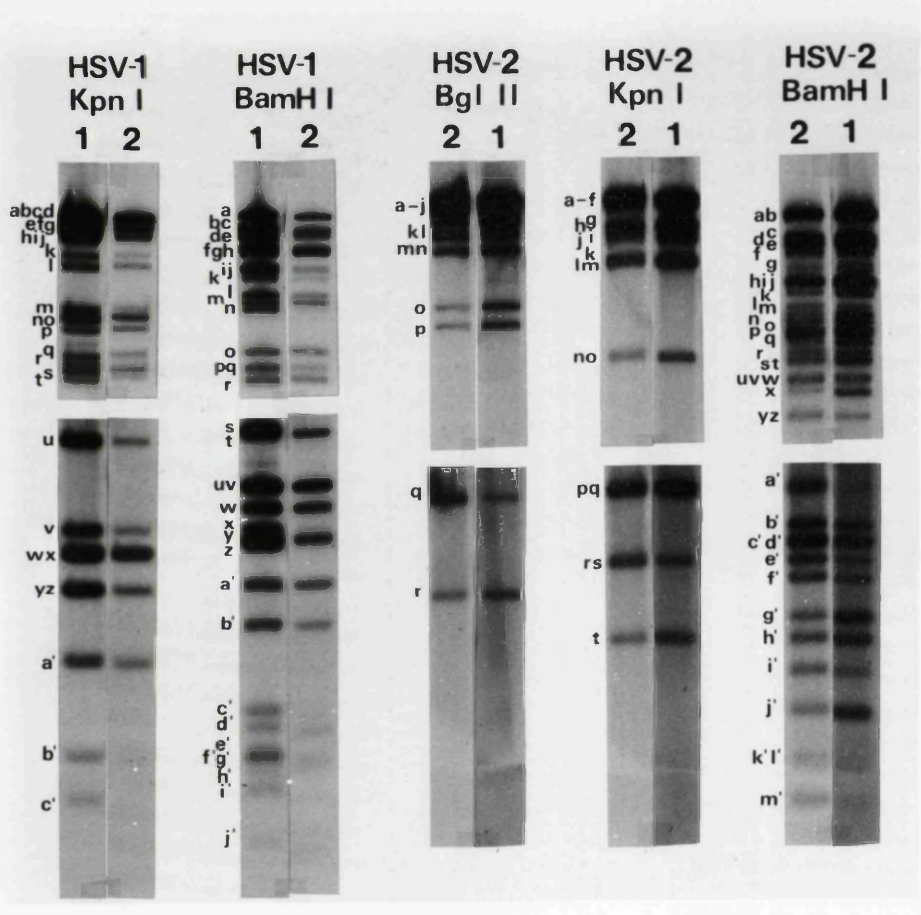

HSV-1
Kpn I
1 2
HSV-1
BamH I
1 2
HSV-2
Bgl II
2 1
HSV-2
Kpn I
2 1
HSV-2
BamH I
2 1

Figure C2.2

LEFT HAND FIVE PAIRS OF TRACKS

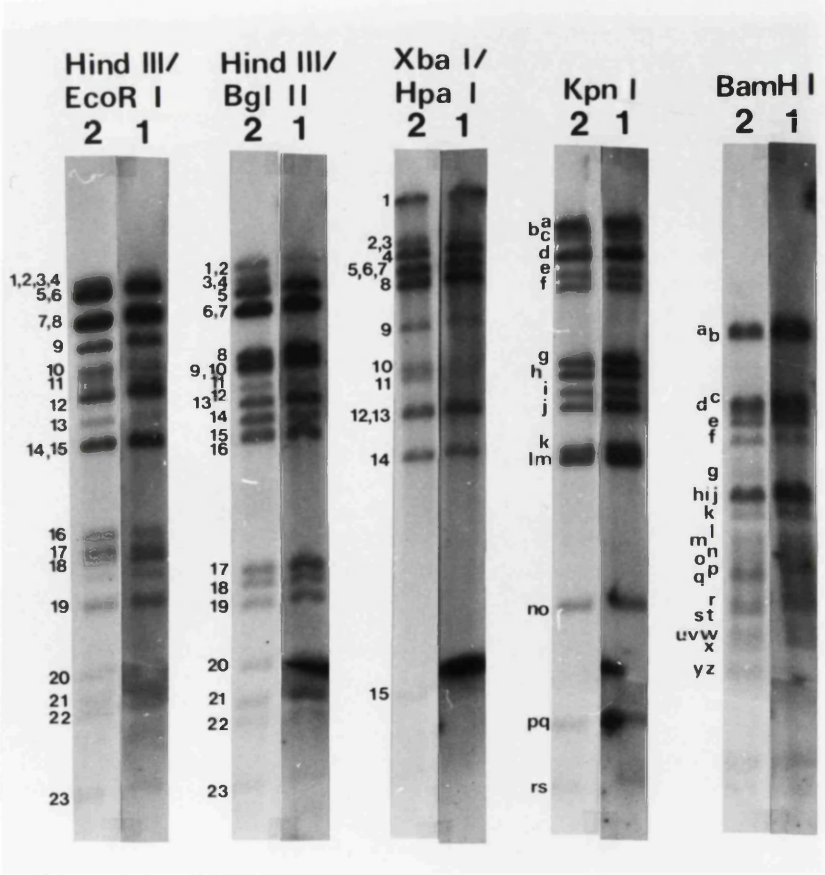
HSV-1/HSV-2 intertypic homology. Restriction digests of HSV-2 DNA, indicated at the top of each pair of tracks, were transferred from a 0.5% agarose gel to nitrocellulose. The blot strips were hybridised with nick translated HSV-1 (1) or HSV-2 (2) DNA as indicated, and autoradiographed. Tracks of a pair were not autoradiographed for equal durations. The temperature of hybridisation was 45°.

RIGHT HAND SIX PAIRS OF TRACKS

Effect of temperature on the hybridisation of nick translated HSV-2 DNA to blot strips containing Kpn I or BamH I digests of HSV-1 DNA.

Blot strips were hybridised with HSV-1 (1) or HSV-2 (2) probe at three different temperatures, then autoradiographed. Tracks of a pair were not autoradiographed for equal durations.

Fragments of the Kpn I digest are indicated on the left, and BamH I on the right.


Hind III/ EcoR I
2 1
1,2,3,4
5,6
7,8
9
10
11
12
13
14,15
16
17
18
19
20
21
22
23
Hind III/ Bgl II
2 1
1,2
3,4
5
6,7
8
9,10
11
12
13
14
15
16
17
18
19
20
21
22
23
Xba I/ Hpa I
2 1
1
2,3
4
5,6,7
8
9
10
11
12,13
14
15
Kpn I
2 1
a
b
c
d
e
f
g
h
i
j
k
lm
no
pq
rs
BamH I
2 1
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n
o
p
q
r
st
uv
w
x
yz

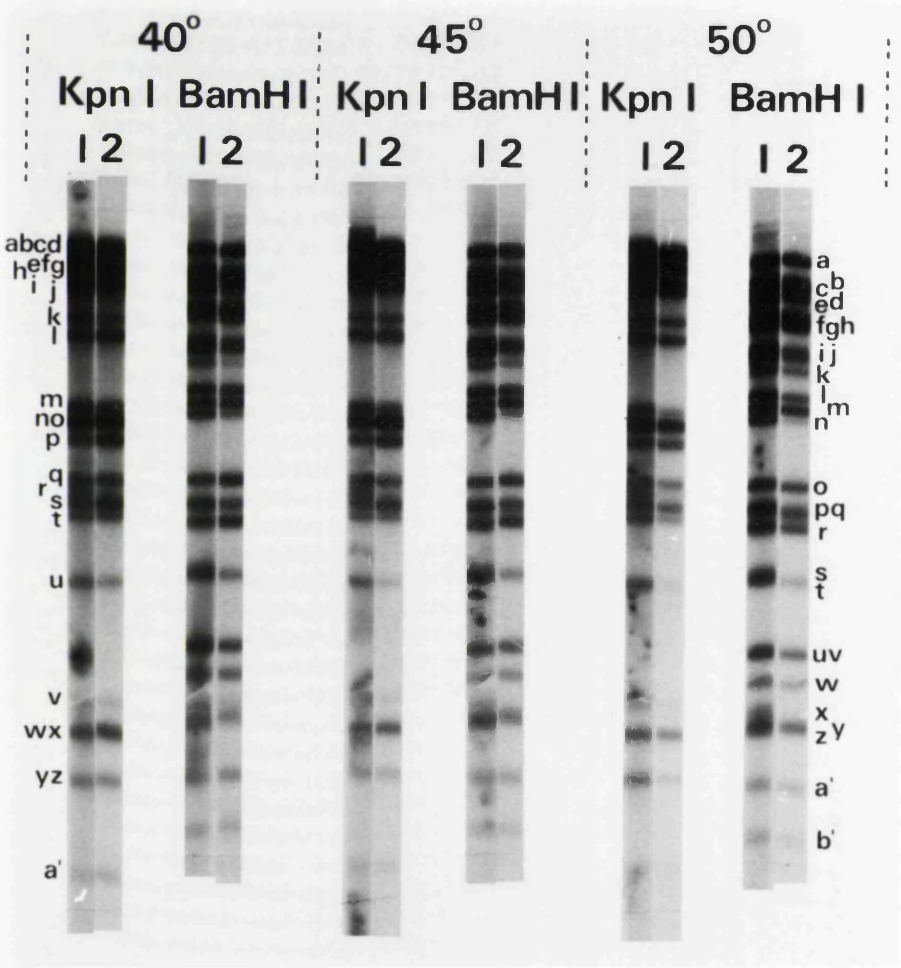

40°
45°
50°
Kpn I
BamH I
1 2
abcd
efg
h
i
j
k
l
m
no
p
q
r
s
t
u
v
wx
yz
a'
a
b
c
d
e
fgh
i
j
k
l
m
n
o
pq
r
s
t
uv
w
x
z
y
a'
b'

Figure C2.3

Summary of HSV-1/HSV-2 intertypic homology data. Autoradiographs of nitrocellulose blot strips of HSV-1 or HSV-2 digests to which nick translated HSV-1 or HSV-2 DNA had been hybridised at 45^{o} were autodensitometrically scanned and R_3 values calculated (see text). In this diagram mean R_3 values are represented histographically with respect to restriction maps of the HSV genome in the conventional orientation (P). Fragments which could not be scored are not included in the histogram. The number of autoradiographs scanned for each digest is shown on the right of each map, and the vertical line on the left indicates an R_3 value of 1.0. An approximate distribution of intertypic homology is given above the genome at the bottom, where large R_3 values indicate regions of higher homology; they are classified into three levels only.

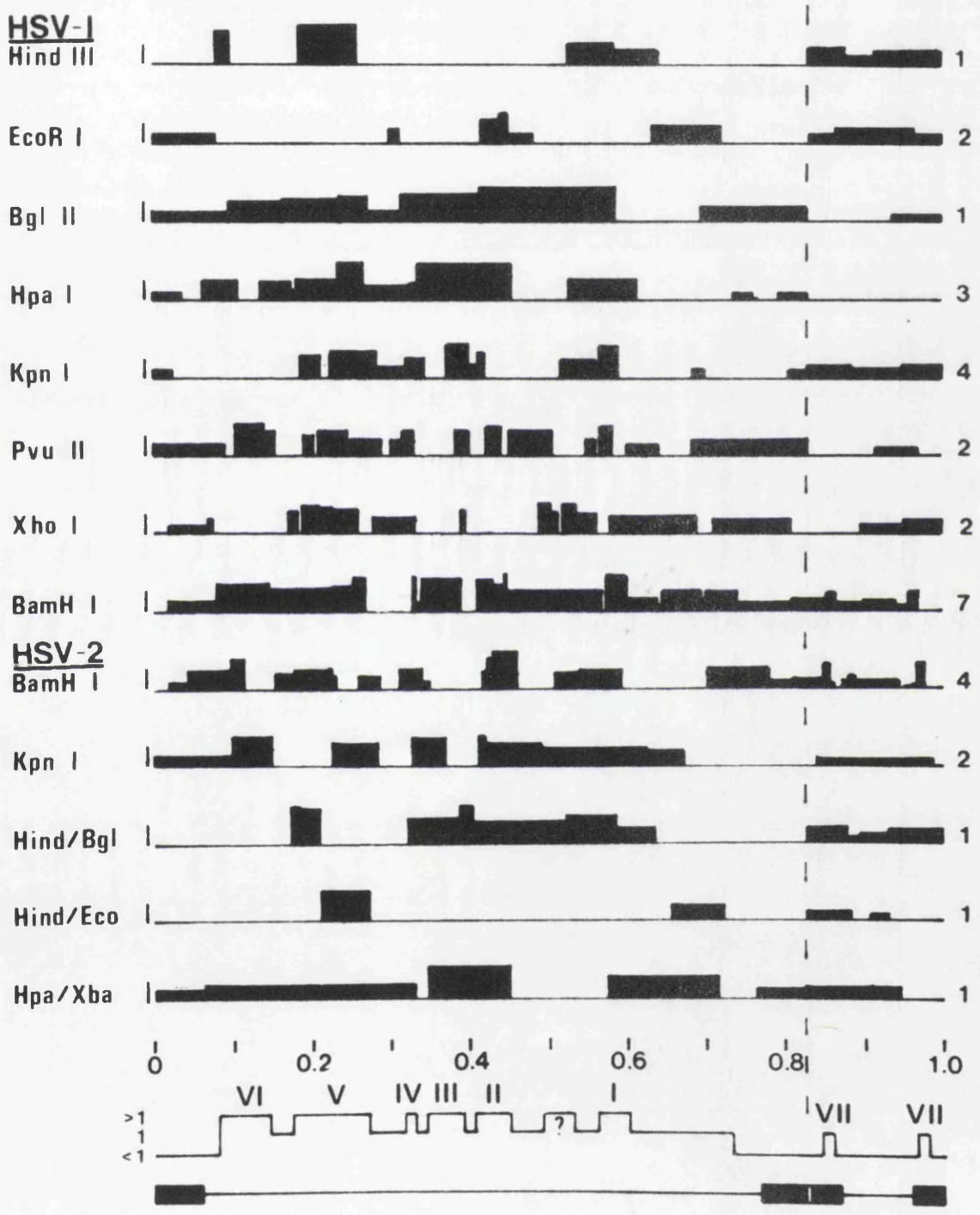

HSV-1
Hind III
1
EcoR I
2
Bgl II
1
Hpa I
3
Kpn I
4
Pvu II
2
Xho I
2
BamH I
7
HSV-2
BamH I
4
Kpn I
2
Hind/Bgl
1
Hind/Eco
1
Hpa/Xba
1
0
0.2
0.4
0.6
0.8
1.0
VI
V
IV
III
II
I
VII
VII
?
>1
1
<1

Figure C2.4

Autoradiographs of the results of hybridisation at 40° of nick translated recombinant pAT153 plasmids containing inserts of HSV-1 or HSV-2 DNA to blot strips of HSV-2 BamH I (left) or HSV-1 BamH I (right). The nature of the HSV DNA insert is indicated at the top of each strip.

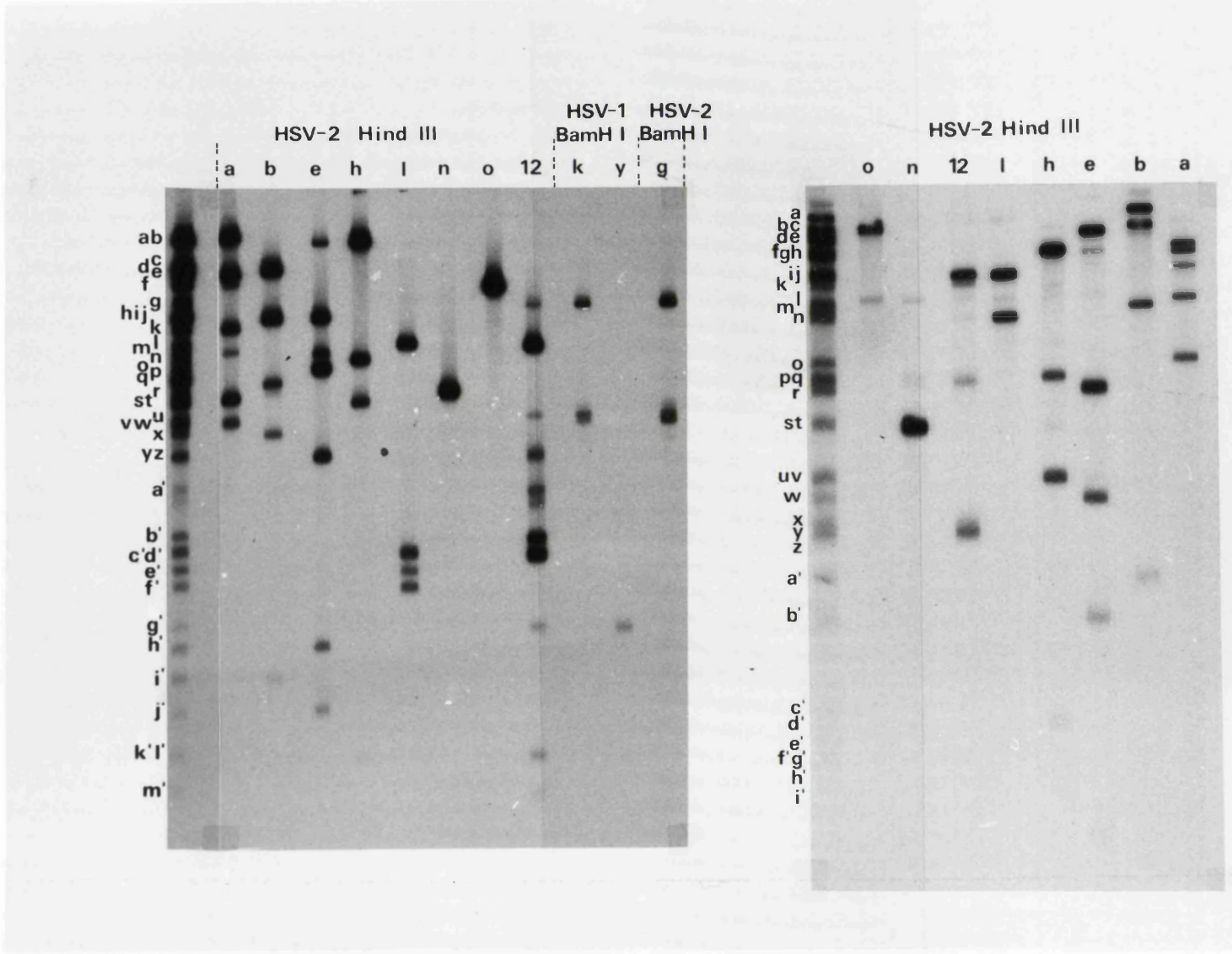

HSV-2 Hind III
HSV-1 BamH I
HSV-2 BamH I
a b e h l n o 12 k y g
ab
dc
e
f
hij
g
k
m
l
n
o
q
p
st
r
vw
u
x
yz
a'
b'
c'
d'
e'
f'
g'
h'
i'
j'
k'
l'
m'
HSV-2 Hind III
o n 12 l h e b a
a
bc
de
fg
h
ij
k
l
m
n
o
pq
r
st
uv
w
x
y
z
a'
b'
c'
d'
e'
f'
g'
h'
i'

Figure C2.5

Cross blots of HSV-1 Kpn I/HSV-1 BamH I (upper) and HSV-1 BamH I/HSV-1 BamH I. The unlabelled dimension is vertical, and the ^{32}P-labelled dimension is horizontal.

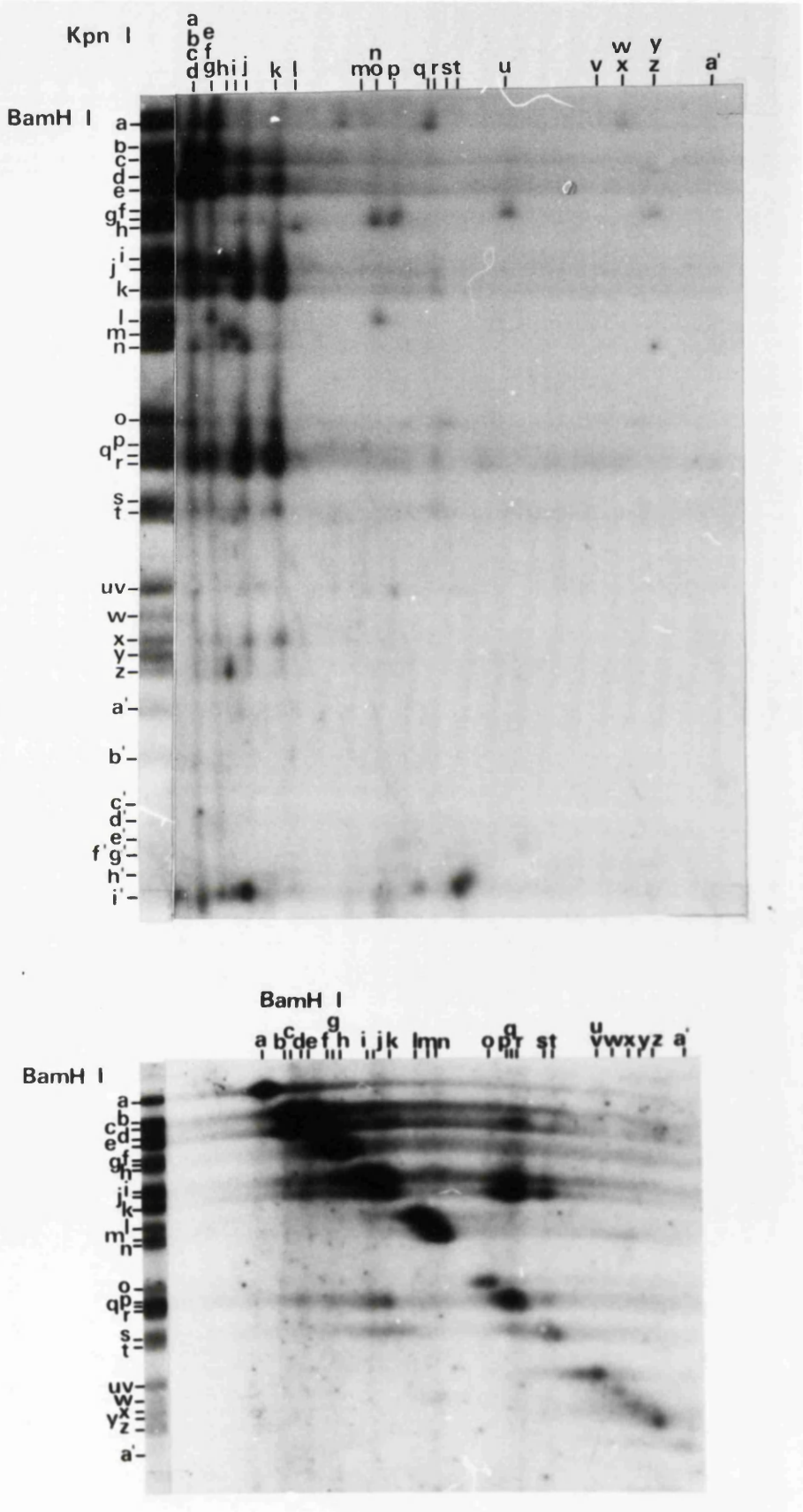
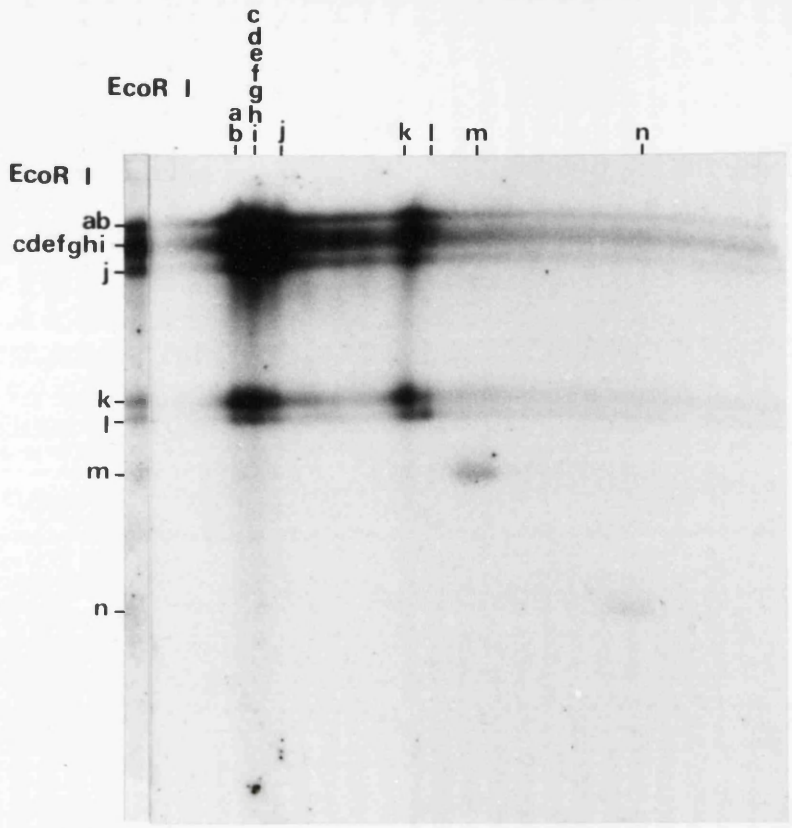

Kpn I
a b c d e f g h i j k l n m o p q r s t u v w x y z a'
BamH I
a b c d e g f h i j k l m n o q p r s t uv w x y z a' b' c' d' e' f' g' h' i'
BamH I
a b c d e f g h i j k l m n o p q r s t u v w x y z a'
BamH I
a b c d e g f h i j k l m n o q p r s t uv w x y z a'

Figure C2.6

Cross blots of HSV-1 EcoR I/HSV-1 EcoR I (upper) and HSV-1 BamH I/HSV-2 BamH I (lower). The unlabelled dimension is vertical, and the ^{32}P-labelled dimension is horizontal.


EcoR I
c
d
e
f
g
a h
b i
j
k
l
m
n
EcoR I
ab
cdefghi
j
k
l
m
n

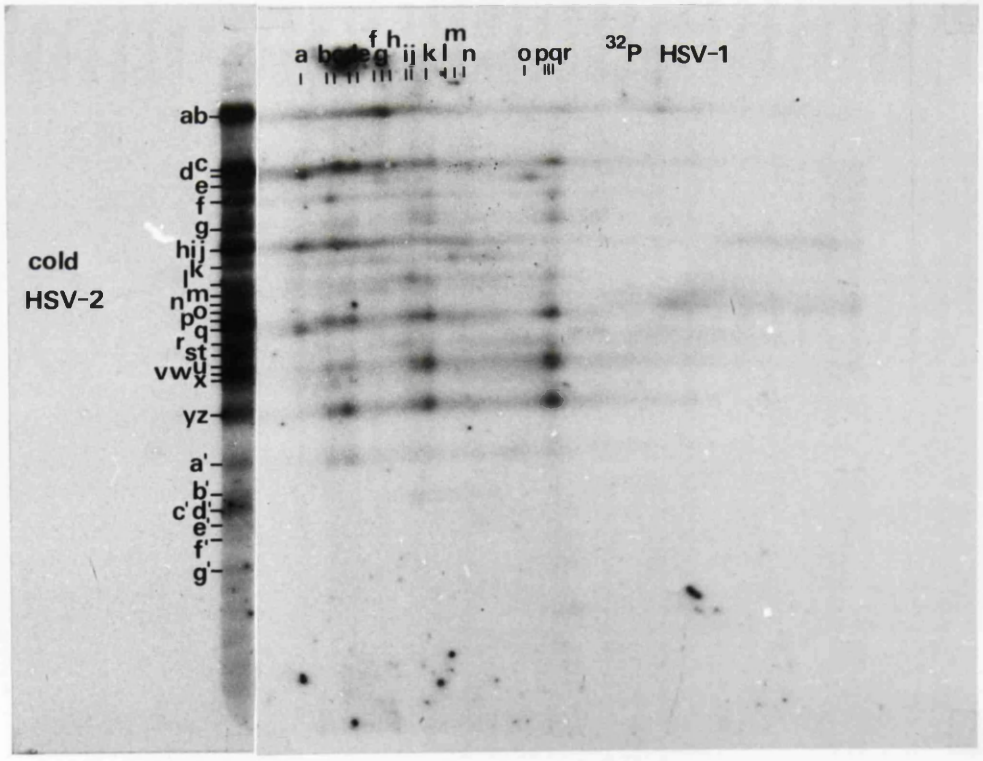

f h
m
a
ij k l
n
o p q r
32P HSV-1
ab
d c
e
f
g
h i j
k
l
n m
o
p
q
r
s t
u
v w x
yz
a'
b'
c' d'
e'
f'
g'
cold
HSV-2

Figure C2.7

Homology between HSV and PRV or EHV-1 DNA. Nick translated HSV-1 (1), HSV-2 (2), PRV (P) or EHV-1 (E) DNA was hybridised at two temperatures to blot strips of HSV-1 BamH I or HSV-2 BamH I, as indicated below the panels. Exposure time differs between tracks.

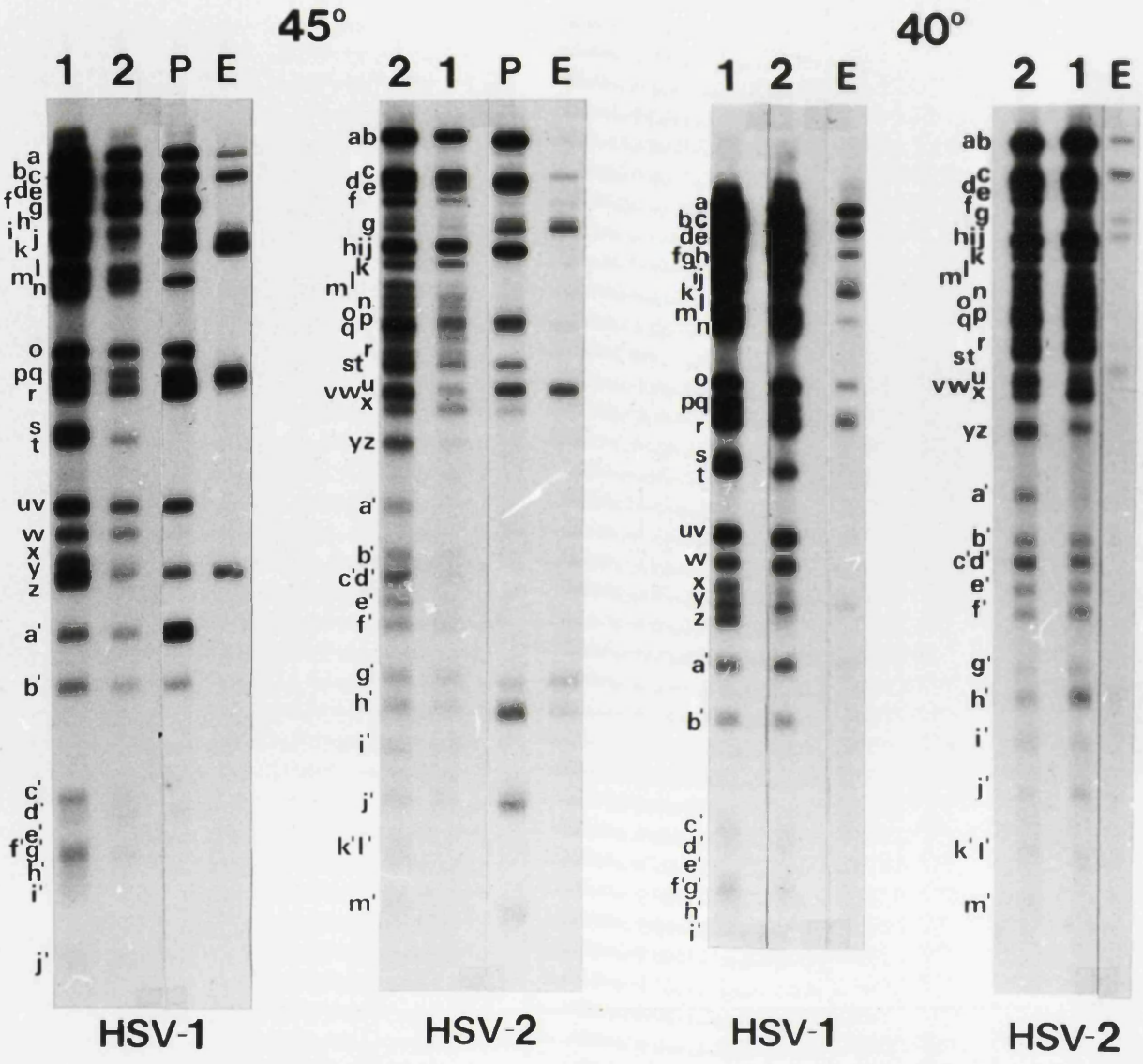

45°
40°
1 2 P E
2 1 P E
1 2 E
2 1 E
HSV-1
HSV-2
HSV-1
HSV-2

Figure C2.8

Autoradiographs of the results of hybridisation at 40° of nick translated recombinant pAT153 plasmids containing inserts of HSV-1 or HSV-2 DNA to blot strips of EHV-1 Bgl II/EcoR I (left) or Bgl II (right) digests. The nature of the HSV DNA insert is indicated at the top of each strip. Experiments were done twice.

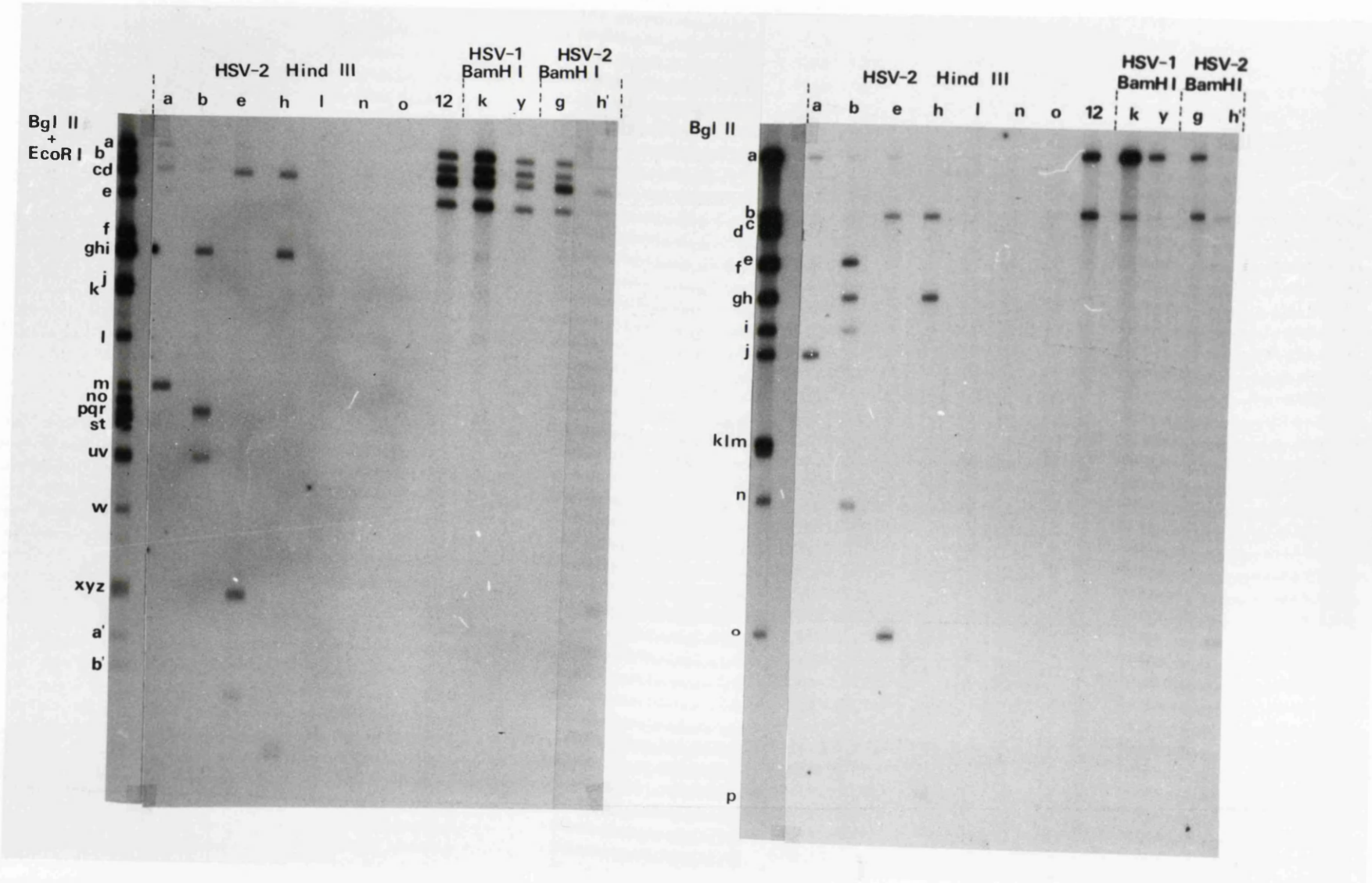
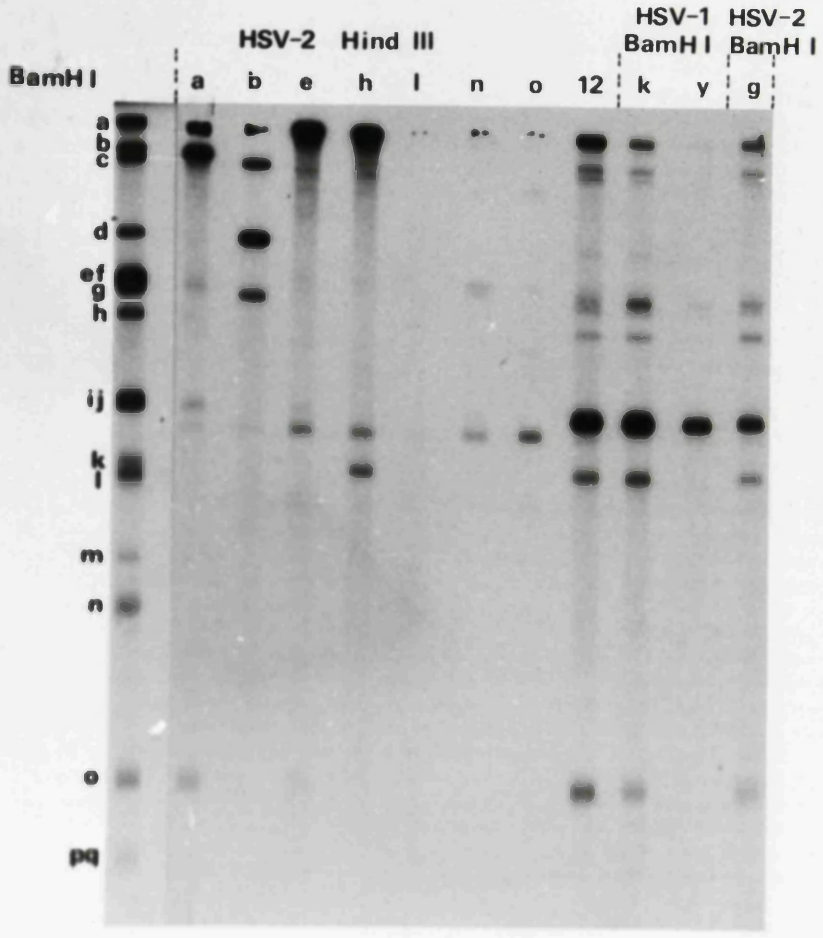

HSV-2 Hind III
HSV-1 BamH I
HSV-2 BamH I
a b e h l n o 12 k y g h'
Bgl II + EcoR I
a b cd e f ghi k l m no pqr st uv w xyz a' b'
HSV-2 Hind III
HSV-1 BamHI
HSV-2 BamHI
a b e h l n o 12 k y g h'
Bgl II
a b d f gh i j klm n o p

Figure C2.9

Autoradiographs of the results of hybridisation at 40° of nick translated recombinant pAT153 plasmids containing inserts of HSV-1 or HSV-2 DNA to blot strips of PRV BamH I (left) or Kpn I (right) digests. The nature of the HSV DNA insert is indicated at the top of each strip. Experiments were done twice.


BamH I
HSV-2 Hind III
a
b
e
h
l
n
o
12
HSV-1 BamH I
k
y
HSV-2 BamH I
g
a
b
c
d
e
f
g
h
ij
k
l
m
n
o
pq

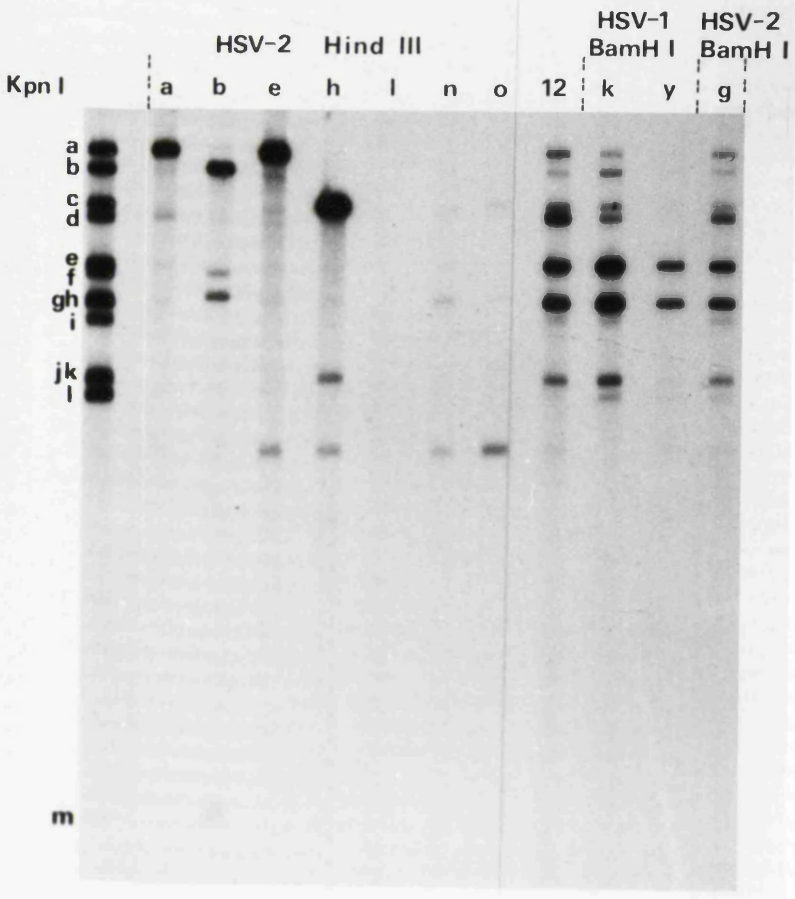

Kpn I
HSV-2 Hind III
a
b
e
h
l
n
o
12
HSV-1 BamH I
k
y
HSV-2 BamH I
g
a
b
c
d
e
f
gh
i
jk
l
m

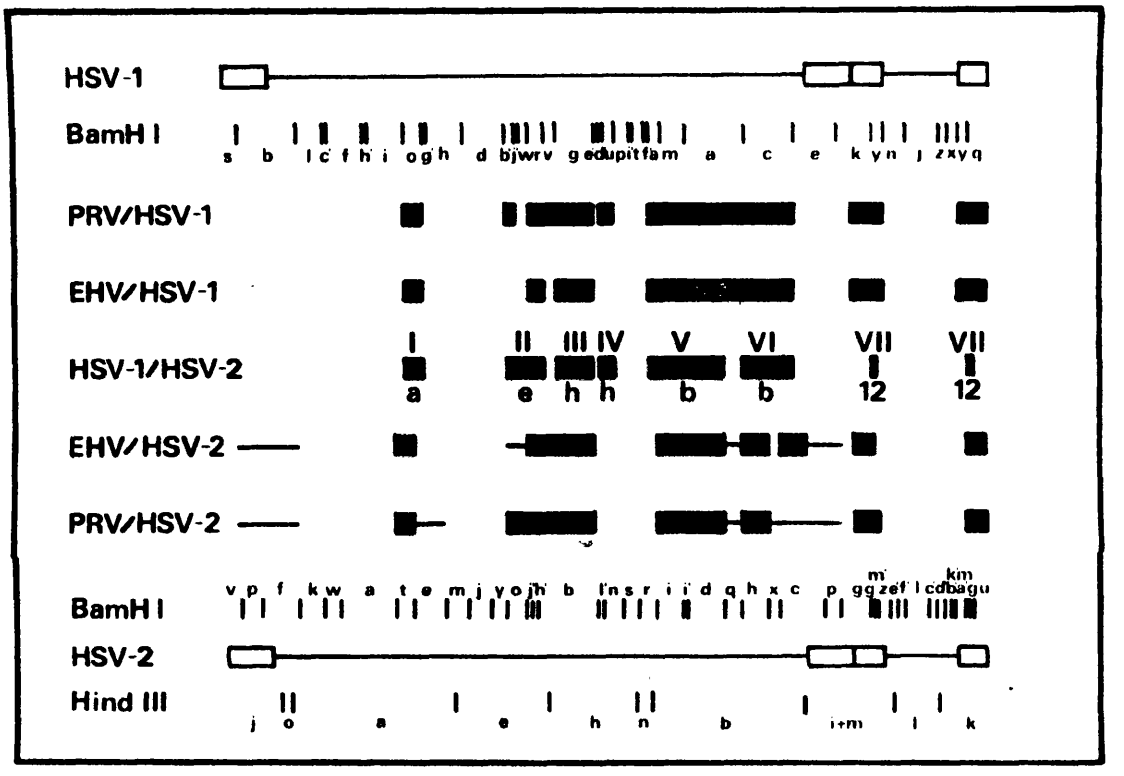
Figure C2.10

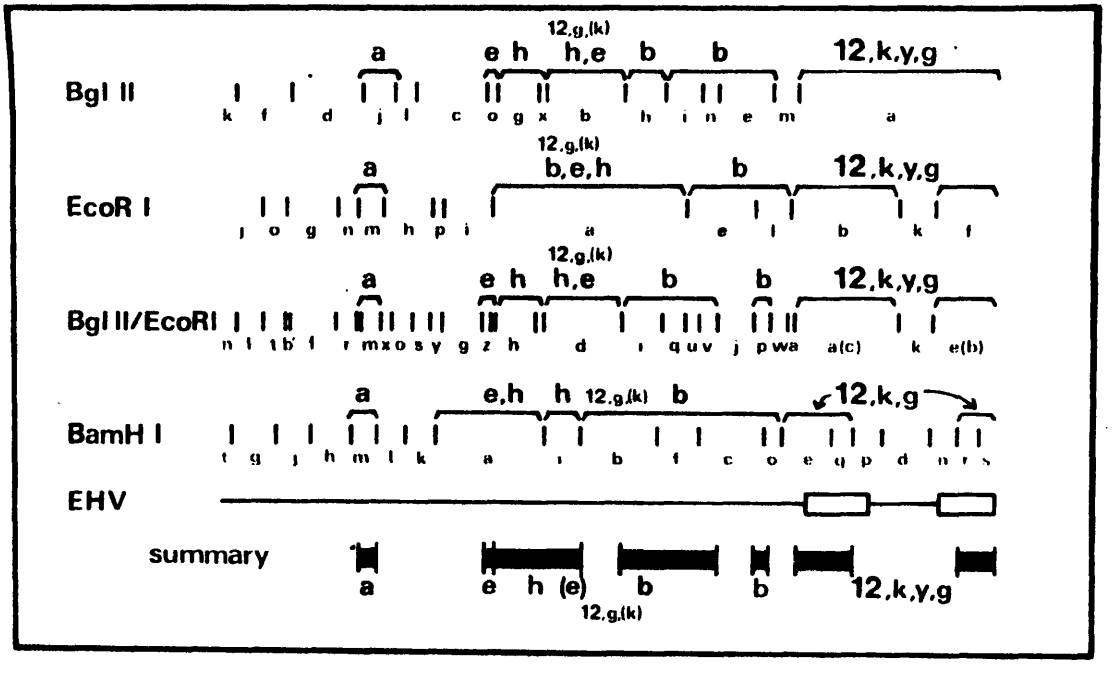
Summary of the distribution of homologous regions between the genomes of HSV-1, HSV-2, EHV-1 and PRV.

The upper panel shows restriction maps of the HSV-1 and HSV-2 genomes in the I_L arrangement. Black rectangles indicate regions of significantly greater homology between the probe and the blot strips to which it was hybridised (probe/blot), and horizontal lines for EHV-1 and PRV probes show regions of weaker but detectable hybridisation. Results for HSV, EHV-1 and PRV probes are taken from Figure C2.3, and Tables C2.2-C2.3, using hybridisation temperatures of 45°, 40° and 45° respectively. The seven regions (I-VII) of greater homology between the HSV-1 and HSV-2 genomes are indicated, and the cloned HSV-2 HindIII fragments within which they are located (a, b, e, h, l2).

The two lower panels show the locations of restriction fragments within the PRV (Powell, 1979) and EHV-1 genomes (J.M. Whalley, personal communication) to which cloned HSV DNA fragments hybridised (for nomenclature see Table B1.3). Data are taken from Tables C2.4-C2.5, and fragments in parentheses demonstrated weaker hybridisation.

Homologous regions in the EHV-1 genome align with respect to those in the HSV genome in the I_L (or I_{SL}) arrangement, whereas the PRV and HSV genomes appear not to be colinear.


HSV-1
BamH I
PRV/HSV-1
EHV/HSV-1
HSV-1/HSV-2
I II III IV V VI VII VII
a e h h b b 12 12
EHV/HSV-2
PRV/HSV-2
BamH I
HSV-2
Hind III


12,g,(k)
a e h h,e b b 12,k,y,g
BgI II
EcoR I
BgI II/EcoRI
BamH I
a e,h h 12,g,(k) b 12,k,g
EHV
summary
a e h (e) b b 12,k,y,g
12,g,(k)

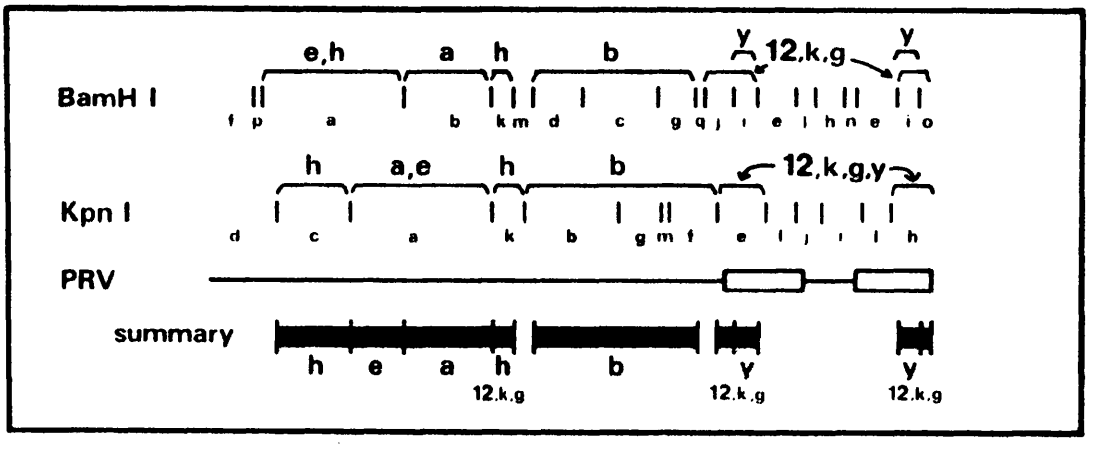
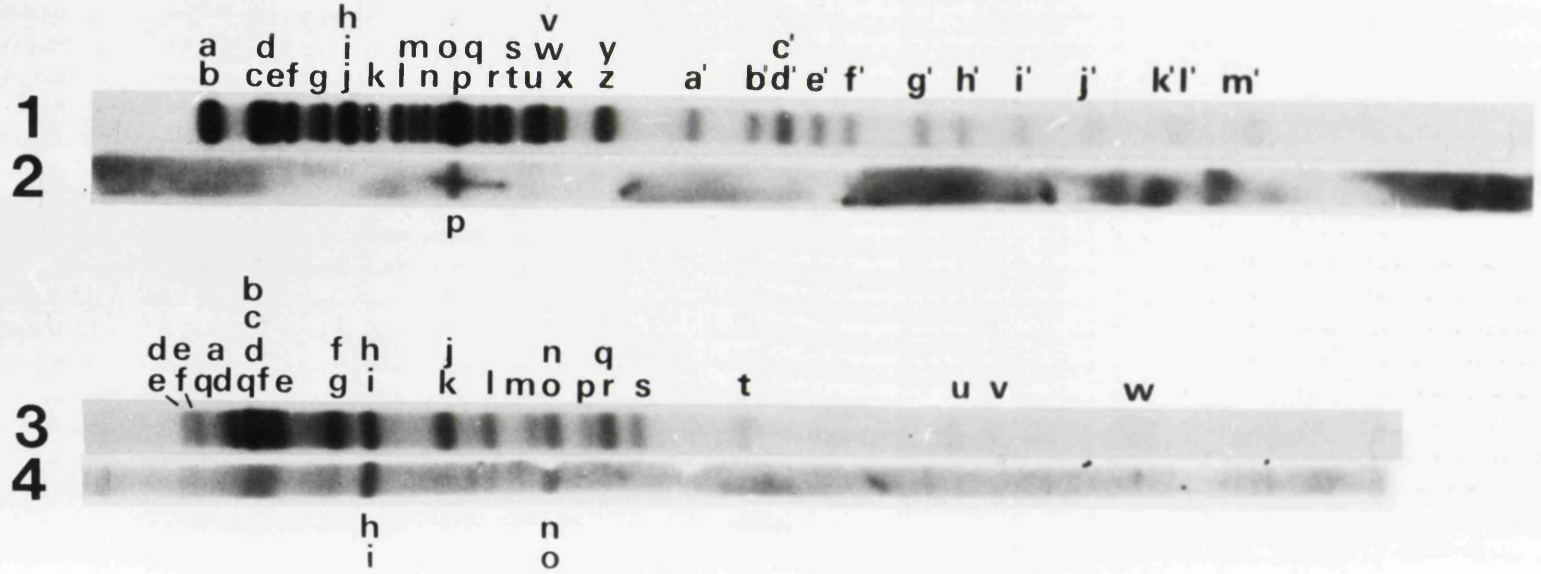

e,h a h b y 12,k,g y
BamH I
h a,e h b 12,k,g,y
Kpn I
PRV
summary
h e a h b y y
12,k,g 12,k,g 12,k,g

Figure C2.11

Autoradiograph of the results of hybridisation at 40° of nick translated HSV-2 (1,4) or CMV (2,3) DNA to nitrocellulose blot strips containing the HSV-2 BamH I digest (1,2) or the CMV Hind III digest (3,4). Relevant restriction fragments are indicated, and restriction maps of CMV (AD169) DNA from Weststrate et al. (1980) are shown, with a molecular weight scale (x 10^{-6} daltons).


1
2
3
4
a d h i m o q s v w y c'
b c e f g j k l n p r t u x z a' b' d' e' f' g' h' i' j' k' l' m'
p
b c
d e a d f h j n q
e f q d q f e g i k l m o p r s t u v w
h i
n o

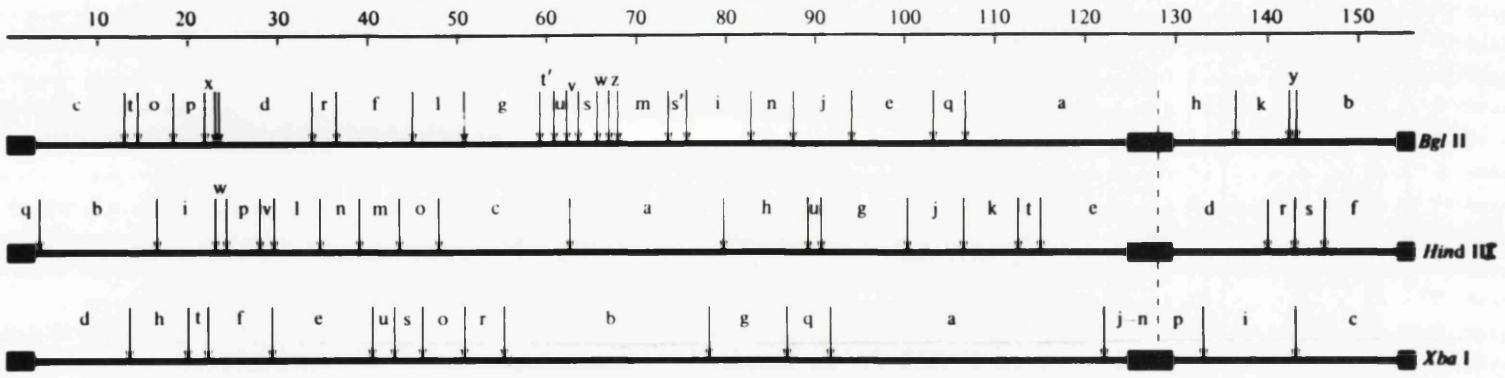

10 20 30 40 50 60 70 80 90 100 110 120 130 140 150
Bgl II
Hind III
Xba I

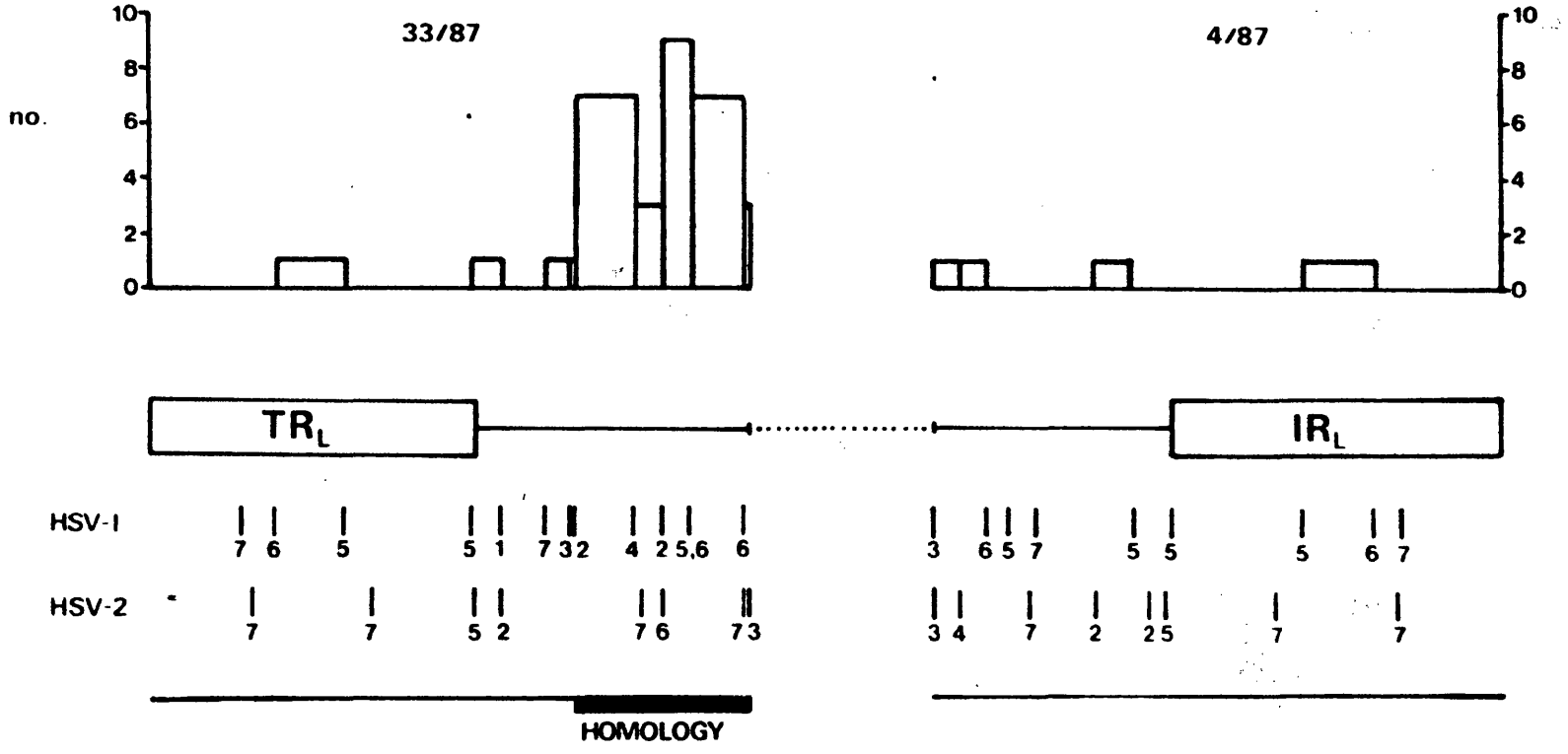
Figure C2.12

Histogram of crossover positions in the L segment of recombinants produced by marker rescue of HSV-1 tsD with HSV-2 EcoRI joint fragments. 33 crossovers in 87 recombinants occurred at the left hand side of L, and 4 crossovers at the right hand side.
HSV-2 restriction sites are shown aligned with respect to those for HSV-1 below the representation of the portion of the L segment covered by the rescuing HSV-2 fragments. The L segment is shown relative to the P orientation of the genome. An estimation of the degree of intertypic homology is shown by the thickness of the black line.

1	XbaI
2	HindIII
3	EcoRI
4	BglII
5	HpaI
6	KpnI
7	BamHI

INTERTYPIC RECOMBINANTS of HSV-1 ts D syn$^+$ / HSV-2 EcoR I joint fragments:

CROSSOVER POINTS IN LONG REGION



SECTION 3

NUCLEOTIDE SEQUENCES OF THE JOINT BETWEEN THE L AND S SEGMENTS OF THE GENOMES OF HSV-1 AND HSV-2

RESULTS

Introduction

The a sequence is present as a direct repetition at the physical termini of the HSV genome, and also in inverted orientation at the joint between L and S. The objective of this work was to determine the DNA sequence of the joint regions of the genomes of HSV-1 and HSV-2, in order to locate sequence arrangements which might be involved in the function(s) of the a sequence, to evaluate the coding potential of this region, and to understand the previously observed size heterogeneity in the HSV-1 joint region (Wagner and Summers, 1978).

SstI restriction maps of the HSV-1 and HSV-2 joint regions

Recleavage experiments were performed to map the restriction sites of SstI (or its isoschizomer, SacI) within the HSV-1 and HSV-2 BamHI joint and terminal fragments. Implicit in the following arguments is the assumption that restriction sites in IR_L or IR_S are present in identical locations in TR_L or TR_S, respectively.

Figure C1.18 shows that HSV-1 BamHI k consists of a fusion of BamHI q and s, and that EcoRI k is an S terminal fragment which entirely contains BamHI q. The sizes of the four SstI fragments produced from HSV-1 BamHI k are given in Table C3.1, and no smaller fragments were detected on polyacrylamide gels. The results shown in Figure C3.1 panel A allow the positions of SstI sites within EcoRI k and BamHI k to be determined. BamHI s gave two fragments, the larger of which comigrated with SstI B (track 2). Therefore the single SstI site within BamHI s defines SstI B at the left hand end of BamHI k, with respect to the conventional

arrangement of the genome. EcoRI k produced three fragments, the smallest of which comigrated with SstI C (track 3). Only the larger of the other two fragments comigrated with a fragment in the HSV-1 SstI digest (not shown), and so this is the S terminal SstI fragment. Therefore SstI A must be the joint fragment, comprising a fusion of the smaller SstI fragment of BamHI s plus the largest SstI fragment of EcoRI k. To the right of SstI A is C, which was common to the BamHI k and EcoRI k digests. To the right of SstI C is D, the right hand end fragment of BamHI k. Therefore the order of SstI restriction fragments within HSV-1 BamHI k is B A C D. Figure C3.3 shows the SstI physical map of HSV-1 BamHI k.

HSV-2 BamHI g is the joint fragment consisting of a fusion of BamHI u and v, EcoRI k and m are the two S terminal fragments, and EcoRI f is an L terminal fragment. Therefore BamHI u is contained entirely within EcoRI k and m, and BamHI v within EcoRI f (Figure C1.19). BamHI g produced eight SstI fragments, of which 7 and 8 were more readily resolved on polyacrylamide gels; fragment sizes are given in Table C3.1. Figure C3.1 shows data for the deduction of SstI sites within BamHI g. BamHI u produced SstI 1, 5, 6, and, taking into account band intensities, a fragment which comigrated with 5 (Figure C3.1 panel B track 2). The latter fragment must be the S terminal SstI fragment. SstI 6 was not produced by EcoRI k or m and therefore must be the right hand fragment of BamHI g, with respect to the conventional representation of the genome. Cleavage of HSV-2 KpnI a, which contains the entire S segment except for the terminal 1 x 10^6 daltons (i.e. KpnI r, see Figure C1.19), gave SstI 5 (Figure C3.1 panel D), whereas KpnI rs gave,

among other products, SstI 8 (panel E). Therefore the order of SstI fragments at the right hand side of BamHI g is 8 1 5 6. SstI 3, 4 and 7 originate, then, from the left hand side of BamHI g, and 2 was not produced by recleavage of larger terminal fragments, suggesting that it is the SstI joint fragment. EcoRI f gave SstI 3 and 7 but not 4, and therefore 4 maps at the left hand end of BamHI g. In conclusion, the order of SstI fragments within BamHI g is 4 (3,7) 2 8 1 5 6, with 2 mapping at the joint. The relative order of 3 and 7 was assigned in the elucidation of the SmaI map, and the full SstI map is shown in Figure C3.3.

Other restriction maps of the HSV-1 and HSV-2 joint regions

Other maps were determined largely using BamHI joint fragments isolated from recombinant plasmids. The major form of joint present in HSV-1 and HSV-2 DNA contains a single a sequence, but minor forms contain two or more tandem repeated a sequences. This distribution was reflected in cloned fragments. The two plasmids used for deduction of restriction maps were k1 (HSV-1) and g1 (HSV-2) (Figure C3.6). The former has a single a sequence and the latter has two. Further experiments, using a plasmid with two a sequences in the HSV-1 DNA insert (k2 in Figure C3.6) and a plasmid with a single a sequence in the HSV-2 DNA insert, confirmed that multiple a sequences are present in each case as tandem direct repeats. This is discussed elsewhere. Restriction maps shown in Figure C3.3 show single a sequences at the joint.

Plasmid DNA was nick translated, cleaved with BamHI, and the relevant joint fragment isolated from an agarose gel. A number of single and double digestions were then done to map restriction sites, as well as some partial digestions and digestions of 5'-terminally labelled DNA. Product sizes

were calibrated using the HinfI and HaeIII digests of pBR322 DNA (Sutcliffe, 1978). The data is shown in Tables C3.2 and C3.3, from which restriction maps were deduced (Figure C3.3). Details of the argument for the SmaI map of HSV-1 BamHI k are described below.

The HincII and Sau3AI sites in BamHI k were located (Table C3.2). Panels A and B of Figure C3.2 show double digestions of HSV-1 BamHI k including SmaI. HincII cleaved SmaI B, SstI cleaved SmaI B, E and N, and Sau3AI cleaved SmaI A and B. Cleavage of isolated SstI and Sau3AI fragments allowed SmaI fragments to be assigned to regions of BamHI k as follows (Table C3.2).

(C,D,J,L) N (G,H,I,K,P) A (F,R) B (M,Q) E O

The order within Sau3AI A was determined by ^{32}P-labelling this fragment at the 5' termini with T4 polynucleotide kinase, digesting with SstI and separating the two uniquely labelled fragments by agarose gel electrophoresis. The two fragments were then partially digested with SmaI and sizes of products determined by comparison with molecular weight markers (Figure C3.2 panel F). The product sizes correspond to sequential additions of SmaI fragments to the labelled terminal SmaI fragment, so that the size increments correspond to SmaI fragment sizes. The partial digestion products are shown in Table C3.2, and give the order of SmaI fragments within Sau3AI A as J C L D N H I K P G, with 430 bp of SmaI A between SmaI G and the Sau3AI site (the Sau3AI site coincides with the BamHI site at the left side of J). The only ambiguity is in the positions of SmaI G and H, since they are similar in size. They were unambiguously assigned by fine mapping of the HinfI fragment containing

SmaI A, G, K, P and part of I, which is described later. The only fragment common to the joint and both termini was SmaI G (Table C3.2), suggesting that this fragment is contained completely within the a sequence.

The order of SmaI F and R was ascertained by partially digesting Sau3AI B (Figure C3.2 panel C). The order of fragments within Sau3AI B from double digestion is, in bp, 640-(24,450)-495, where the unordered fragments SmaI F and R are 450 and 24 bp respectively (as calibrated on agarose; F migrated as 410 bp on polyacrylamide). The predicted partial digestion products from the two possible arrangements are shown in Table C3.2 part I. The three larger partial digestion products were 1115, 970 and 945 bp (Figure C3.2 panel C), supporting the order of the two fragments as SmaI R F. The other two expected partial digestion products of 665 and 475 bp were not distinguished probably because 665 bp did not resolve from 640 bp, and 475 bp did not resolve from 495 bp. The intensities of the bands at 640 and 495 bp were not decreased to the same extent as the 450 bp (SmaI F) band by using less SmaI in the digestion. The order was confirmed as R F by partial digestion of SstI A, when F-R was detected but not 750-R, where 750 bp is the part of SmaI B contained within SstI A (Figure C3.2 panel E).

SmaI M and Q were ordered by partial digestion of SstI C. The order of fragments within SstI C from double digestion is, in bp, 78-(91,38)-260, where the unordered fragments SmaI M and Q are 91 and 38 bp, respectively. The partial digestion products predicted from the two possible arrangements

are shown in Table C3.2 part J. The actual products, shown in Figure C3.2 panel D, give the order as SmaI M Q. The full order of SmaI fragments in HSV-1 BamHI k is shown in Figure C3.3. The other restriction maps shown in this Figure were determined similarly by consideration of the data in Tables C3.2 and C3.3.

Size variability in the joint region

Recently plaque-purified stocks of HSV-1 strain 17 and HSV-2 strain HG52 were plated on BHK cells, grown under EMu2, and a single plaque of each picked. Ten subplaques of each were isolated, plaque-purified twice more, and a virus stock prepared at 37° for each parent and progeny plaque. Virion DNA was ^{32}P-labelled in vivo and BamHI restriction profiles compared (Figure C3.4). Terminal and joint fragments of HSV-1 DNA were the most variable in size (BamHI k, q, s), and variations in the joint and S terminal fragments (BamHI k and q, respectively) seemed correlated. Variation was shown to be in q and not p, which comigrated in some cases, by digestion with other restriction endonucleases. Less obvious variation was observed in the mobilities of HSV-1 BamHI n and x, which span the junctions between U_S and TR_S/IR_S (e.g. compare tracks 2 and 5), and in BamHI z, which maps in U_S (e.g. compare tracks 5 and 6). In contrast, HSV-2 isolates varied only in BamHI z and a', which span the junctions between U_S and TR_S/IR_S, and no variation in HSV-2 joint (BamHI g) or terminal fragments (BamHI u and v) was detected. Variability was shown to reside in z and not y by digestion with other restriction endonucleases.

The region of variability in the HSV-1 joint region was located more precisely by SmaI digestion of BamHI

joint fragments isolated from *in vivo* ^{32}P-labelled virion DNA (Figure C3.5). The joint region of clonally related HSV-2 isolates was analysed similarly (Figure C3.6). HSV-1 SmaI G varied in size between 275 and 400 bp seemingly by the insertion or deletion of multiples of approximately 11-12 bp. HSV-1 SmaI A exhibited size variability of up to 400 bp which was independent of that in SmaI G. Digestion of BamHI k from five of the HSV-1 isolates with Sau3AI and TaqI allowed the variable region in SmaI A to be more accurately located between the Sau3AI A-B and TaqI A-D sites (Figure C3.5, maps in Figure C3.3). Recleavage of BamHI fragments containing the genomic termini showed, for each isolate, the same size distribution of variable fragments as in the joint region. No variation in the BamHI joint fragments of the HSV-2 isolates was detected by cleavage with SmaI (Figure C3.6) or SstI.

HSV-1 BamHI joint fragments isolated from plasmids, like those isolated directly from virion DNA, showed size variation in SmaI A and G (Figure C3.6), whereas no variation was observed in cloned HSV-2 joint fragments, except that due to the presence of an extra a sequence as described below. One of the HSV-1 clones, k2, had a smaller SmaI I fragment, although variability in this fragment was not observed in virion DNA.

Although HSV-1 and HSV-2 differ in that the former showed size variability in the joint region and the latter did not, they both possess a second type of heterogeneity in the joint region and at the L terminus which consists of insertions of approximately 300 bp thought to contain the a sequence. The majority of HSV DNA molecules has a single a sequence at the joint, while a smaller proportion has two

or more. This is reflected in cloned HSV-1 joint fragments, most of which had a single a sequence (k1, k2, k3 in Figure C3.6), but some of which had two ($\bar{k}$1, $\bar{k}$2). Plasmids $\bar{k}$1 and $\bar{k}$2 each had two SmaI G fragments, the latter with two of the same size but the former with two of different sizes. SmaI profiles were consistent with the presence of the two a sequences as tandem direct repeats (see Figure C3.13). Similar results were obtained with cloned HSV-2 joint fragments, and an isolate with two a sequences ($\bar{g}$1) is included in Figure C3.6.

Homology between the joint regions of HSV-1 and HSV-2 DNA

Nitrocellulose blots containing SmaI fragments of HSV-1 and HSV-2 BamHI joint fragments were prepared from plasmid DNA (k1 and $\bar{g}$1 in Figure C3.6) by transfer from 2% agarose or 5% polyacrylamide gels. Strips were probed with nick translated plasmid DNA containing the joint fragments. Results from polyacrylamide gels are shown in Figure C3.7. Fragments larger than 1000 bp and smaller than 150 bp did not transfer efficiently, and therefore only HSV-1 SmaI B-J and HSV-2 SmaI 2-6 were resolved after hybridisation. The larger fragments transferred satisfactorily from agarose gels. Homologous probes failed to show expected hybridisation to fragments mapping in the a sequences (HSV-1 SmaI G, HSV-2 SmaI 5 and J) and so it was not possible to compare homology between the HSV-1 and HSV-2 a sequences. This effect could have been due either to inefficient transfer of these fragments to nitrocellulose or to ineffecient hybridisation. HSV-1 plasmid probe hybridised to HSV-2 SmaI 1 (agarose gels) and 4, and HSV-2 probe hybridised to HSV-1 SmaI B, E, F and H (the SstI/SmaI double digest showed that hybridisation did not occur to D). These results are

interpreted in terms of a region of intertypic homology extending through HSV-1 SmaI F, B and E (HSV-2 SmaI 1) in the c sequences, and a region of less homology between HSV-1 SmaI H and HSV-2 SmaI 4 in the b sequences.

Fine restriction maps of the joint regions of HSV-1 and HSV-2

Before attempting to determine the nucleotide sequences of the joint regions of HSV-1 and HSV-2 DNA it was necessary to map further restriction sites in these regions. Nick translated HSV-1 BamHI k Sau3AI A from plasmid kl was cleaved with a number of endonucleases and also double digested with SmaI. HinfI apparently did not cleave SmaI G, K, P and 430 bp (part of SmaI A) but did cleave SmaI I (Figure C3.8 panel A). Isolation of the largest Sau3AI/HinfI fragment of 1130 bp and digestion with SmaI showed that it contained SmaI G, K, P and two fragments of 410 bp (430 bp on agarose; part of SmaI A) and 160 bp (part of SmaI I). The largest HinfI/BamHI fragment from plasmid kl (a HinfI fragment of 1760 bp) contained SmaI G, K, P and two fragments of 1040 bp (nearly all of SmaI A) and 160 bp (part of SmaI I). A number of single and double digests were done to map this fragment, which contains the HSV-1 joint a sequence. Data are summarised in Table C3.4, and restriction maps are shown in Figure C3.9. HSV-2 BamHI g SstI 2 and SmaI 2 were mapped similarly (see Figure C3.3 for the locations of these fragments). Data are summarised in Table C3.5, and maps are shown in Figure C3.9.

Location of the variable region within the HSV-1 a sequence

Figure C3.10 shows a comparison of digests of the Sau3AI/HinfI joint fragment from plasmids kl and k2, in the

latter of which SmaI G is larger (Figure C3.6). The fragments which are larger in k2 than in k1 are, in bp, SmaI - 340, AvaII - 680, BstNI - 1000, AvaI - 340, HaeII - 980, BglI - 930, SstII - 320 (k1 sizes given). The size difference is about 40 bp. The SstII result places the region of variability within the a sequence in the 320 bp fragment. Taken in conjunction with the SmaI result, the variable region resides in the 270 bp between the SmaI site about 40 bp from the left hand end of the a sequence and the SstII site about 100 bp from its right hand end, according to the maps shown in Figure C3.9.

Definition of the genomic termini and location of the a sequence

Figure C3.9 shows the strategies adopted to determine the nucleotide sequences shown in Figure C3.11. Sequences were determined for the joint regions of HSV-1 strains 17 and USA-8 and HSV-2 strain HG52, and also for the region spanning the junction between two directly repeated a sequences in HSV-1 strain 17 and HSV-2 strain HG52.

The a sequence is defined as the sequence present as direct repeats at the genomic termini and as an inverted repeat at the the joint between the L and S segments. Hence the termini may be aligned with the joint region as shown in Figure C3.13. The position of the a sequence at the joint cannot be determined directly from the joint nucleotide sequence, but it can be determined by locating the termini with respect to the joint nucleotide sequence, using their common restriction sites. It is assumed, although it has not been proven, that the terminal and joint a sequences are identical.

HSV-1 DNA was ^{32}P-labelled specifically at the 5'

termini and sizes of genomic terminal restriction fragments determined (Figure C3.12). This method locates the 5' termini of the genome, and not the 3' termini, with respect to the joint sequence. The sizes in nucleotides for the S terminus of strain 17 were: SmaI 36-37, HaeIII 23-24. The difference in size of these two fragments is two nucleotides less than would be predicted from the joint nucleotide sequence in Figure C3.11, in which the SmaI and HaeIII sites are located after nucleotides 367 and 352 respectively. This discrepancy is probably a result of a high proportion of C moieties (10/15) in the labelled strand between the two sites, which increases the overall charge to mass ratio of the larger fragment, causing it to migrate faster in the gel. If this is so, the smaller HaeIII terminal fragment provides the more accurate estimate, placing the S terminus at 329-330 on the joint sequence. The sizes in nucleotides for the L terminus in strain 17 were: SstII 15-16, HaeIII 9-10, SmaI 6-7. These restriction sites are located in the joint sequence after nucleotides 711, 719 and 722 respectively, bearing in mind that in this case the labelled strand is complementary to that shown in Figure C3.11. For the reason described above the smaller fragments might provide a better estimate of the nucleotides defining the L terminus (728-729). The HSV-1 a sequence in plasmid k1 therefore has a size of 398-400 bp (between 329-330 and 728-729).

The genomic termini of HSV-1 strain USA-8 were defined by the same criteria (Figure C3.12) and are shown in Figure C3.11 at 330-331 and 728-729 with respect to the strain 17 sequence. From the autoradiograph reproduced in Figure

C3.12 it could be seen that S terminal fragments from strain USA-8 DNA were two nucleotides smaller than those from strain 17 DNA, whereas L terminal fragments were the same size in both strains. The S and L termini of HSV-2 strain HG52 were located at 62-64 and 309-319 respectively (Figure C3.11) by SmaI cleavage of nick translated HSV-2 BamHI u and v isolated from virion DNA (Table C3.3 parts B and C). The HSV-2 a sequence therefore has a size of 245-257 bp.

In both HSV-1 strains and the HSV-2 strain the genomic termini are located close to the right hand end of direct repeats in the joint sequences. The direct repeat in strain USA-8 comprises the first 17 nucleotides of the 21 nucleotide direct repeat in strain 17 at 311-331 and 710-730. However, the primary sequences of the HSV-1 direct repeats are different from that of HSV-2.

The nucleotide sequence of plasmids containing joint fragments of HSV-1 strain 17 or HSV-2 strain HG52 with two a sequences showed that the a sequences are tandem direct repeats with a single copy of the appropriate 21 or 17 nucleotide direct repeat at the a-a junction (Figure C3.15).

Tandem reiterations in the joint region

The HSV-1 strain 17 sequence shown in Figure C3.11 was determined using plasmid k1, but k2 was also sequenced in the regions where it differed in size from k1. Three regions of reiteration were found in the HSV-1 strain 17 joint region, one located in the a sequence and two in adjacent c sequences (reiterations I, II and III in Figures C3.9 and C3.11). The three repeated units of 12, 16 and 17 nucleotides of which the reiterations consist have different primary sequences, although they all contain a predominance

of C residues in the same strand of the duplex. The sequence of k2 in two of the reiterations (I and III) differed from that of k1 only in the number of reiterated units. Reiteration I (408-623) in k1 contained 18 copies of CCGCTCCTCCCC whereas k2 contained 21 copies (Figure C3.14). Similarly, reiteration III (1167-1489) consisted of 19 copies of CCGCCCCTCGCCCCCTC in k1 and 11 copies in k2 (Figure C3.15). These two reiterations are contained within SmaI G and SmaI A respectively, and provide an explanation for the observed size variability in the a and c sequences of different plaque isolates of HSV-1 strain 17.

Reiteration II (928-1055) did not contain a simple repeated unit. Furthermore, the difference in sequence between k1 and k2 was not reflected in different copy numbers but in the organisation of the repeated units, thus:

k1 $(CCCTCCCCAGCCCCAG)_2$ $(CCCTCCCCGGCCCCAG)_6$

k2 $(CCCTCCCCAGCCCCAG)_1$ $(CCCTCCCCGGCCCCAG)_7$.

The two 16 nucleotide repeated units differ in a single nucleotide, and the sequences of reiteration II in k1 and k2 differ by a single nucleotide (Figure C3.14).

Reiterations I and II were also identified in the joint region of HSV-1 strain USA-8. Reiteration II comprised at least 15 copies of the first 16 nucleotide variant of the repeated unit of strain 17 (shown above). Reiteration I is related to that of strain 17 in a more complex way (Figure C3.14). Figure C3.11 shows that it contains 8 copies of a unit which consists of the first 11 nucleotides of the strain 17 12 nucleotide repeated unit. Adjacent to this are 6 copies of a unit which comprises the

11 nucleotides with the sequence TCTGTGGGTGGGG inserted after the third nucleotide. Reiteration III was also identified in strain USA-8, but the sequence was not analysed in detail.

A partial overlap of the repeated unit of reiterations I, II and III is present immediately to the right of each reiteration defined by the brackets in Figure C3.11. The location of each partial overlap is arbitrary and depends on the definition of each repeated unit. The partial overlaps consist of the first 7, 11 and 10 nucleotides of the repeated units of reiterations I, II and III respectively.

No tandem reiterations equivalent to those present in the HSV-1 a sequence were found in the the HSV-2 sequence (Figure C3.11). It is not known whether reiterations equivalent to II and III exist in HSV-2 DNA.

Comparison of nucleotide sequences

The region of greatest difference in the joint sequences of HSV-1 strains 17 and USA-8 is in the c sequence immediately adjacent to the a sequence. The differences in this region, which is AC rich on the strand shown in Figure C3.11, represent both substitution of nucleotides and deletion or insertion of short sequences. Only one nucleotide differs between the 310 analysed nucleotides of the b sequences to the left of the first 17 nucleotide repeat at 311-327, and strains 17 and USA-8 are identical in 166 comparable nucleotides at the right hand end of the c sequence shown (1601-1766). Reiteration I is the region of greatest difference between the a sequences of the two HSV-1 strains, and there are ten base substitutions and one deleted nucleotide in the remainder of the a sequence. Since reiterations I, II and III were identified in both strains, although these differ

widely in time and geography of isolation, it seems likely that they will be a general feature of HSV-1 isolates.

The a sequences of HSV-1 and HSV-2 both have a high GC content (83% and 84% respectively), but the sequences show little similarity. Within the a sequences the longest similar sequence, which is also highly asymmetric between the two strands of the duplex, is 362-289 of HSV-1 strain 17 and 94-123 of HSV-2 strain HG52. Unlike HSV-1, HSV-2 does not possess reiteration I, but both serotypes have a direct repeat at the b-a and a-c junctions.

Expression of the HSV-1 joint region

There is as yet no evidence that transcription starts in the nucleotide sequence of the HSV-1 joint region shown in Figure C3.11, and no sequences consistent with the consensus "Hogness box" (Gannon et al., 1979) are present. The hexanucleotide AAUAAA has been found in close proximity to the 3' termini of the majority of polyadenylated eukaryotic mRNAs yet analysed (Benoist et al., 1980). A few exceptions, which possess a sequence closely related to AAUAAA, have been noted (MacDonald et al., 1980; Hobart et al., 1980; Goeddel et al., 1981). The DNA sequence AATAAA is located at two positions in the strain 17 joint region, at 1500-1505 and on the opposite strand at 1546-1541. Benoist et al. (1980) have observed a conserved sequence related to TTTTCACTGC (more accurately $\overset{TT}{GG}T\overset{TC}{GA}AX\overset{TGC}{ATG}$) located 3' to the AATAAA close to the polyadenylation site of several, but not all, mRNAs, and the related sequence TTTGCAGTAG is present at 1520-1511 3' to the AATAAA at 1546-1541 (considering the other strand of the duplex from that shown). No such sequence is present 3' to the other

AATAAA.

These results suggest that a mRNA is transcribed from right to left and is polyadenylated in the region 1530-1520. Experiments were carried out in collaboration with Dr F.J. Rixon to ascertain whether this is indeed the case. The TaqI D fragment (Figures C3.3 and C3.9), consisting of nucleotides 1498-1608, was labelled at the 3' termini by incorporation of α-^{32}P-dCTP using the Klenow fragment of E.coli DNA polymerase. The two strands were separated by polyacrylamide gel electrophoresis under denaturing conditions (8 M urea). HSV-1-infected cell mRNA was produced under immediate early conditions (cycloheximide block; Clements et al., 1977), from which the 4.7 kb mRNA was isolated from methylmercuric hydroxide agarose gels (Watson et al., 1979). Aliquots of either total immediate early RNA or the 4.7 kb mRNA were hybridised separately with the two uniquely-labelled DNA single strands (57^{o} in 3 x SSC and 90% formamide for 5 hr) according to the method of Berk and Sharp (1977). The samples were treated with S1 nuclease to degrade single-stranded nucleic acids, and then subjected to polyacrylamide gel electrophoresis under denaturing conditions (8 M urea). Only one of the DNA strands was protected by RNA, so this was compared with the nucleotide sequence of that fragment on the same gel (Figure C3.16). This result shows that the 3' terminal coded nucleotide of the mRNA coding for V_{mw} IE 175 is located in the region 1530-1525. No transcripts from the other strand terminating close to the AATAAA at 1500-1505 were detected, either in immediate early RNA or in RNA isolated from cells harvested 6 hr post infection at 37^{o}.

It is not yet known whether joint sequences other

than those coding for V_{mw} IE 175 mRNA are represented in stable transcripts, or where primary transcription terminates. The region contains several potential splice donor (PuGTXXG) and acceptor (PyPyXPyAG) signals (Seif et al., 1979), but these cannot be interpreted in the absence of transcript mapping data. Three features of the DNA sequence make it likely that a considerable part of the joint region does not code for polypeptide, notably the a and adjacent c sequences. First, translation is unlikely to occur through reiteration I in the a sequence. Variation in the copy number of the 11 nucleotide repeated unit of this reiteration in strain USA-8 could result in translation of each of the three possible downstream reading frames. Similar arguments apply to the c sequence adjacent to a, which contains reiterations II and III. Second, if translation of mRNA representing reiteration I of strain 17 occurred, the polypeptide would contain the unusual structure of 18 tandem repeats of 4 amino acids (plasmid k1). Similarly, reiteration III would code for a polypeptide containing 6 tandem repeats of 17 amino acids. Third, the absence of nucleotides from one strain compared with the other would generate translational frameshifts. Strain USA-8 has one nucleotide inserted between 328 and 329 of strain 17, one nucleotide deleted at 340, and five nucleotides inserted between 740 and 741 which would produce this effect.

DISCUSSION

Features of reiterations

Determination of the DNA sequences at the joint region between the L and S segments of the genomes of HSV-1 and HSV-2 has revealed several features. The presence of reiterations in HSV-1 provides a ready explanation for the widely observed size variability in the a and c sequences. The HSV genome is known readily to undergo recombination during replication, and unequal crossover events between tandem reiterations would give rise to size variability in these regions. Similar tandem reiterations might be responsible for such variability elsewhere in the genome. A functional role for the reiterations has not yet been identified, and it is possible that they encode no essential functions but represent regions in which errors in DNA replication can accumulate. The absence of reiteration I in the a sequence of HSV-2 strain HG52 argues against an essential role for that tandem reiteration in virus replication. The 11 nucleotides CCCGCTCCTCC which form the first repeated unit of reiteration I in HSV-1 strain USA-8 (in this case redefining the unit shown in Figure C3.11 by moving the brackets one nucleotide to the left) are also present at 871-881 (with respect to the strain 17 sequence), showing that this sequence is not amplified of necessity in the HSV-1 genome. The repeated units of the reiterations, although different, have similarities such as a preponderance of C residues on one strand of the duplex and G residues on the other, and it is possible that such sequences are more readily amplified than others. This similarity extends also to reiterations IV and V which are located elsewhere in the HSV-1 genome, as summarised in Figure C3.18. It is of interest to note that the sequence CPyCCXCCC is present in

reiterations I and III of both HSV-1 strains, and in reiteration IV; indeed it is present on both strands of the 24 nucleotide repeated unit of reiteration I in strain USA-8. GGCCTCCC is present in reiteration II and CCCCACTC (CPyCCXCTC) in reiteration V. The SV40 genome possesses six copies of PyPyCCGCCC close to the origin of DNA replication (Dhar et al., 1977), two of which are within T-antigen binding site III (Tjian, 1978). However, the presence of this binding site is not essential for SV40 DNA replication (Subramanian and Shenk, 1978). T-antigen binding site II, the presence of which is required for DNA replication (Shenk, 1978; Gluzman et al., 1980), possesses two palindromes of GCCTC (Tjian, 1978), and reiterations I-IV contain PyCCTC and reiteration V contains CACTC. Such sequences, however, are sufficiently short to be present commonly in HSV-1 DNA (e.g. PyPyCCGCCC at 1688-1695 and GCCTC at 1686-1690), and so it remains to be shown that the sequences of the repeated units are specific for preferential amplification.

Coding potential at the 3' end of V_{mw} IE 175 mRNA

The genes coding for dPyK (McKnight, 1980), V_{mw} IE 175 (this study; M-J. Murchie, personal communication), V_{mw} IE 12 (M-J. Murchie and F.J. Rixon, personal communications) and V_{mw} 136'(143) (J. McLauchlan, personal communication) have 5' and 3' sequences consistent with conserved features of equivalent regions of other eukaryotic genes coding for polyadenylated mRNAs. The only introns yet described for HSV-1 reside in TR_S/IR_S in the genes coding for V_{mw} IE 12 and 68 (Watson et al., 1981; F.J. Rixon and M-J. Murchie, personal communications).

The sequence at the 3' terminus of the V_{mw} IE 175 mRNA (Figure C3.11) contains two possible termination codons in

different frames, at positions corresponding to 1562-1560 (UGA) and 1591-1589 (UAA). The hypothetical amino acid sequence of the carboxy terminus of V_{mw} IE 175 utilising each of these termination codons is given in Table C3.6. Also shown is the occurrence in each case of the four amino acids coded by triplets containing combinations of G and C moieties only, and the distribution of the four nucleotides in the third codon position. Two observations from these data suggest that, if either termination codon is used, the UAA frame is the more likely. First, this frame contains far fewer arginine residues in this region and therefore might be thought to code for a more reasonable protein structure. Second, the great majority of codons in this frame have G or C residues in the third position. This is consistent with the expectation that this region of the DNA would possess a high GC content especially in the third codon position, since it is under selective pressures to maintain both a high GC content (for unknown reasons) and a functional V_{mw} IE 175 gene. Full elucidation of the coding region of this gene awaits complete sequence determination.

Role of the a sequence in coding for polypeptide

It seems likely that the HSV-1 a sequence does not code for polypeptide. Previously, the lesion in an HSV-1 ts mutant (tsc75) had been incorrectly mapped in the a sequence, and examination of the polypeptides induced by this mutant at the non-permissive temperature prompted the conclusion that the a sequence forms part of the V_{mw} IE 175 gene (Knipe et al., 1979). This study shows that the a sequence is not included in the gene. Preston (in press) has shown that the mutation maps in the c sequence some 2 kbp away from the a sequence. These findings have repercussions detrimental to the hypothesis that

all a sequences within a genome are obligatorily identical, as discussed in Section 4.

Homology between the joint regions of the HSV-1 and HSV-2 genomes

Hybridisation experiments (Figure C3.7) demonstrated homology between HSV-1 BamHI k and the c sequence of HSV-2 BamHI g, and between HSV-2 BamHI g and the c sequence of HSV-1 BamHI k. These regions correspond in location to the 3' portions of the genes coding for HSV-1 V_{mw} IE 175 and HSV-2 V_{mw} IE 182. A more localised region of homology in the b sequences (HSV-1 SmaI H and HSV-2 SmaI 4) was also detected. It was not shown that homology exists between these two fragments, although this is the simplest explanation of the results. HSV-1 SmaI H is adjacent to sequences coding for the 5' terminus of V_{mw} IE 110 mRNA (Mackem and Roizman, 1980), and therefore it is possible that HSV-1 and HSV-2 share homology in transcriptional control sequences at the 5' termini of the genes coding for V_{mw} IE 110 (HSV-1) and V_{mw} IE 118 (HSV-2). Fragments containing the a sequence did not hybridise well even to homologous probe, and it was therefore not possible to estimate the extent of homology between the HSV-1 and HSV-2 a sequences.

Role of the a sequence in maturation of genome length DNA

In both HSV-1 and HSV-2 the S terminus is equivalent to a position close to the right hand end of the 17 and 21 nucleotide direct repeat at the b-a junction, and the L terminus is equivalent to the right hand end of the other direct repeat at the a-c junction. Although the locations of the termini with respect to the joint sequence have not been defined to single nucleotides, Figure C3.11 shows that the HSV-1 strain USA-8 S terminus is one nucleotide to the right of that in HSV-1 strain 17, whereas the L termini coincide.

A minority of virion DNA molecules contain more than one copy of the a sequence at the L terminus and at the joint. Determination of the nucleotide sequences between two tandem HSV-1 or HSV-2 a sequences has shown that the a sequences are arranged as head-to-tail dimers which have one copy of the direct repeat at their point of fusion (Figure C3.17). This arrangement could arise either by misaligned genetic recombination at the direct repeats, or by direct ligation of the L and S termini. The fact that multiple a sequences have not been observed at the S terminus of HSV-1 strain 17 and HSV-2 strain HG52 presumably reflects the mechanism of DNA replication and maturation. It is possible that the direct repeats serve as recognition sites for the maturation of unit length DNA from concatemers. Since the HSV genomic termini are specifically located, it is likely that concatemeric DNA is specifically cleaved at alternate joint regions to produce DNA of genome length, but there is as yet insufficient evidence upon which to base detailed models. Nevertheless, the consequence of the maturation mechanism is that the L terminus essentially contains both copies of the direct repeat, whereas the S terminus contains only the a-c copy (Figure C3.17).

Role of the a sequence in segment inversion

Data which suggest that the a sequence has a role in segment inversion by a site-specific mechanism are discussed in Section 4. Precisely which sequences are involved in the proposed site-specific recombination events is unknown. One possibility is that the region which is conserved between the HSV-1 and HSV-2 a sequences (362-389 in Figure C3.11A and 94-123 in Figure C3.11B) is a recombinator sequence analogous to chi in prokaryotes (Smith et al., 1980) and cog in fungi (Catcheside and Corcoran, 1973), promoting high local rates of

recombination, or that it is equivalent to att in lambda (Landy and Ross, 1977), promoting recombination in a specific sequence. These hypotheses could be tested by transferring this sequence to any other readily studied genome. Further aspects of the role of the a sequence in DNA replication are discussed in Section 4.

Table C3.1

Sizes (bp) of restriction fragments of HSV-1 BamH I k and HSV-2 BamH I g. These fragments contain one a sequence and, since there are regions of size variability in HSV-1 BamH I k, sizes refer specifically to the recombinant plasmid kl shown in Figure C3.6 . Total sizes of the fragments are given, and restriction maps are shown in Figure C3.3 .

Table C3.1

HSV-1 BamH I k			
Sst I		Sma I	
A	3500	A	1100
B	1600	B	840
C	510	C	700
D	330	D	560
		E	560
		F	410
		G	340
		H	350
		I	275
		J	240
		K	150
		L	143
		M	91
		N	86
		O	48
		P	43
		Q	38
		R	24
	5940		6015

HSV-2 BamH I g			
Sst I		Sma I	
1	2020	1	2650
2	1310	2	1050
3	960	3	540
4	740	4	510
5	390	5	440
6	290	6	300
7	160	7	112
8	130	8	87
		9	76
		10	67
		11	63
		12	14
	6000		5909

Table C3.2

Data for the restriction mapping of HSV-1 BamHI k.

Most experiments used this fragment isolated from plasmid kl (Figure C3.6), except in parts B, C, F and G, when the fragments were isolated from virion DNA. Nick translated DNA was used except in part H, when a fragment ^{32}P-labelled at the 5' termini was used. Fragments in parentheses were not resolved, and so indicate predicted rather than actual sizes. Electrophoresis was carried out on 2% agarose, 7.5% polyacrylamide or 15% polyacrylamide gels. Agarose gels consistently gave larger values for sizes of fragments greater than 500 bp than polyacrylamide gels. Therefore sizes above 500 bp are quoted from agarose gels, and below 500 bp from polyacrylamide gels. The deduced restriction maps are shown in Figure C3.3.

Table C3.2

A. Fragment sizes (bp) of HSV-1 BamH I k.

Sst I	3500 1600 510 330
Sau3A I	3300 1600 900 170
Taq I	3700 1270 900 110 26
Hinc II	4600 1400
Sma I	1100 840 700 560 560 410 340 350 275 240 150 143 91 86 48 43 38 24

B. Fragment sizes (bp) of HSV-1 BamH I q.

Sst I	2700 510 330
Sma I	1100 840 560 410 340 91 48 38 37 24

C. Fragment sizes (bp) of HSV-1 BamH I s.

Sst I	1600 1300
Sma I	700 560 350 340 275 240 150 143 86 43

D. Fragment sizes (bp) of double digests of HSV-1 BamH I k.

Sma I/Sst I	1100 760 700 560 410 350 340 275 260 250 240 150 143 91 86 78 70 (48 43 38 24)
Sma I/Sau3A I	700 660 560 560 510 420 410 350 340 275 240 170 150 150 143 91 86 (48 43 38 24)
Sma I/Taq I	820 680 700 560 560 410 350 340 275 240 150 150 143 140 110 91 86 43 38 26 24 22
Sma I/Hinc II	1100 700 680 560 560 410 350 340 275 240 160 150 143 91 86 48 43 38 24
Sst I/Sau3A I	1700 1600 1600 510 330 170 70
Sst I/Taq I	(2000 1600, 1270) 510 300 110 70 26
Sst I/Hinc II	2900 1600 600 510 330
Sau3A I/Taq I	(3300 1100 900) 400 170 110 26

Table C3.2

E. Fragment sizes (bp) of digests of fragments of HSV-1 BamH I k.

fragment	digested with	products
Sst I A	Sma I	1100 720 410 350 340 275 150 80 43 24
Sst I B	Sma I	700 560 240 143
Sst I C	Sma I	260 91 78 38
Sst I D	Sma I	250 48
Sau3A I A	Sma I	700 560 430 350 340 275 240 150 86 43
Sau3A I B	Sma I	640 495 410 24
Sau3A I C	Sma I	560 150 91 48 38
Sst I A	Sau3A I	(1650 840) 560 75
Sst I C	Sau3A I	510
Sst I A	Taq I	(2000 840) 110 70
Sst I C	Taq I	510
Sau3A I A	Taq I	(3300)
Sau3A I B	Taq I	(1100) 350 110

F. Fragment sizes (bp) of digests of fragments of HSV-1 BamH I q.

fragment	digested with	products
Sst I X	Sma I	1100 720 410 340 40 24
Sst I C	Sma I	260 91 78 38
Sst I D	Sma I	250 48

Table C3.2

G. Fragment sizes (bp) of digests of fragments of HSV-1 BamH I s.

fragment	digested with	products
Sst I B	Sma I	700 560 240 143
Sst I X	Sma I	350 340 275 150 80 43

H. Sma I partial digestion products (bp) from each of the two halves of HSV-1 BamH I k Sau3A I A, which had been 5'-labeled at the Sau3A I sites and the two halves separated by digestion with Sst I, and their interpretation as Sma I fragments.

left hand fragment of Sau3A I A	right hand fragment of Sau3A I A
240 (J)	430 (part of A)
950 (J-C)	770 (430-G)
1100 (J-C-L)	820 (430-G-P)
1650 (J-C-L-D)	1000 (430-G-P-K)
	1290 (430-G-P-K-I)
	1630 (430-G-P-K-I-H)
	1700 (430-G-P-K-I-H-part of N)

I. Sma I partial digestion products (bp) of HSV-1 BamH I k Sau3A I B which would be predicted from the two possible arrangements.

fragment order	predicted partial digestion products
640-R-F-495	1115 970 945 665 475
640-F-R-495	1115 1090 970 520 475

Table C3.2

J. Sma I partial digestion products (bp) of HSV-1 BamH I k Sst I C which would be predicted from the two possible arrangements.

fragment order	predicted partial digestion products				
78-M-Q-260	389	298	207	169	129
78-Q-M-260	389	351	207	129	116

Table C3.3

Data for the restriction mapping of HSV-2 BamHI g.

Most experiments used this fragment isolated from plasmid $\bar{g}$1 (Figure C3.6), except in parts B, C, F, G and H, when the fragments were isolated from virion DNA. The plasmid fragment contains two tandem repeated a sequences instead of the single a sequence present in the major joint fragment from virion DNA. This extra 250 bp results, for example, in two additional fragments in the SmaI digest which are not produced from the normal joint (230 and 14 bp). This was taken into account in deducing the restriction maps shown in Figure C3.3.
Nick translated DNA was used, and fragments in parentheses indicate predicted rather than actual sizes since they were not resolved on the gel used. Electrophoresis was carried out on 2% agarose, 7.5% polyacrylamide or 15% polyacrylamide gels. Agarose gels consistently gave larger values than polyacrylamide gels for sizes of fragments greater than 500 bp. Therefore sizes above 500 bp are quoted from agarose gels, and below 500 bp from polyacrylamide gels.

Table C3.3

A. Fragment sizes (bp) of HSV-2 BamH I $\bar{g}$, which contains two tandemly repeated a sequences.

Sst I	2020 1550 960 740 390 290 160 130
Sau3A I	4100 1250 900
Taq I	1800 1600 1050 770 660 85 25
Hinc II	2600 1800 1300 250
Kpn I	4700 1500
Sma I	2650 1050 540 510 440 300 230 112 87 76 67 63 14 14

B. Fragment sizes (bp) of HSV-2 BamH I u.

Sst I	2020 390 390 290 130
Sma I	2650 440 87 22 14

C. Fragment sizes (bp) of HSV-2 BamH I v.

Sst I	1550 1200 960 160
Sma I	1050 540 510 300 215 112 76 67 63 14

D. Fragment sizes (bp) of double digests of HSV-2 BamH I $\bar{g}$.

Sma I/Sst I	2020 1000 540 460 390 350 290 230 210 112 87 87 76 67 63 62 40 23
Sma I/Sau3A I	1600 1050 900 540 460 440 300 230 112 87 76 67 63 53
Sma I/Taq I	510 480 440 290 250 230 112 85 76 67 63 57 44 25
Sma I/Hinc II	1400 1100 1050 540 510 440 300 230 112 87 76 67 63
Sma I/Kpn I	1400 1100 1050 540 510 440 300 230 112 87 76 67 63

Table C3.3

D. Fragment sizes (bp) of double digests of HSV-2 BamH I g (continued).

Sst I/Sau3A I	1600	1550	740	600	450	390	290	200	
	160	130							
--Sst I/Taq I	560	390	280	160	130	85	48	41	25
Sst I/Hinc II	1300	1000	960	740	460	390	360	290	250
	160	130							
Sst I/Kpn I	1550	1050	960	750	740	390	290	160	
	130								
--Sau3A I/Hinc II	290	250							
--Sau3A I/Kpn I	500								
--Taq I/Hinc II	540	460	400	250	85	25			
--Taq I/Kpn I	85	25							
--Hinc II/Kpn I	2600	1800	1300	250	250				

Fragments smaller than 20 bp were not resolved. For those digests marked -- fragments larger than 600 bp were not resolved.

E. Fragment sizes (bp) of digests of Sst I fragments of HSV-2 BamH I g.

fragment	digested with	products
1	Sma I	2020
2	Sma I	1000 350 230 67 14 14
3	Sma I	460 290 112
4	Sma I	540 63
5	Sma I	390
6	Sma I	210 87
7	Sma I	76 62 23
8	Sma I	87 40

Table C3.3

E. Fragment sizes (bp) of digests of Sst I fragments of HSV-2 BamH I g (continued).

fragment	digested with	products
1	Taq I	(1050 1000) 45
2	Taq I	(870) 530
3	Taq I	(920) 39
4	Taq I	540 85
5	Taq I	390
6	Taq I	260 25
7	Taq I	160
8	Taq I	130

F. Fragment sizes (bp) of Sma I digests of Sst I fragments of HSV-2 BamH I u.

Sst I 1	2020
Sst I 5X	390 350 25 14
Sst I 6	210 87
Sst I 8	87 40

G. Fragment sizes (bp) of Sma I digests of Sst I fragments of HSV-2 BamH I v.

Sst I X	1000 220 67 14
Sst I 3	460 290 112
Sst I 4	540 63
Sst I 7	76 62 23

Table C3.3

H. Fragment sizes (bp) of Sma I partial digestion of Sst I fragments of HSV-2 BamH I g.

Sst I 2	1050	365	80
Sst I 3	570	410	
Sst I 4	600	67	

Table C3.4

Data for the restriction mapping of the 1760 bp Hinf I fragment which contains the joint between HSV-1 L and S. Also used was the Sau3A I/Hinf I 1130 bp fragment which spans the joint, and comprises the left hand end of the 1760 bp fragment.
Nick translated DNA was prepared from recombinant plasmid kl (Figure C3.6). Electrophoresis was carried out on 2% agarose or 7.5% polyacrylamide gels, and fragment sizes greater than 500 bp are quoted from agarose gels and less than 500 bp from polyacrylamide gels. The deduced restriction maps are shown in Figure C3.9.

Table C3.4

A. Fragment sizes (bp) of the HSV-1 BamH I k Sau3A I/Hinf I 1130 bp fragment, which contains the a sequence.

Sma I	410	340	160	150	43	
Ava II	680	450				
BstN I	1000	130				
Ava I	360	340	160	150	50	43
Hae II	980	150				
Bgl I	930	170	25			
Sst II	420	320	190	85	80	18
Taq I	1130					

Underlined fragments are those which were labelled when the 1130 bp fragment was prepared from 5'-labelled HSV-1 BamH I k Sau3A I A.

B. Fragment sizes (bp) of the HSV-1 BamH I k Hinf I 1760 bp fragment, which contains the a sequence.

Sma I	1040	340	160	150	43		
Ava II	1080	680					
BstN I	1500	130	130				
Ava I	680	350	340	160	150	43	
Hae II	1000	430	150	125	70		
Bgl I	1460	170	70	26	25		
Sst II	950	320	190	95	85	80	18
Taq I	1500	150	110				

Underlined fragments were definitely not present in the Hinf I digest of nick translated HSV-1 BamH I k.

Table C3.4

C. Fragment sizes (bp) of Sau3A I double digests of the HSV-1 BamH I k Hinf I 1760 bp fragment.

Sau3A I/Sma I	630	410	340	160	150	43	
Sau3A I/Ava II	680	630	450				
Sau3A I/BstN I	1000	500	130	130			
Sau3A I/Ava I	630	350	340	160	150	50	43
Sau3A I/Hae II	980	430	150	125	70		
Sau3A I/Bgl I	930	530	170	70	26	25	
Sau3A I/Sst II	570	420	320	190	95	85	80	18
Sau3A I/Taq I	1130	370	150	110			

D. Fragment sizes (bp) of double digests of the HSV-1 BamH I k Sau3A I/Hinf I 1130 bp fragment.

Sma I/Ava II	410	310	160	150	45	43	
Sma I/BstN I	410	340	150	130	43	32	
Sma I/Hae II	410	340	150	150	43		
Sma I/Bgl I	410	340	160	125	43	27	
Sma I/Sst II	260	130	85	80	58	43	18
Ava II/BstN I	550	450	130				
Ava II/Hae II	530	450	150				
Ava II/Bgl I	530	400	170	25			
Ava II/Sst II	420	320	190	85	41	32	18
BstN I/Bgl I	930	130	35	25			
BstN I/Sst II	420	320	170	85	80	27	18
Bgl I/Hae II	930	150	25	13			
Bgl I/Sst II	420	320	120	85	80	62	18
Sst II/Ava I	350	250	135	85	80	61	48	43	18
Sst II/Hae II	420	320	140	85	80	50	18
Bgl I/Ava I	350	340	160	125	50	43	27

Table 03.4

E. Fragment sizes (bp) of double digests of HSV-1 BamH I & Hinf I 1760 bp fragment.

Taq I/Hae II	980	350	150	125	62	38	26		
Taq I/Bgl I	1300	170	110	70	53	26	25		
Taq I/Sst II	800	320	190	110	95	85	80	50	18
Bgl I/Hae II	930	430	150	70	70	29	26	25	

Table C3.5

Data for the restriction mapping of the 1310 bp HSV-2 Sst I fragment which contains the joint between L and S (HSV-2 BamH I g Sst I 2 in Figure C3.3). Also used was the Sma I 900 bp fragment which does not span the joint, but overlaps almost entirely the 1310 bp fragment at its left hand end (HSV-2 BamH I g Sma I 2 in Figure C3.3). Nick translated DNA was prepared from recombinant plasmid g1 (Figure C3.6) which contains two tandem repeated a sequences instead of the one a sequence present in the major joint fragment of virion DNA, and so the Sst I fragment is 1550 bp in this case (see Table C3.3). This fact has been taken into account in deducing the restriction maps shown in Figure C3.9.

Electrophoresis was carried out on 2% agarose or 7.5% polyacrylamide gels, and fragment sizes greater than 500 bp are quoted from agarose gels and less than 500 bp from polyacrylamide gels. The deduced restriction maps are shown in Figure C3.9

Table C3.5

A. Fragment sizes (bp) of HSV-2 BamH I g Sst I 2, which contains two tandemly repeated a sequences.

Dde I	1200	360						
Alu I	1440	125						
Hinc II	960	350	250					
Sma I	880	360	230	67	14	14		
Ava I	500	360	280	230	130	67	14	14
Ava II	830	550	100	70	27			
BstN I	1000	250	170	70	29	15		
Sst II	480	340	310	210	200	28	28	14	14
Hinf I	600	360	300	220	29	29	26		

B. Fragment sizes (bp) of Sma I double digests of HSV-2 BamH I g Sst I 2.

Dde I	500	360	360	230	67				
Alu I	880	240	230	125	67				
Hinc II	880	360	230	67					
BstN I	360	330	250	230	170	70	67	29	
Sst II	340	310	290	200	110	110	78	67	28	28
Hinf I	600	360.	250	210	44	26	25	25	

Fragments smaller than 20 bp were not resolved.

Table C3.6

Data concerning the possible amino acid sequences at the carboxy terminus of V_{mw} IE 175.

Table C3.6

A. CODING POTENTIAL OF 3' TERMINUS OF V_{mw} IE 175 mRNA

UGA stop codon

val-ser-leu-gly-leu-ala-his-gly-pro-gln-arg-his-arg-ala-gly-gly-gly-gly-gly-arg-gly-gly-ala-gly-gly-gly-gly-gly-leu-gly-his-ala-pro-ala-ala-gly-ser-cys-gly-leu-gly-arg-arg-leu-gly-arg-arg-arg-arg-arg-arg-val-arg-gly-gly-arg-gly-ala-val-thr-gly-arg-asp-gly-ala-gly-arg-leu-COOH

UAA stop codon

ile-arg-trp-ala-ser-ala-thr-ala-arg-ser-gly-thr-val-leu-ala-ala-ala-gly-ala-val-glu-val-leu-gly-ala-glu-ala-gly-leu-ala-thr-pro-pro-arg-arg-glu-val-val-asp-trp-glu-gly-ala-trp-asp-glu-asp-asp-gly-gly-ala-phe-glu-gly-asp-gly-val-leu-COOH

B. AMINO ACID USAGE

amino acid	UGA stop codon	UAA stop codon
pro	2	2
arg	15	4
ala	8	12
gly	24	9
others	29	31
total	68	58

(continued overleaf)

Table C3.6 continued

C. THIRD CODON POSITION USAGE

nucleotide	UGA stop codon	UAA stop codon
G	24 } 44	30 } 52
C	20 }	22 }
A	13 } 25	4 } 8
T	12 }	4 }

Figure C3.1

Data for Sst I restriction maps of HSV-1 BamH I k and HSV-2 BamH I g.

A
1 HSV-1 BamH I k
2 HSV-1 BamH I s
3 HSV-1 EcoR I k

Digested with Sst I; 2% agarose gel.

B
1 HSV-2 BamH I g
2 HSV-2 BamH I u
3 HSV-2 EcoR I k
4 HSV-2 EcoR I m
5 HSV-2 BamH I uvw
6 HSV-2 EcoR I f

Digested with Sst I; 2% agarose gel.

C
1 HSV-2 BamH I g
2 HSV-2 BamH I u
3 HSV-2 EcoR I k
4 HSV-2 EcoR I m

Digested with Sst I; 7.5% polyacrylamide gel.

D
1 HSV-2 BamH I u
2 HSV-2 Kpn I a

Digested with Sst I; 1.5% agarose gel.

E
1 HSV-2 BamH I g
2 HSV-2 Kpn I rs

Digested with Sst I; 2% agarose gel.

Each of the above fragments was prepared from nick translated HSV-1 or HSV-2 DNA.

Sst I fragments of BamH I joint or terminal fragments are indicated. λ denotes a genome terminal fragment.

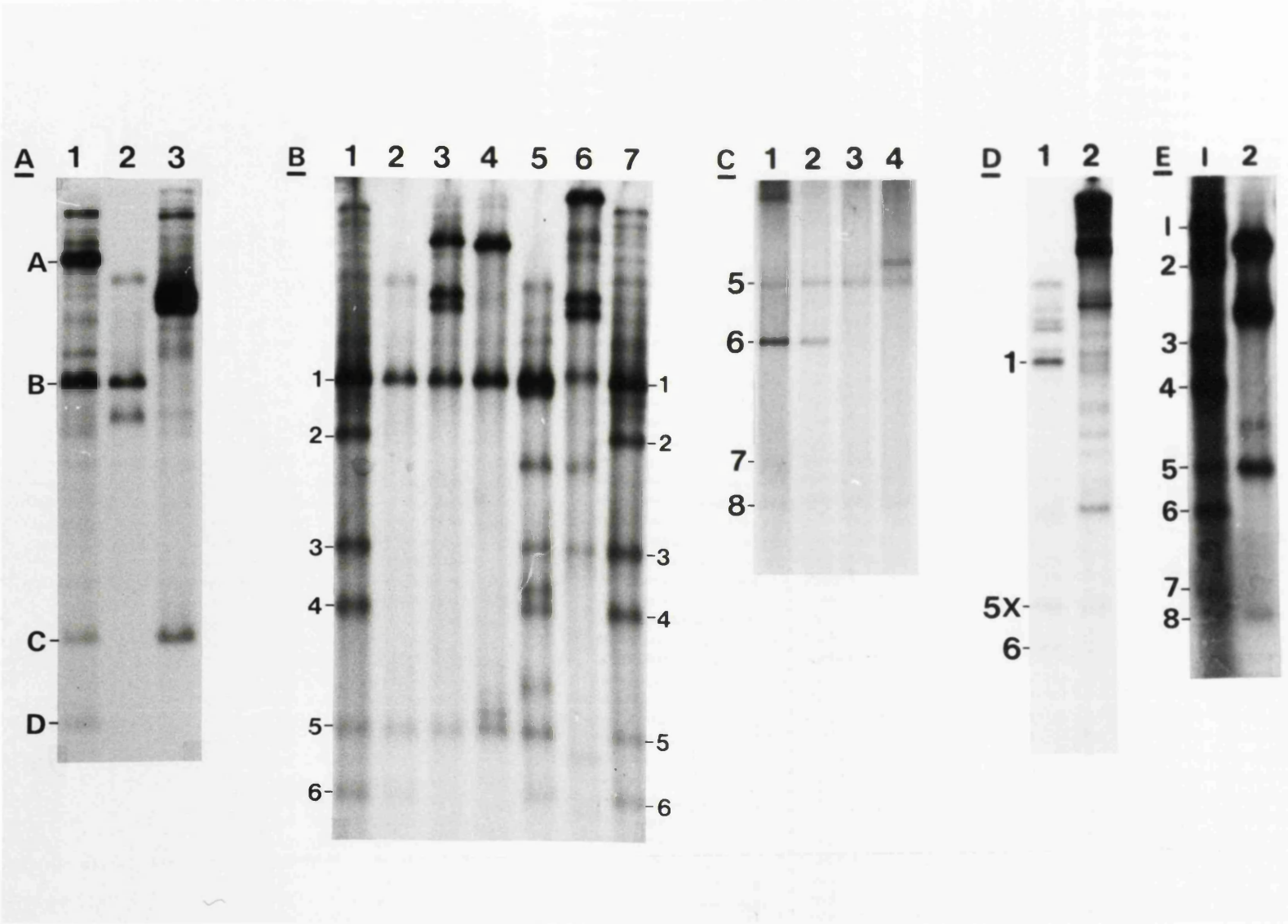

A 1 2 3
A
B
C
D
B 1 2 3 4 5 6 7
1
2
3
4
5
6
C 1 2 3 4
5
6
7
8
D 1 2
1
5X
6
E I 2
I
2
3
4
5
6
7
8

Figure C3.2

Some data for Sma I restriction maps of HSV-1 BamH I k.

A
1 HSV-1 BamH I k digested with Sma I
2 HSV-1 BamH I k digested with Sma I/Sau3A I
3 HSV-1 BamH I k digested with Sma I/Sst I
4 HSV-1 BamH I k Sau3A I A digested with Sma I
5 HSV-1 BamH I k Sau3A I B digested with Sma I
6 HSV-1 BamH I k Sau3A I C digested with Sma I

2% agarose gel.

B
1 pBR322 Hinf I markers
2 HSV-1 BamH I k digested with Sma I
3 HSV-1 BamH I k Sau3A I A digested with Sma I
4 HSV-1 BamH I k Sau3A I B digested with Sma I
5 HSV-1 BamH I k Sau3A I C digested with Sma I
6 HSV-1 BamH I k Sst I B,D digested with Sma I
7 HSV-1 BamH I k Sst I A digested with Sma I
8 HSV-1 BamH I k Sst I C digested with Sma I

5% polyacrylamide gel.

C
1 pBR322 Hinf I markers
2-6 HSV-1 BamH I k Sau3A I A digested with increasing amounts of Sma I

2% agarose gel.

D
1 HSV-1 BamH I k Sst I C partially digested with Sma I

5% polyacrylamide gel.

E
1 HSV-1 BamH I k Sau3A I B partially digested with Sma I

5% polyacrylamide gel.

Sma I restriction fragments of HSV-1 BamH I k are indicated in panels A and B. Sizes of partial digestion products in bp are indicated in panels C-E with Sma I restriction fragments in letters. Nick translated plasmid DNA was used. Sma I fragments of HSV-1 BamHI k Sau3AI A (430 bp is part of SmaI A) and a 1631 bp marker are indicated in panel F.

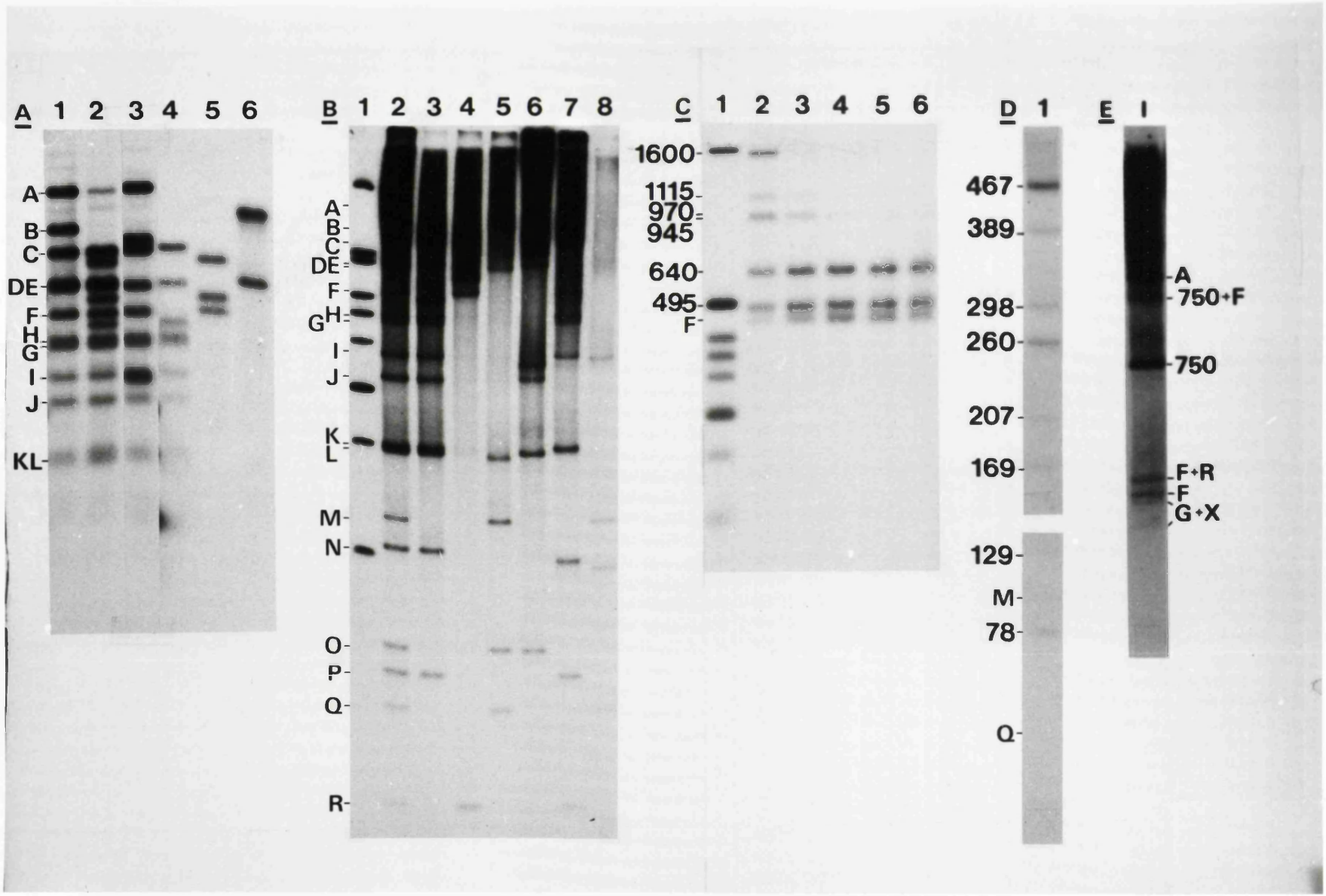
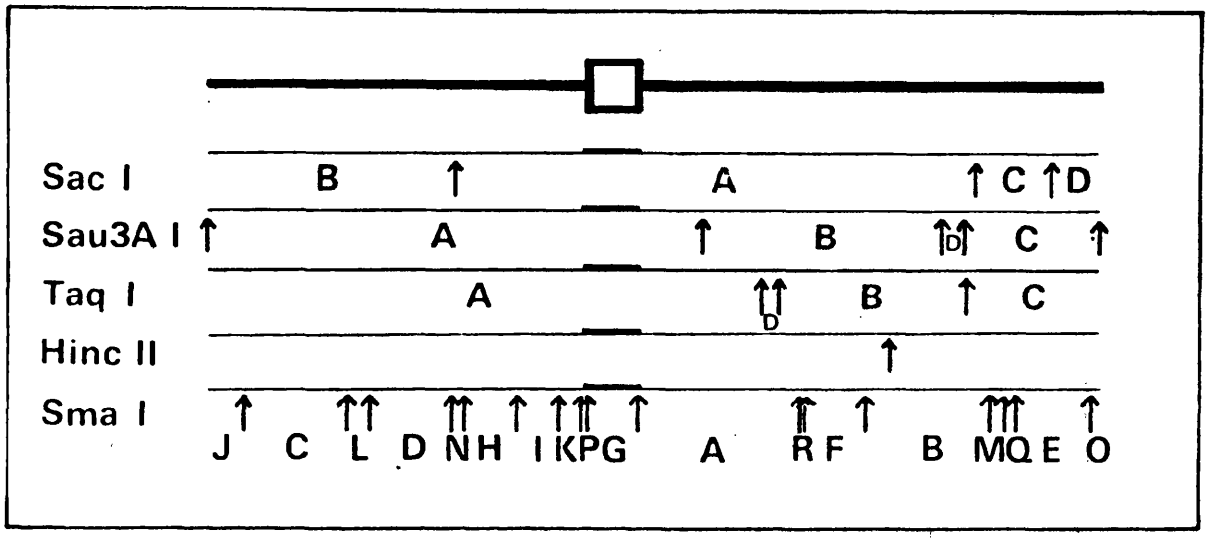

A
1 2 3 4 5 6
A
B
C
DE
F
H
G
I
J
KL
B
1 2 3 4 5 6 7 8
A
B
C
DE
F
G
H
I
J
K
L
M
N
O
P
Q
R
C
1 2 3 4 5 6
1600
1115
970
945
640
495
F
D
1
467
389
298
260
207
169
129
M
78
Q
E
I
A
750+F
750
F+R
F
G+X

Figure C3.3

Restriction maps of HSV-1 BamH I k (upper panel) and HSV-2 BamH I g (lower panel), which contain the joint of L and S. The rectangle indicates the position of the a sequence, and both fragments are about 6000 bp in size..
Nomenclature has been assigned to the fragments of some digests. Sac I and Sst I are isoschizomers. Restriction maps for genome termini, each of which has a terminal a sequence, may readily be deduced from those of the joint regions (see Figure C3.13).


Sac I
B
A
C
D
Sau3A I
A
B
D
C
Taq I
A
D
B
C
Hinc II
Sma I
J C L D N H I K P G A R F B M Q E O

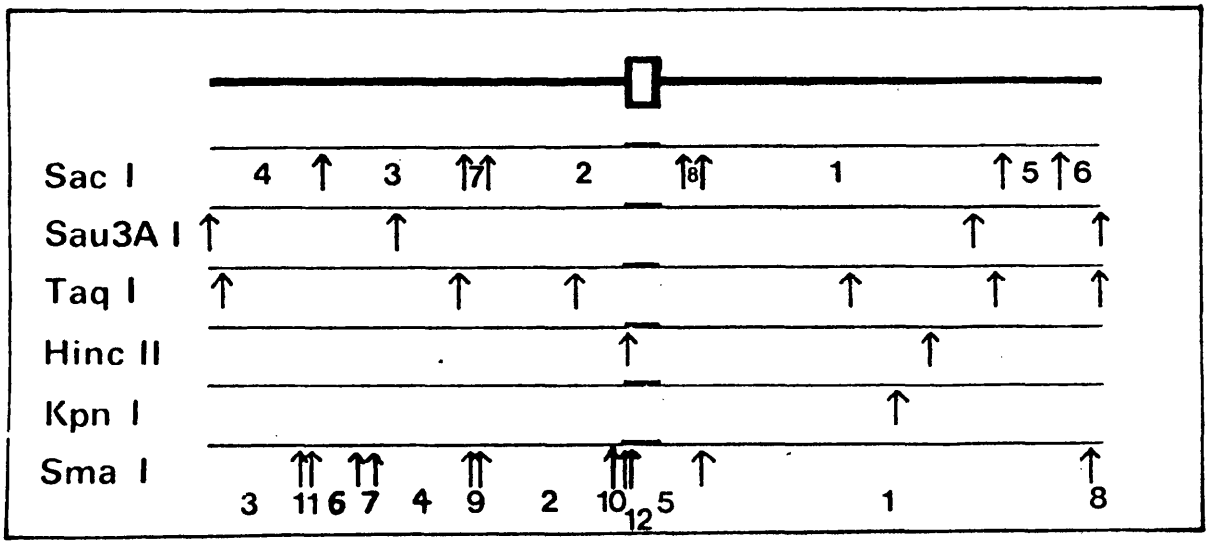

Sac I
4
3
7
2
8
1
5
6
Sau3A I
Taq I
Hinc II
Kpn I
Sma I
3 11 6 7 4 9 2 10 12 5 1 8

Figure C3.4

Autoradiograph showing BamHI restriction profiles of *in vivo* ^{32}P-labelled virion DNA from ten stocks of HSV-1 strain 17 and their parent stock (P), and from ten stocks of HSV-2 strain HG52 and their parent stock (P). Gel electrophoresis was carried out on 0.7% (HSV-1) and 1% agarose (HSV-2). Joint and terminal fragments, and fragments exhibiting size variability, are indicated. Restriction maps are shown in Figures C1.18 and C1.19.

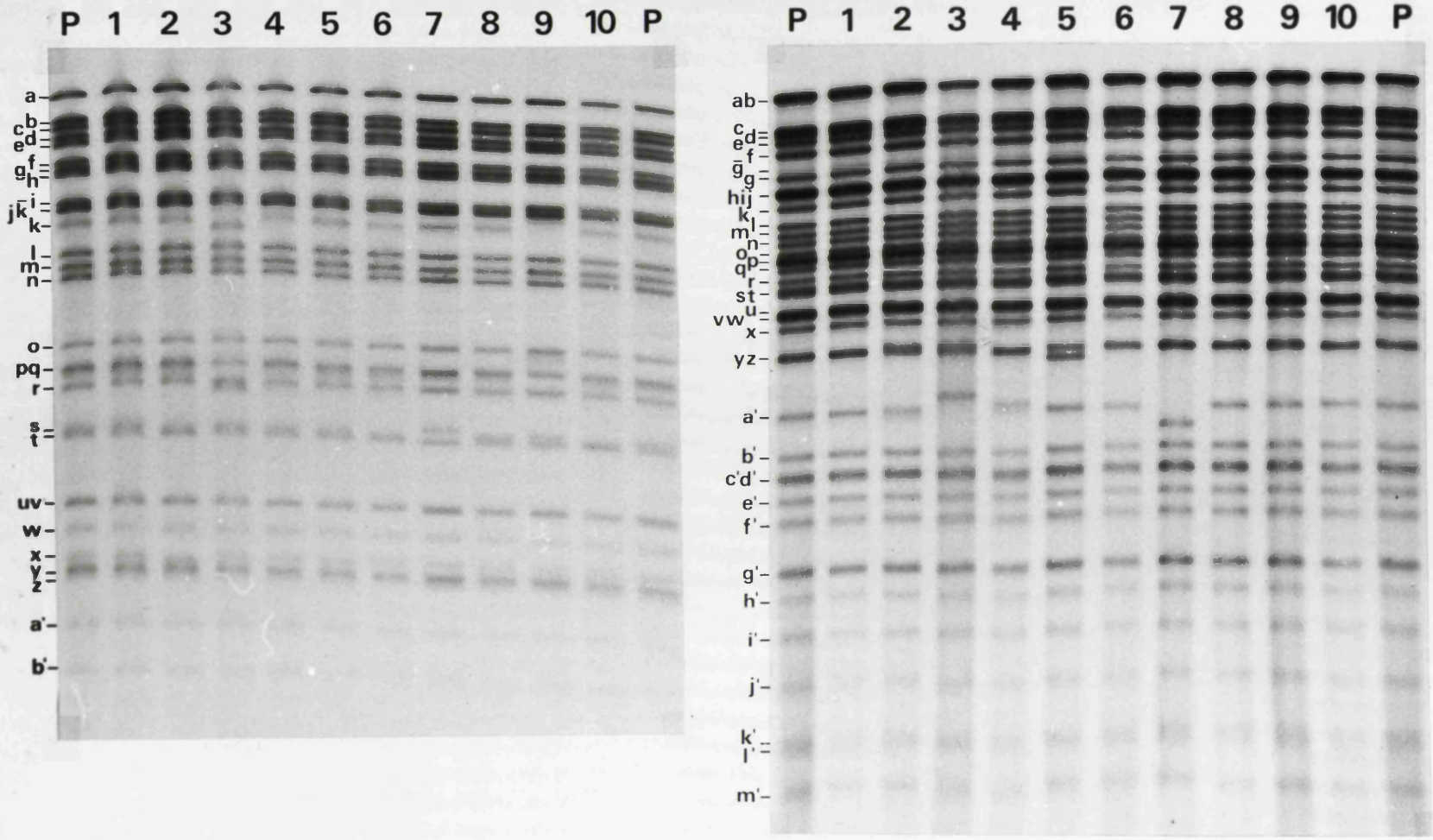

P 1 2 3 4 5 6 7 8 9 10 P
a
cb
ed
gf
h
jk i
k
l
m
n
o
pq
r
st
uv
w
x
y
z
a'
b'
P 1 2 3 4 5 6 7 8 9 10 P
ab
cd
e
f
g
hi
j
k
l
m
n
o
p
q
r
st
vw u
x
yz
a'
b'
c'd'
e'
f'
g'
h'
i'
j'
k'
l'
m'

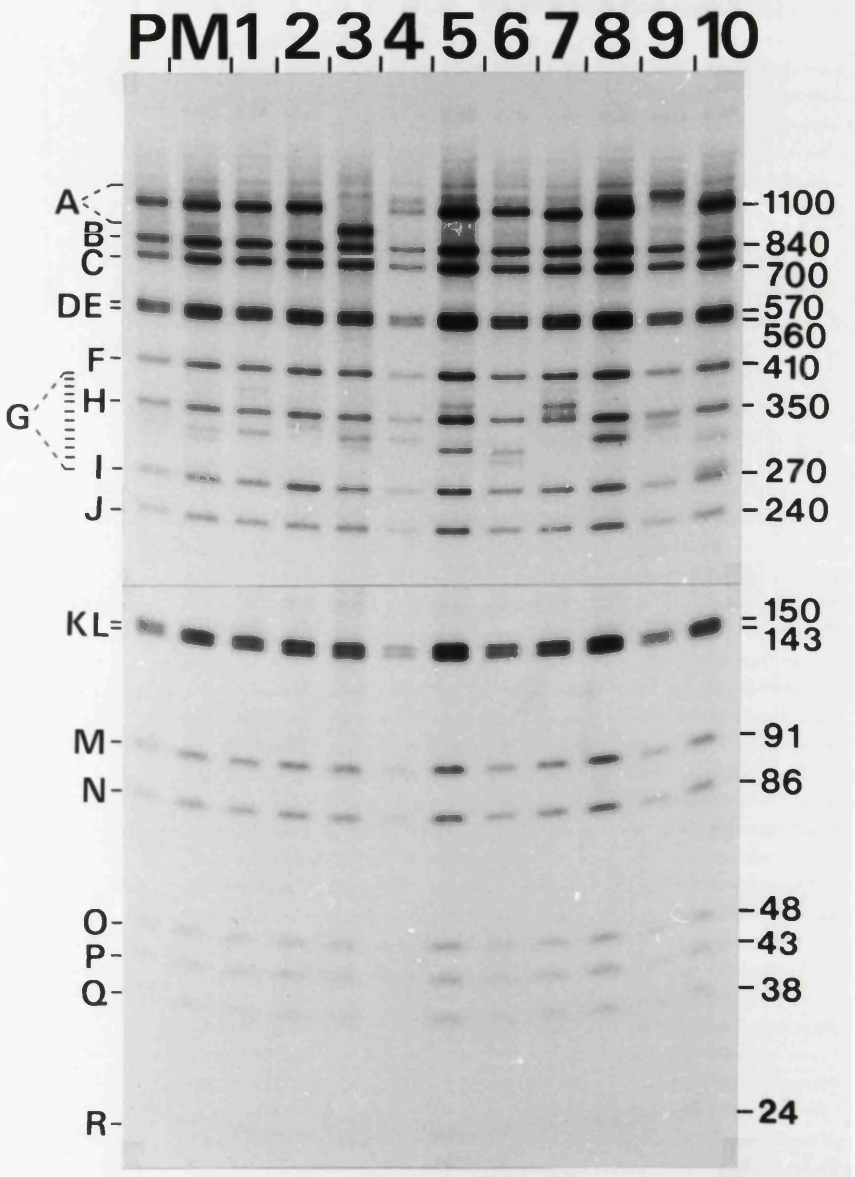
Figure C3.5

LEFT HAND PANEL

Autoradiograph showing SmaI restriction profiles of BamHI joint fragments isolated from in vivo ^{32}P-labelled DNA from ten HSV-1 strain 17 stocks, their parent (P), and a mixture of the ten fragments (M). Gel electrophoresis was carried out on a 7.5% polyacrylamide gel, and the portion below J represents a longer exposure of the gel. Fragment sizes (bp) were calibrated with HaeIII and HinfI digests of pBR322 DNA (Sutcliffe, 1978), and size ranges of the variable fragments SmaI A and G are indicated. The SmaI map is shown in Figure C3.3.

RIGHT HAND PANEL

Autoradiograph showing TaqI and Sau3AI profiles of BamHI joint fragments isolated from in vivo ^{32}P-labelled DNA from five of the ten HSV-1 strain 17 stocks, with their parent (P). Electrophoresis was carried out on a 1.5% agarose gel. TaqI fragments are indicated on the left and Sau3AI fragments on the right. The positions of molecular weight markers of 1631 and 516 bp are shown. Maps are given in Figure C3.3.


PM 1 2 3 4 5 6 7 8 9 10
A
B
C
DE
F
G
H
I
J
KL
M
N
O
P
Q
R
1100
840
700
570
560
410
350
270
240
150
143
91
86
48
43
38
24

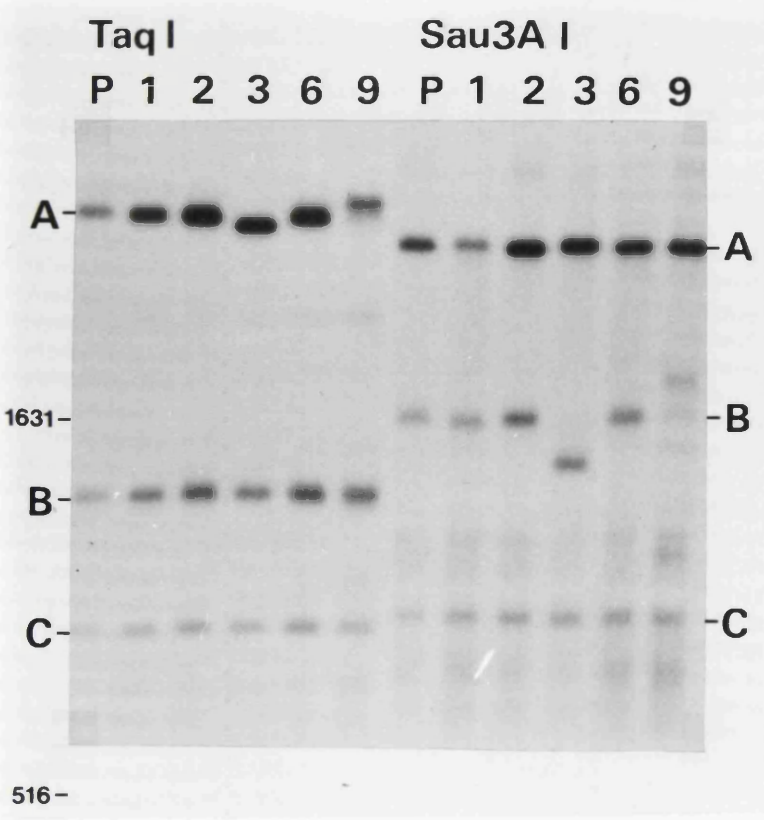

Taq I
Sau3A I
P 1 2 3 6 9 P 1 2 3 6 9
A
1631
B
C
516
A
B
C

Figure C3.6

LEFT HAND PANEL

Autoradiograph showing SmaI restriction profiles of BamHI joint fragments isolated from in vivo ^{32}P-labelled DNA from ten HSV-2 strain HG52 stocks, their parent (P), and a mixture of the ten fragments (M). Electrophoresis was carried out on a 7.5% polyacrylamide gel, and the portion below 6 represents a longer exposure of the gel. Fragment sizes are given in Table C3.1, and the SmaI map in Figure C3.3.

RIGHT HAND PANEL

SmaI/BamHI restriction profiles of recombinant plasmids bearing the BamHI joint fragment of HSV-1 strain 17 or HSV-2 strain HG52 DNA. 2 μg DNA was applied to each track of a 4% polyacrylamide gel, which after electrophoresis was stained with ethidium bromide and photographed under ultraviolet illumination. The plasmid vector pAT153 contains no SmaI sites and therefore migrates as a large fragment in each track. Clones k1, k2 and k3 contain the HSV-1 joint fragment with a single a sequence, $\bar{k}$1 and $\bar{k}$2 contain the same fragment with two a sequences, and $\bar{g}$1 contains the HSV-2 joint fragment with two a sequences. HSV-1 SmaI fragments are indicated on the left, with size ranges of A and G, and sizes in bp of ØX174 HaeIII markers on the right. Standard reference numbers of the clones are given in Table B1.3.

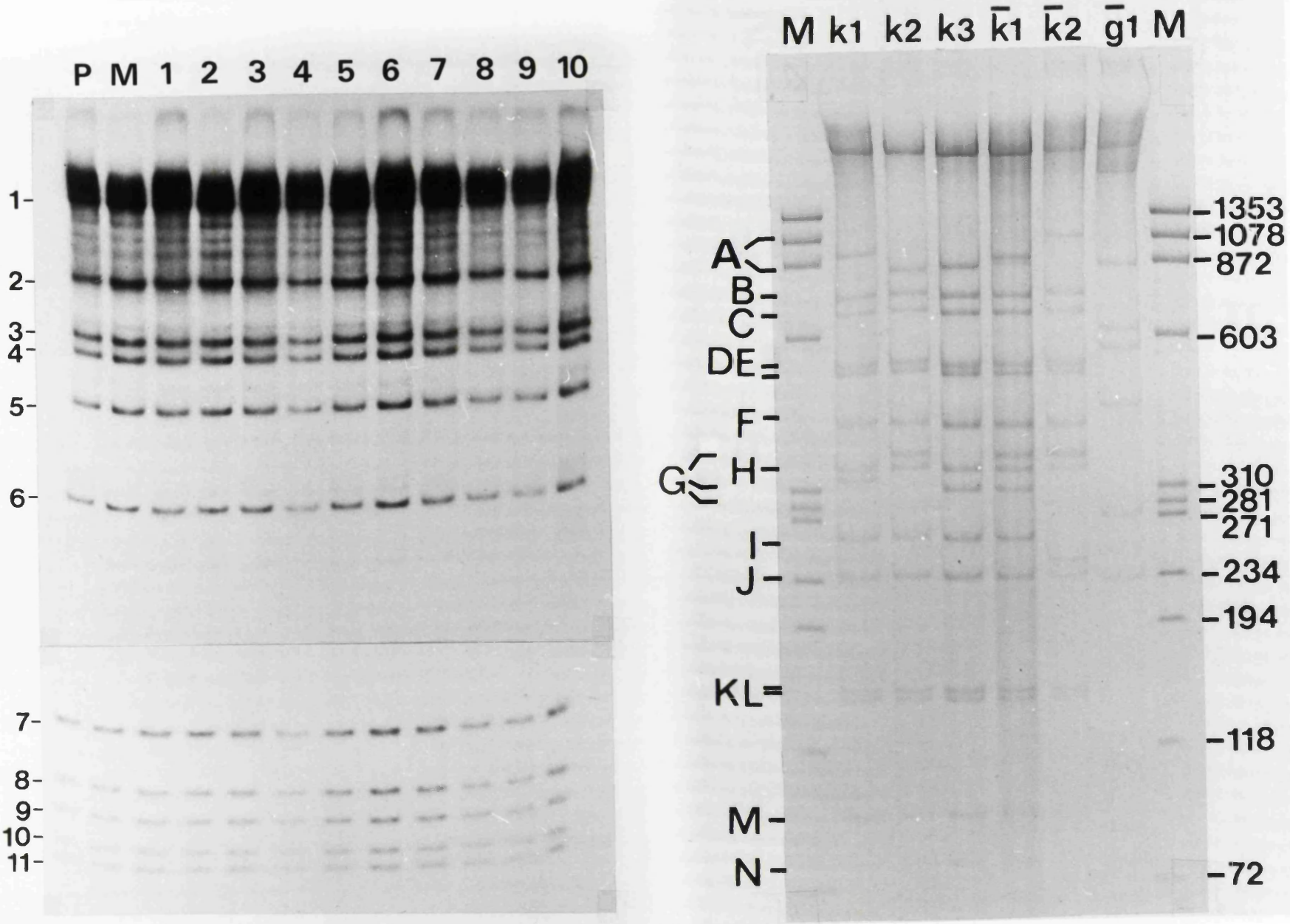

P M 1 2 3 4 5 6 7 8 9 10
1-
2-
3-
4-
5-
6-
7-
8-
9-
10-
11-
M k1 k2 k3 k̄1 k̄2 ḡ1 M
A
B
C
DE
F
G
H
I
J
KL
M
N
1353
1078
872
603
310
281
271
234
194
118
72

Figure C3.7

Homology between the joint regions of the genomes of HSV-1 and HSV-2.
Recombinant plasmids containing the HSV-1 or HSV-2 BamH I joint fragments were digested and transferred to nitrocellulose from a 4% polyacrylamide gel. Blot strips were then hybridised with nick translated recombinant plasmid DNA.

1 kl BamH I/Sma I hybridised with kl
2 kl BamH I/Sma I hybridised with $\bar{g}$l
3 kl BamH I/Sma I/Sst I hybridised with kl
4 kl BamH I/Sma I/Sst I hybridised with $\bar{g}$l
5 $\bar{g}$l BamH I/Sma I hybridised with $\bar{g}$l
6 $\bar{g}$l BamH I/Sma I hybridised with kl

HSV-1 BamH I k Sma I fragments are indicated for tracks 1-4, those fragments in parentheses indicating the new positions of migration of portions of Sma I fragments after cleavage with Sst I. HSV-2 BamH I $\bar{g}$ Sma I fragments are indicated for tracks 5-6. SmaI maps are shown in Figures C3.3 and C3.13. Fragment J of $\bar{g}$l, since this plasmid has two a sequences, maps in a location spanning the a-a junction (Figure C3.13).

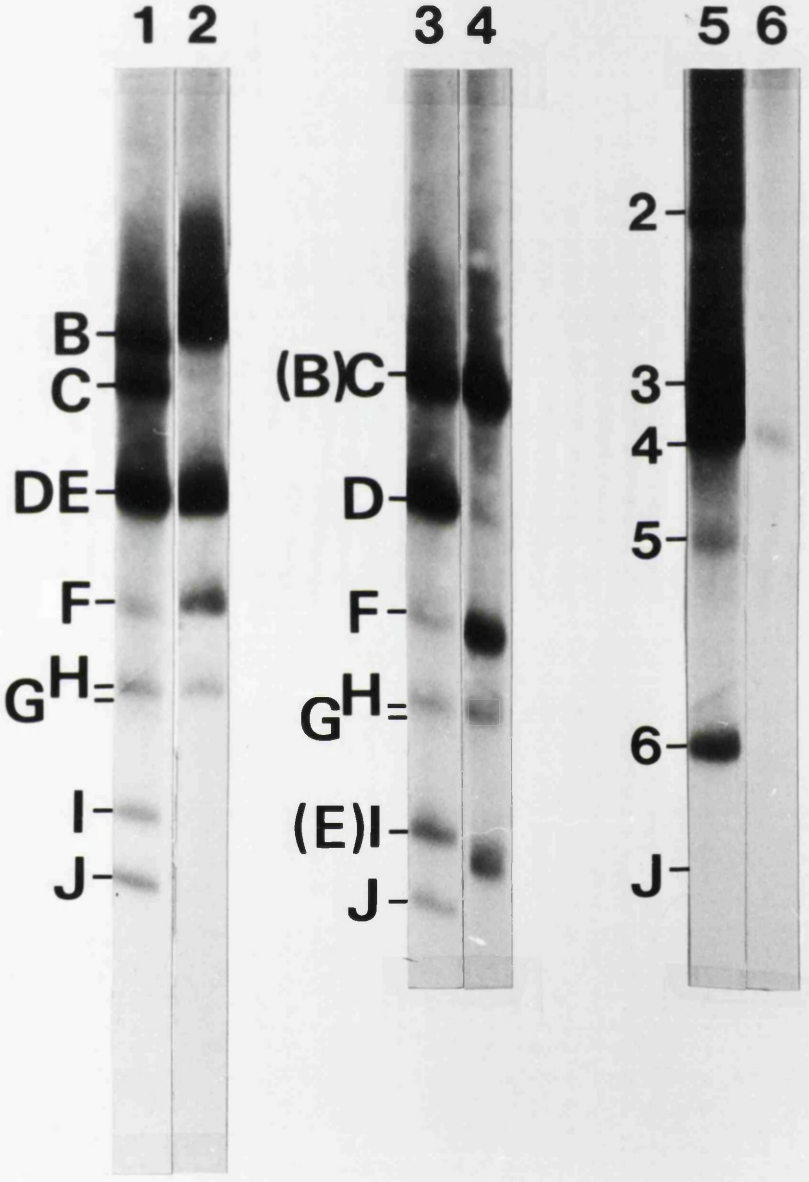

1 2
3 4
5 6
B
C
DE
F
G
H
I
J
(B)C
D
F
G
H
(E)I
J
2
3
4
5
6
J

Figure C3.8

Some data for the restriction mapping of the 1760 bp Hinf I fragment which contains the joint between HSV-1 L and S.

A
1 Sau3A I A digested with Hinf I
2 Sau3A I A digested with Hinf I/Sma I
3 Sau3A I A digested with Sma I

B
1 BamH I k digested with Sma I
2 S/H digested with Sma I
3 Sau3A I A digested with Sma I
4 S/H digested with Ava I
5 S/H digested with Ava II
6 S/H digested with Ava II/Sma I
7 S/H digested with BstN I
8 S/H digested with BstN I/Sma I
9 S/H digested with Hae II
10 S/H digested with Hae II/Sma I
M pBR322 Hae III markers

C
M pBR322 Hae III markers
1 S/H digested with Sma I
2 S/H digested with Sst II/Sma I
3 S/H digested with Sst II
4 S/H digested with Bgl I/Sma I
5 S/H digested with Bgl I

All the fragments were prepared from nick translated plasmid DNA containing HSV-1 BamI I k (kl in Figure C3.6). S/H refers to the Sau3A I/Hinf I 1130 bp fragment which contains the joint between L and S in the HSV-1 genome. 7.5% polyacrylamide gels were used.
In panel A the Sma I fragments of Sau3A I A are indicated, including the 430 bp fragment which is part of Sma I A. The Sma I fragments of BamH I k are indicated in panel B, and the sizes of pBR322 Hae III markers in bp in panels B and C.

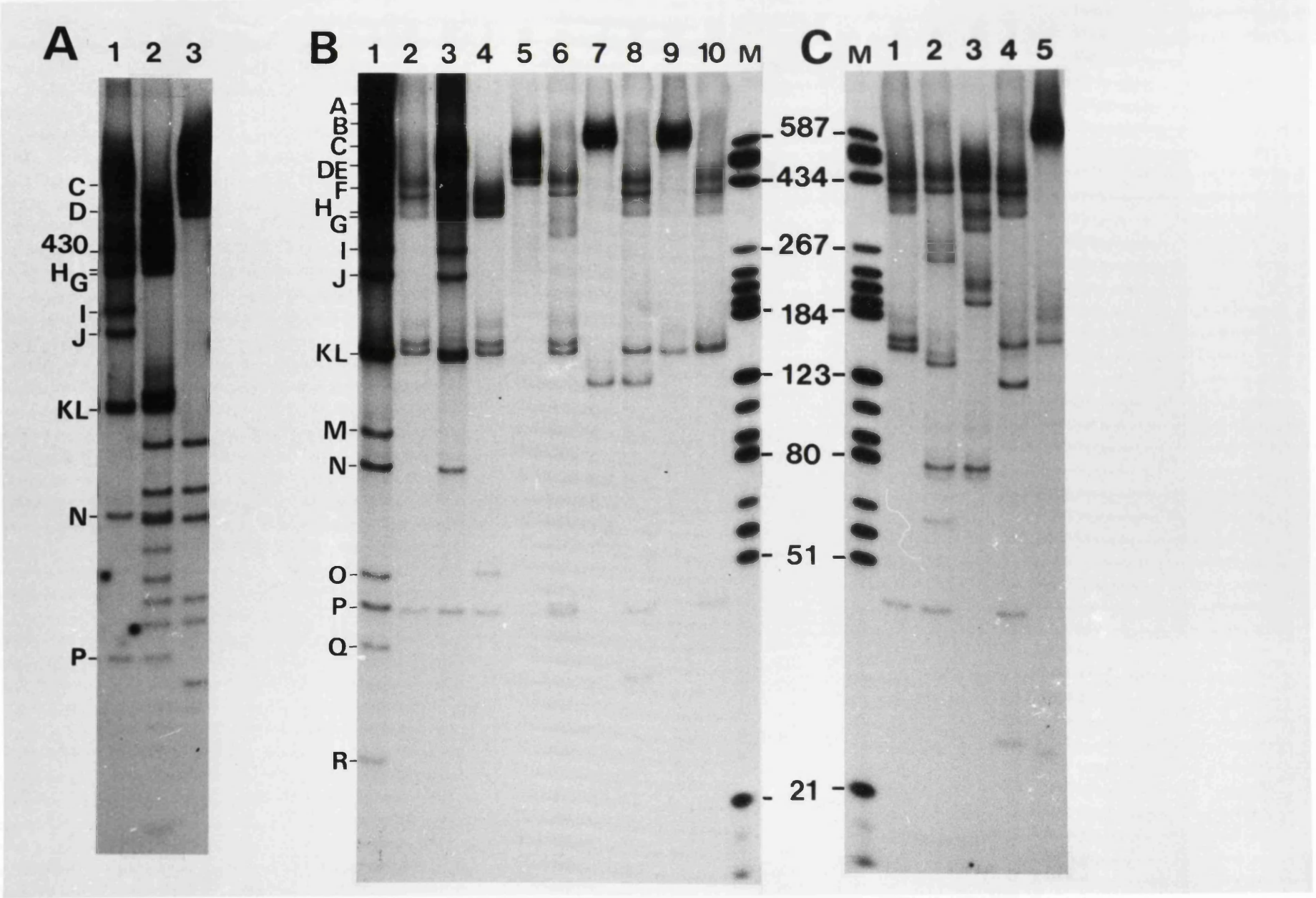
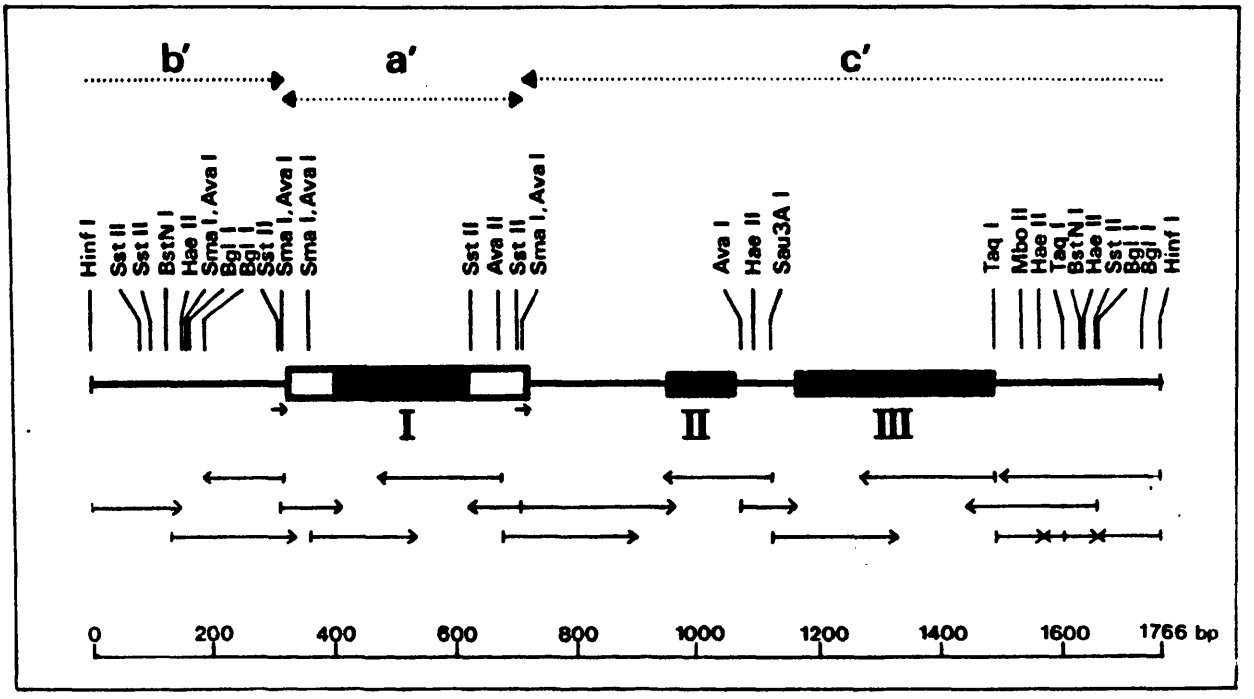

A
1 2 3
C
D
430
HG
I
J
KL
N
P
B
1 2 3 4 5 6 7 8 9 10 M
A
B
C
DE
F
HG
I
J
KL
M
N
O
P
Q
R
587
434
267
184
123
80
51
21
C
M 1 2 3 4 5

Figure C3.9

Restriction endonuclease maps of the joint regions of HSV-1 strain 17 and HSV-2 strain HG52, and the nucleotide sequencing strategies employed. The HSV-1 fragment (HinfI 1760 bp) contains three reiterations shown in black (I, II, III). Restriction sites in this region of the DNA of HSV-1 strain USA-8 were identical to those in strain 17, except for an extra HinfI site in the c sequence 50 bp from the a sequence. The HSV-2 fragment is SstI 1310 bp (BamHI g SstI 2).
Locations of the direct repeats at the b-a and a-c junctions are indicated.


b'
a'
c'
Hinf I
Sst II
Sst II
BstN I
Hae II
Sma I, Ava I
Bgl I
Bgl I
Sst II
Sma I, Ava I
Sma I, Ava I
Sst II
Ava II
Sst II
Sma I, Ava I
Ava I
Hae III
Sau3A I
Taq I
Mbo II
Hae II
Taq I
BstN I
Hae III
Sst II
Bgl I
Bgl I
Hinf I
I
II
III
0
200
400
600
800
1000
1200
1400
1600
1766 bp

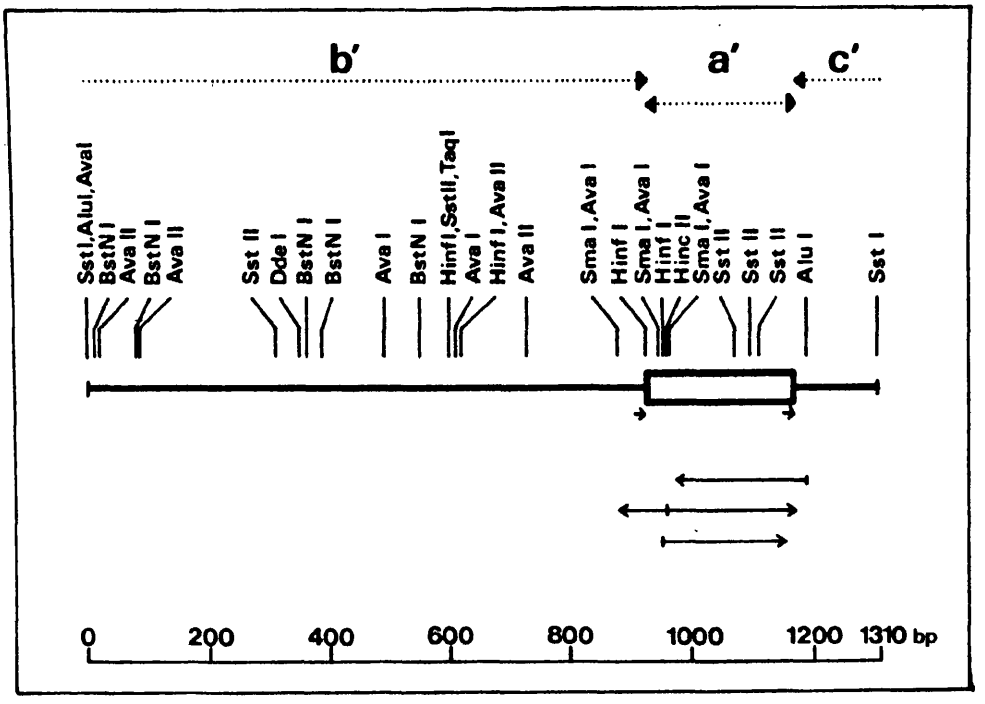

b'
a'
c'
Sst I, Alu I, Ava I
BstN I
Ava II
BstN I
Ava II
Sst II
Dde I
BstN I
BstN I
Ava I
BstN I
Hinf I, Sst II, Taq I
Ava I
Hinf I, Ava II
Ava II
Sma I, Ava I
Hinf I
Sma I, Ava I
Hinf I
Hinc II
Sma I, Ava I
Sst II
Sst II
Sst II
Alu I
Sst I
0
200
400
600
800
1000
1200
1310 bp

Figure C3.10

Finer definition of the location of HSV-1 a sequence variability.

Sau3A I A was isolated from nick translated plasmids k1 (1) and k2 (2), which contain inserts of HSV-1 BamH I k, and the latter of which has a larger Sma I G fragment (Figure C3.6).

The two Sau3A I A fragments were cleaved as indicated, and in each case the fragment which is larger in k2 is shown. The pBR322 Hae III marker fragments (M) are included.

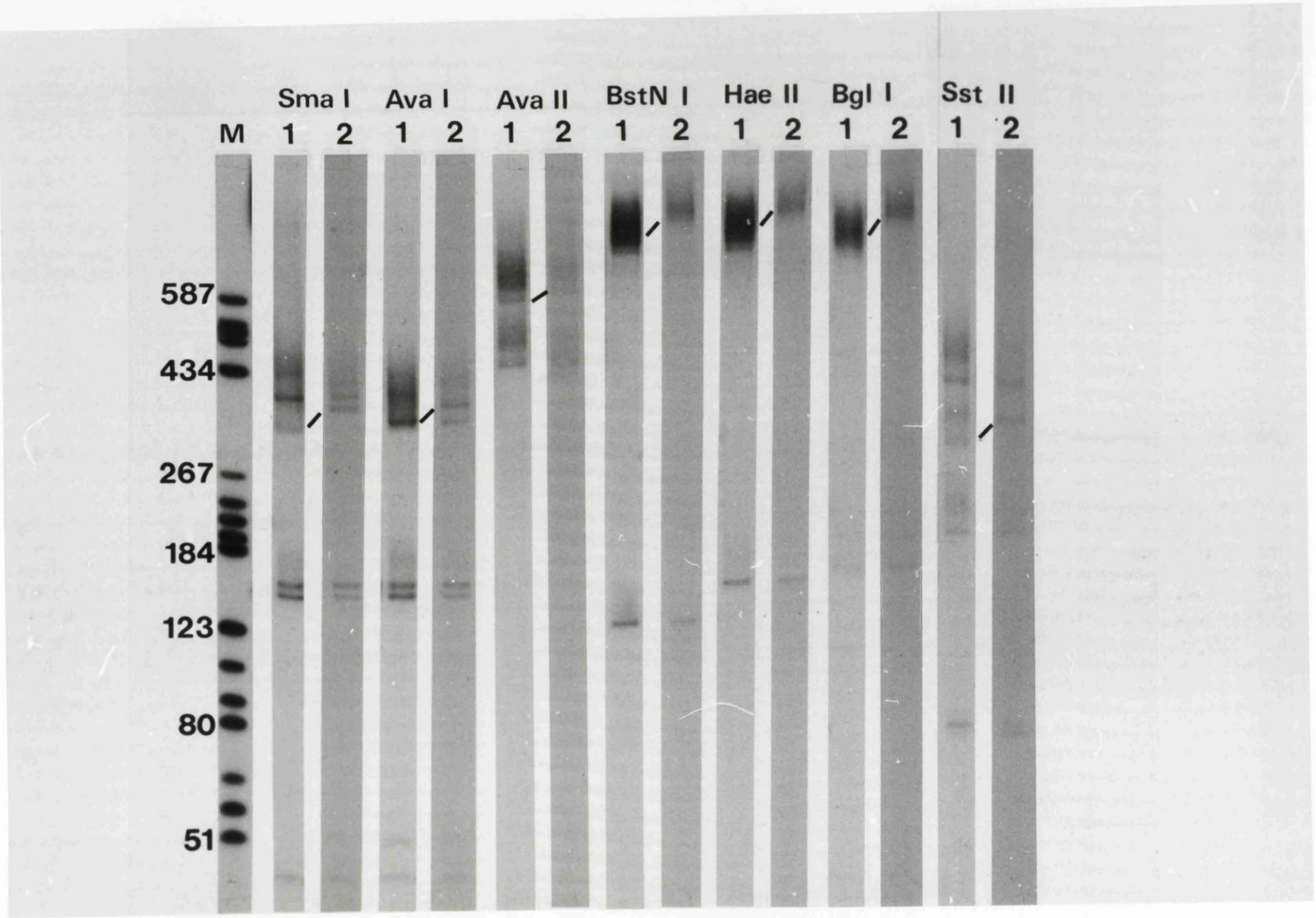

Sma I
Ava I
Ava II
BstN I
Hae II
Bgl I
Sst II
M
1
2
587
434
267
184
123
80
51

Figure C3.11

Nucleotide sequences of the joint regions of HSV-1 and HSV-2 DNA, shown conventionally 5'-3' in the b'-a'-c' orientation.

A HSV-1 strain 17 clone kl, with the positions where HSV-1 strain USA-8 differs indicated below the sequence. A dash represents the absence of a nucleotide, and strain USA-8 was not sequenced between the two sets of three dots. The locations of the genomic termini with respect to the joint sequences are shown (strain 17 above and strain USA-8 below the sequence). These define the b-a and a-c junctions and are close to the right hand ends of direct repeats (21 nucleotides in strain 17, 17 in strain USA-8), the common 17 nucleotides of which are boxed (311-327 and 710-726). Reiterations I, II and III are indicated, and the two AATAAA hexanucleotides are boxed (1500-1505 and 1546-1541). The region in which the terminal coded nucleotide at the 3' terminus of V_{mw} IE 175 mRNA was mapped is shown (1530-1525). A number of repeated structures of unknown significance are present but are not discussed in the text (e.g. an almost exact 18 nucleotide inverted repeat at 243-260 and 281-299 and a direct repeat of 20 nucleotides in strain USA-8 terminating at 778 and 861). The entire sequence has a GC content of 78%.

B HSV-2 strain HG52. The locations of the genomic termini with respect to the joint sequence are shown. They are close to the right hand ends of 17 nucleotide direct repeats (boxed) and define the b-a and a-c junctions. The entire sequence has a GC content of 80%.

A

```
GACTCGGGAA CGTGGAGCCA CTGGCGCAGC AGCAGCGAAC AAGAAGGCGG GGGCCCACCG GCGGGGGGCG          70

GCGGCGGGGC GGCCGCGGGC GCGCTCCTGA CCGCGGGTTC CGAGTTGGGC GTGGAGGTTA CCTGGGACTG         140

TGCGGTTGGG ACGGCGCCCG TGGGCCCGGG CGGCCGGGGG CGGCGGGGGC CGCGATGGCG GCGGCGGCGG         210

GCCATGGAGA CAGAGAGCGT GCCGGGGTGG TAGAGTTTGA CAGGCAAGCA TGTGCGTGCA GAGGCGAGTA         280

                                                        b'  a'
GTGCTTGCCT GTCTAACTCG CTAGTCTCGG CCGCGGGGGG CCCGGGCT-GC CCGCCGCCAC CGCTTTAAAG         350
                          C                          GC          G-

GGCCGCGCGC GACCCCCGGG GGGTGTGTTT TGGGGGGGGC CCGTTTTCGG GGTCTGG                        407
      C                          C                T

reiteration I
(CCGCTCCTCCCC)18 ------------------------  CCGCTCC CGCGGCCCCG CCCCCCACGC                650
(CCGCTCCTCCC-)8(CCGTCTGTGGGTGGGGCTCCTCCC)6

CCGCCGCGCG CGCGCACGCC GCCCGGACCG CCGCCCGCCT TTTTTGCGCG CGCGCGCGCC CGCGGGGGGC         720
           G                                              CGC C
Sma I  a'  c'
CCGGGCTGCC ACAGGTGAAA----- CCAACAG----------AGC ACGGCGCACT CCGCACGT------------CA    770
       C          C T  CAACA         AACACCAAC              C GGCG  TCCAGTTCCTCATC

CACGTCACGT CATCCACCAC ACCTGCCCAA CAACACAACT CACAGCGACA ACTCACCGCG CAACAACTCC         840
```

```
TGTTCCTCAT CCACACGTCA CCGCGCACCT CCCGCTCCTC CAGACGTACC CCGGCGCAAC ACACCGCTCC         910
                            C                A    C                G

                                reiteration II
TGCTACACAC CACCGCC---- (CCCTCCCCAGCCCCAG)2   (CCCTCCCCGGCCCCAG)6 CCCTC               1060
  TC             CCAG (CCCTCCCCAGCCCCAG)15+ ...

CCCGGCGCGT CCCGCGCTCC CTCGGGGGGG TTCGGGCATC TCTACCTCAG TGCCGCCAAT CTCAGGTCAG        1130

                                reiteration III
AGATCCAAAC CCTCCGGGGG CGCCCGCGCA CCACCA (CCGCCCCTCGCCCCCTC)19 C CGCCCCTCGA          1500
                                V_mw IE 175 mRNA 3'-end
ATAAACAACG CTACTGCAAA ACTTAATCAG GTTGTTGCCG TTTATTGCGT CTTCGGGTCT CACAAGCGCC        1570

CCGCCCCGTC CCGGCCCGTT ACAGCACCCC GTCCCCCTCG AACGCGCCGC CGTCGTCTTC GTCCCAGGCG        1640
                                ...

CCTTCCCAGT CCACAACTTC CCGCCGCGGG GGCGTGGCCA AGCCCGCCTC CGCCCCCAGC ACCTCCACGG        1710

CCCCCGCCGC CGCCAGCACG GTGCCGCTGC GGCCCGTGGC CGAGGCCCAG CGAATC                        1766
```

B

```
                                                        b'  a'
TGGCGTGCGC AGCCCGGGCC GTGTTGCGGG CCCTCTTAAG GGGCGGCGGC AGGACGGGGA CTCCCGCCCC         70

GCCTCTTTTC CCCCGGGGAG TCAACCCCCG GGGGGGGTGT TTTTTGGGGG GGGGCGCGAA GGCGGGCGGC        140

GGCGGCGGGC GGGCGGCAGG GCAGCCCCGC GCGCCCCCTT CCCCGTCCCT CCCCCGGAGC CGGCCGCTCC        210

CCCGCGGGCG CCGCCCCTCC CCCCGCGCGC CGCGGGCTGC CTTCCGCGGC GCCCCCGCGC GGCTTTTTTC        280
                                a'          c'
CCGCGCCCGC CCCCGCGCGG CAGGACGGGG ACTAGCAGGC TGTGCCGCAG ACCACCACAC ACTCCCAA          348
```

Figure C3.12

Autoradiograph showing sizes of genomic terminal restriction fragments of HSV-1 strains 17 and USA-8. 20 μg HSV-1 DNA was ^{32}P-labelled at 5' termini in a volume of 20 μl, and the BamHI S and L terminal fragments isolated. These were recleaved as indicated and electrophoresis carried out on a 16% polyacrylamide (8 M urea) sequencing gel, using as size markers G-A and C-T chemical degradation nucleotide sequencing reactions (kindly provided by M-J. Murchie), the fragment sizes of which are given in nucleotides. The S terminal BamHI fragment was contaminated with the L terminal fragment possessing an additional a sequence.

S
L
Sma I
Hae III
Sma I
Hae III
Sst II
CT
17
USA-8
17
USA-8
GA
CT
USA-8
17
USA-8
17
USA-8
17
GA
40
30
25
20
15
10
5

Figure C3.13

Restriction maps of the BamHI fragments of HSV-1 DNA (upper panel) and HSV-2 DNA (lower panel) which contain the joint (6 kbp) and terminal regions. The two termini are aligned with the joint so that the polarities of the three regions, including the a sequences (rectangles), are equivalent. The remainder of the genome is indicated by a dotted line. The SstI map is shown above the genome, and the SmaI map below. X_L and X_S denote SstI and SmaI terminal fragments of the genome.

The presence of two tandem directly repeated a sequences at the HSV-1 joint, as shown at the bottom of the upper panel, introduces a second SmaI G fragment, and a fragment P' of 43 bp which is the same size at SmaI P. The presence of two tandem directly repeated a sequences at the HSV-2 joint, as shown at the bottom of the lower panel, introduces a second SmaI I2 fragment, and a fragment J of 240 bp.

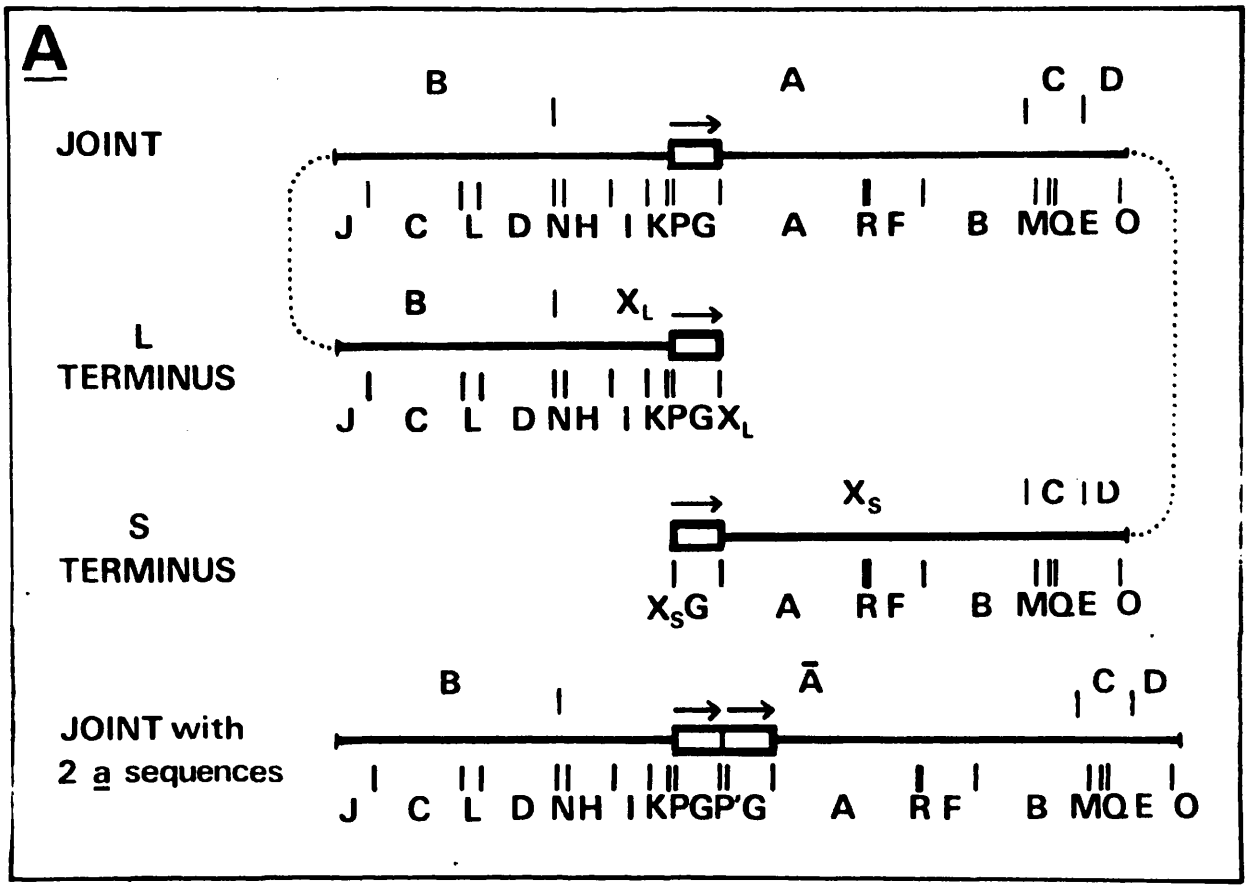

A
JOINT
L TERMINUS
S TERMINUS
JOINT with 2 a sequences
B
A
C D
J C L D NH I KPG A RF B MQE O
X_L
X_S
Ā
P'G
B
4 3 7 2 8 1 5 6
3 11 6 7 4 9 2 10 12 5 8
X_L
X_S
1 5 6
X_S 12 5
2̄
10 J 5
12 12

Figure C3.14

LEFT HAND TWO PANELS

Autoradiograph of 8% polyacrylamide sequencing gels showing reiteration I of HSV-1 strains 17 and USA-8.

From left to right the fragment sequence shown in each group is as follows.

1. Strain 17 (plasmid k1) from AvaI 370 to AvaII 670.
2. Strain 17 (plasmid k1) from AvaII 670 to BstNI 130.
3. Strain 17 (plasmid k2) from AvaII 670 to BstNI 130.
4. Strain USA-8 from AvaII 670 to AvaI 370.

The reiterated sequence is shown in each case, and their copy number in plasmids k1 and k2.

RIGHT HAND TWO PANELS

Autoradiograph of 6% polyacrylamide sequencing gels showing reiteration II of HSV-1 strain 17.

From left to right the fragment sequence in each group is as follows.

1. Strain 17 (plasmid k1) from SstII 710 to Sau3AI 1130.
2. Strain 17 (plasmid k2) from AvaII 670 to Sau3AI 1130.

Copy numbers of the two repeated units, which differ by a single nucleotide, are shown by letters and numbers. The main reiterated sequence is noted.

The tracks are in groups of five as follows.

G - cleaved at G residues
A - cleaved at G and A residues
AC - cleaved at A and, to a lesser extent, C residues
T - cleaved at C and T residues
C - cleaved at C residues.

The restriction sites and nucleotide positions (to the nearest 10 bp) referred to are shown in Figure C3.9.

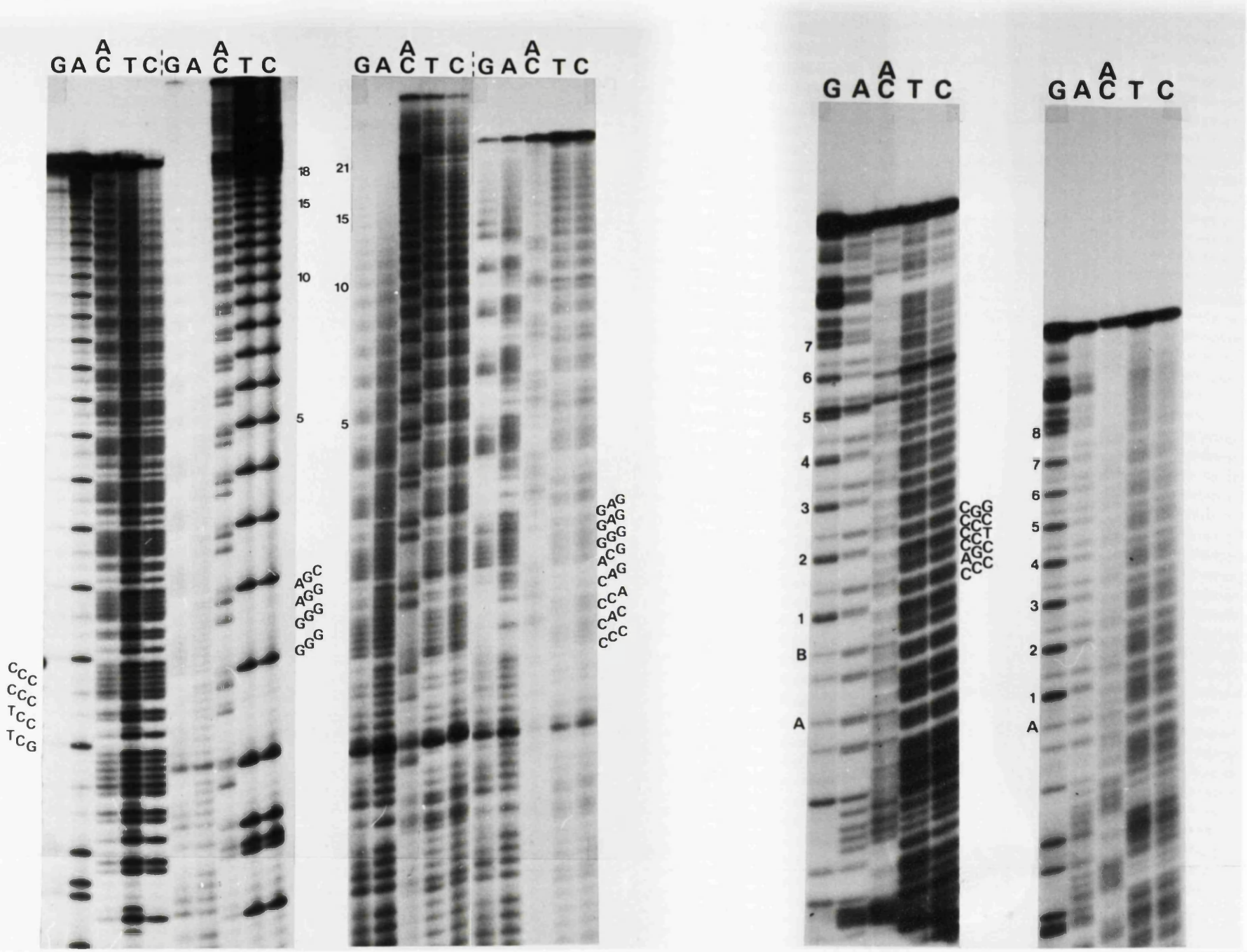

G A C T C G A C T C
18
15
10
5
21
15
10
5
G A C T C G A C T C
G A C T C
G A C T C
7
6
5
4
3
2
1
B
A
8
7
6
5
4
3
2
1
A

Figure C3.15

LEFT HAND TWO PANELS

Autoradiograph of 8% polyacrylamide sequencing gels showing reiteration III of HSV-1 strain 17.

From left to right the fragment sequence in each group is as follows.

1. Strain 17 (plasmid k1) from TaqI 1500 to Sau3AI 1130.
2. Strain 17 (plasmid k1) from Sau3AI 1130 to TaqI 1500.
3. Strain 17 (plasmid k2) from Sau3AI 1130 to TaqI 1500.

The reiterated sequence is noted, and the copy numbers in plasmids k1 and k2.

RIGHT HAND THREE PANELS

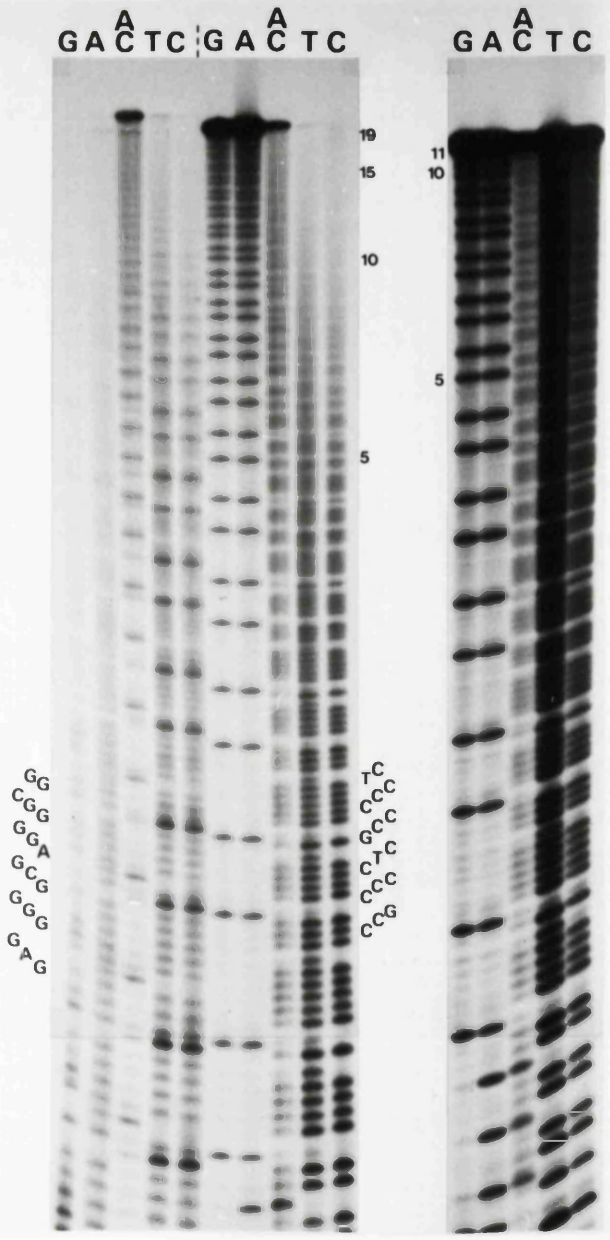
Autoradiograph of 8% polyacrylamide sequencing gels showing the direct repeats at the a-c and a-a junctions of HSV-1 strain 17 and at the c-a junction of HSV-1 strain USA-8.

From left to right the fragment sequence in each group is as follows.

1. Strain 17 (plasmid k1) from AvaII 670 to Sau3AI 1130.
2. Strain 17 (plasmid k2) from AvaII 670 to MnlI 420. This plasmid has two a sequences, so the nucleotide sequence proceeds from the AvaII site in one a sequence, across the junction between the two a sequences, to the MnlI site in the next.
3. Strain USA-8 from HinfI 770 to HaeII 160.

The repeated sequence is noted; that in (3) is from the complementary strand of the duplex to that shown in Figure C3.11, from right to left. The locations of MnlI sites were deduced from Figure C3.11. Strain USA-8 has an additional HinfI site at 770 compared with strain 17.

Track designation is the same as for Figure C3.14. The restriction sites and nucleotide positions (to the nearest 10 bp) referred to are shown in Figure C3.9.


G A C T C G A C T C
G A C T C
19
15
10
5
11
10
5

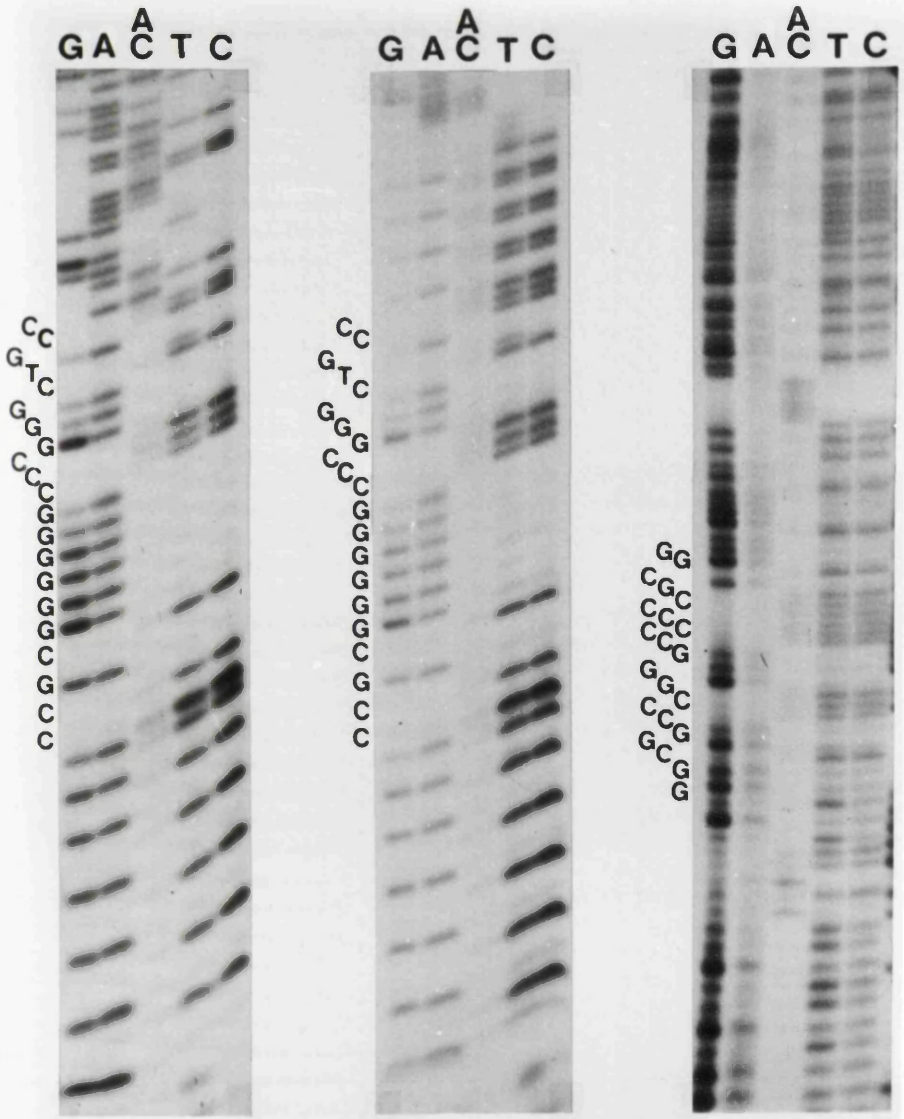

G A C T C
G A C T C
G A C T C

Figure C3.16

Location of the 3' terminus of HSV-1 V_{mw} IE 175 mRNA with respect to the DNA sequence.

The TaqI D fragment consisting of nucleotides 1498-1608 was labelled at the 3' termini and separate strands were isolated. The strand complementary to V_{mw} IE 175 mRNA (that shown in Figure C3.11) was hybridised to RNA as described in the text, treated with S1 nuclease and electrophoresed alongside the DNA sequence of the same fragment (3'-5' reading from the bottom) on an 8% polyacrylamide (8 M urea) gel. The tracks are as follows.

1 - untreated single stranded DNA fragment
2 - annealed with 10 μg yeast RNA (control)
3 - annealed with 5 μg HSV-1 polyadenylated IE mRNA
4 - annealed with 10 μg HSV-1 polyadenylated IE mRNA
5 - annealed with 15 μg HSV-1 polyadenylated IE mRNA
6 - annealed with isolated 4.7 kb IE mRNA which codes for V_{mw} IE 175.

Tracks G, A, T and C indicate the DNA sequencing reactions as designated in Figure C3.14.

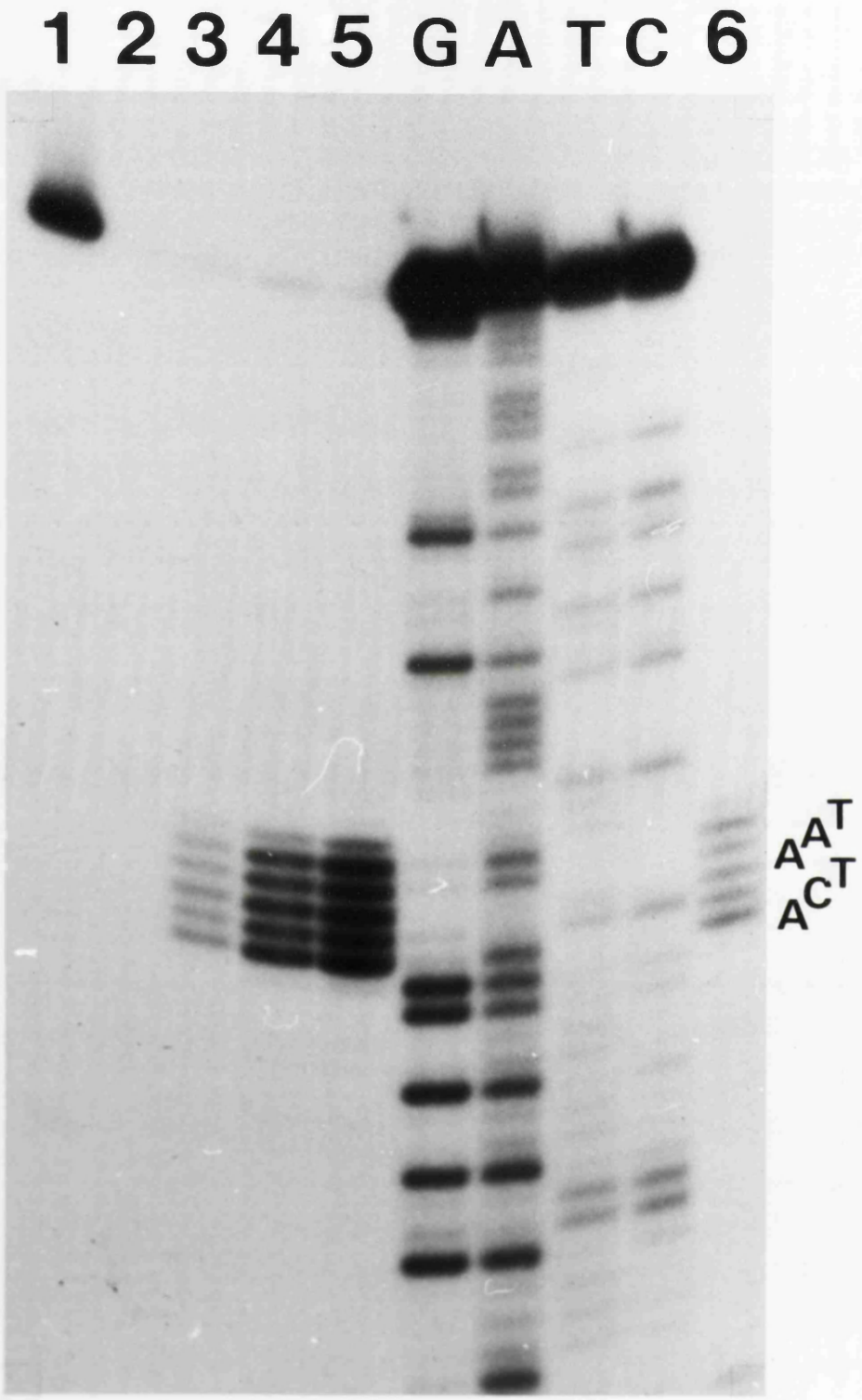
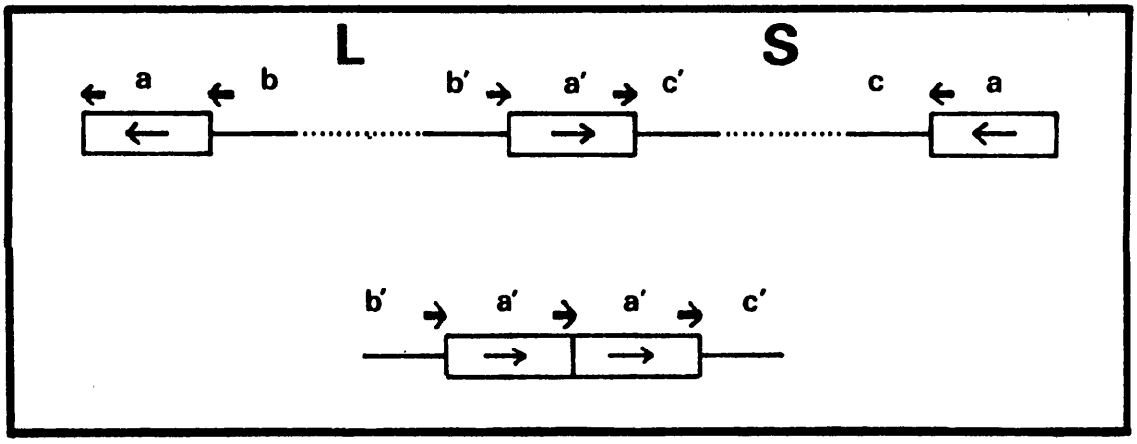

1 2 3 4 5 G A T C 6
AAT
ACT

Figure C3.17

Structural arrangements at the joint and termini of the HSV genome (above), and at joints containing two a sequences (below). The a sequences are shown as rectangles and their orientation and the locations of the direct repeats at the b–a and a–c junctions are indicated.


L
S
a
b
b′
a′
c′
c
a
b′
a′
a′
c′

SECTION 4

INVERSION OF THE TWO SEGMENTS OF THE HSV GENOME

RESULTS

Introduction

Both orientations of the L and S segments of HSV-1 and HSV-2 virion DNA are present in equal amounts, giving rise to four genome arrangements. This is also a feature of HSV-1/HSV-2 intertypic recombinants. The mechanism by which segment inversion occurs is unknown. Virion DNA of the recombinant Bxl(28), a result of genetic recombination between HSV-1 tsB and HSV-2 ts1, was shown by Preston et al. (1978) to be atypical in possessing L predominantly in one orientation, whereas S was present equally in both orientations (Figure C4.3). This phenotype was defined as "fixed", normal inversion being termed "free". It must be stressed that in fixed recombinants the majority, but not the entirety, of virion DNA molecules possess the appropriate segment in the major orientation.

Preston et al. (1978) concluded from gross restriction analysis that Bxl(28) possesses HSV-2 sequence in TR_L and HSV-1 sequence in IR_L. One possibility, therefore, is that a region of type-specific homology between TR_L and IR_L is necessary for normal inversion of L. However, other recombinants also seemed heterotypic for TR_L/IR_L and yet inverted normally. It is not possible by gross restriction analysis to distinguish between HSV-1 and HSV-2 DNA sequences in regions closer to the joint or termini than 4000 bp, so in order to locate previously undetected crossovers the joint and terminal regions were analysed in detail for Bxl(28), for Bxl(28) subclones, and for a number of other recombinants.

The recombinant RE4 was generated by marker rescue of HSV-1 tsE DNA by HSV-2 DNA fragments (Wilkie et al., 1979b). RE4 was analysed in detail in the work described here and shown to be fixed in L and S, allowing inversion of both

segments to be investigated.

The genes coding for V_{mw} IE 110 (HSV-1) and its counterpart in HSV-2, V_{mw} IE 118, map entirely within TR_L and IR_L. Preston et al. (1978) showed that recombinants which are heterotypic for this diploid gene induced both IE 110 and IE 118. Two subclones of Bxl(28) are shown here to be deleted in TR_L or IR_L, and these allowed investigation of the expression of this gene by genomes with only one full copy.

In the following descriptions it is apparent that the interpretation of detailed restriction analyses of recombinant genome structures is very complex. Therefore, although a significant proportion of data has been included in the Figures, its interpretation has been limited to a few examples. Emphases are placed on structures critical to the conclusions drawn, on demonstrating the limits of the analysis, and on showing the structural subtleties of which HSV recombinant genomes are capable.

Structures of the joints and termini of Bxl(28-1)

The working stock used in these experiments was named Bxl(28-1), the term Bxl(28) being retained for the putative original recombinant as described below. The DNAs of tsB, tsl and Bxl(28-1) were ^{32}P-labelled by nick translation and cleaved with SstI or SmaI and the products separated by electrophoresis on 2% agarose, 7.5% polyacrylamide or 15% polyacrylamide gels. The resulting profiles were analysed using the relevant restriction maps of these regions of the genome (Figure C3.3). Cleavage products of termini which do not correspond to fragments from the joint region, and are therefore the terminal SstI or SmaI fragments of the genome, are referred to as X (see Figure C3.13). It was sometimes necessary to cleave comigrating fragments (e.g. HSV-2 BamHI vw), and in these

instances attention is drawn only to cleavage products from joint or terminal regions. Faint bands arising from contaminating sequences, a feature of the use of nick translated DNA, are also not discussed.

Figure C4.1 panel A shows the SstI cleavage profiles of Bxl(28-1) joint fragments. Track 2 shows the major joint fragment, which is type 1, and also HSV-2 SstI fragments from the b and c sequences in lesser abundance. The larger joint fragment (track 3) has bands characteristic of both the HSV-1 and HSV-2 joint regions, and probably comprises mainly the type 1 joint with an extra a sequence (HSV-1 SstI A is larger) and type 1 IR_L joined to type 2 IR_S (the novel joint fragment possibly migrated above HSV-2 SstI 1, perhaps with two a sequences). Two rarer forms may also be present: one with type 2 IR_L joined to type 1 IR_S, and the other with HSV-2 DNA throughout the joint. These results show that the major joint species is type 1 throughout, but that other species due to inversion are also present. Panel B track 2 shows a SmaI digest of the major joint of Bxl(28-1), in which again the major bands are type 1. HSV-1 SmaI G, being a fragment variable in size, is smaller in this recombinant that in tsB. Track 3 of panel C and track 3 of panel D show that the major L terminus is type 2, including the a sequence. The HSV-1 minor L terminus is barely detectable in panel C track 4, since L is fixed.

Preston et al. (1978) demonstrated that the HSV-2 KpnI site in TR_S/IR_S was to some extent segregated between both TR_S and IR_S in Bxl(28-1), and so the two S termini were isolated separately. The unique EcoRI S terminus of Bxl(28-1), conventionally the right hand side of S, contains HSV-1 and

HSV-2 SstI fragments consistent with it being a mixture of the normal HSV-2 S terminal EcoRI fragment and a fragment of similar size with a crossover in TR_S (Figure C4.2 panel A; summarised in Figure C4.18). Small size differences between some fragments from parental and recombinant genomes (e.g. HSV-2 SstI 1) are probably the result of size variability in these regions. The SstI profile of the BglII S terminus, conventionally the left hand side of S, is consistent with it being a mixture of the normal HSV-2 S terminal BglII fragment and a fragment with a crossover in TR_S in an equivalent location to that described above (Figure C4.2 panel C). Panels B and D of Figure C4.2 demonstrate the presence of the KpnI site in HSV-2 TR_S/IR_S in at least a proportion of both S terminal fragments of Bxl(28-1). SmaI digestion of the BamHI S terminal fragments of Bxl(28-1) (Figure C4.2 panel E) shows the presence of an HSV-1 a sequence at the type 1 terminus and an HSV-2 a sequence at the type 2 terminus, deduced in this case from the presence of HSV-2 SmaI 5.

These results confirm that Bxl(28-1) is fixed in L and show that, in the major orientation of L, TR_L and IR_L, including the a sequences, are heterotypic. S consists of a mixture of at least two structures, the crossover in one of which is probably most accurately located within HSV-1 BamHI m', because subclones of Bxl(28-1) which possess HSV-1 BamHI q lack this fragment (Figure C4.11 panel C). A summary of the structures present in Bxl(28-1), and that of the putative original recombinant Bxl(28), are illustrated in Figure C4.18.

Gross structures of Bxl(28-1) subclones

Twenty subclones of Bxl(28-1) were isolated by Mrs M. Murphy by three cycles of plaque purification at 31°,

and in vivo ^{32}P-labelled DNA was analysed using XbaI, HindIII, EcoRI, BglII, HpaI, KpnI and BamHI. Results for several of the subclones with four endonucleases are shown in Figures C4.4 and C4.5. Genome structures deduced from this gross analysis are shown in Figure C4.6.

More than half of the subclones are free in L, and all are free in S. Those subclones fixed in L are readily identified, as is Bxl(28-1), by the overabundance of the two joint fragments HSV-2 EcoRI c and e over the other two, b and d, and by the excess of the L terminal fragment HSV-2 HpaI g over HSV-1 HpaI m (Figure C4.4). The original crossover in U_L is conserved in all subclones, and all except perhaps subclone 27 have symmetric S segments: that is, either S consists entirely of HSV-2 DNA sequence, or it has HSV-1 DNA at both S termini with crossovers within the two HSV-1 BamHI m' fragments (Figure C4.11 panel C). The data suggest that two subclones are deleted in TR_L/IR_L: subclone 14 with 3 x 10^6 daltons deleted in type 1 IR_L, and subclone 22 with 5 x 10^6 daltons deleted in type 2 TR_L. Restriction profiles of these two subclones show novel bands interpreted as joint and terminal fragments resulting from the deletions. Only subclone 27 has identical restriction patterns to Bxl(28-1).

The analysis also suggests that some free subclones such as 10, 11 and perhaps 9 have additional crossovers in TR_L or IR_L. Therefore the joint and terminal fragments of certain subclones were analysed in detail by cleavage with SstI and SmaI.

Structures of the joints and termini of Bxl(28-1) subclones

Figures C4.7 and C4.8 show data for joint regions, Figures C4.9 and C4.10 for L termini, and Figures C4.11 and C4.12 for S termini. To a large extent only the major genome

forms were analysed in these experiments.

The major joint of subclone 1, which is fixed in L, is type 1 throughout (Figure C4.7 panel A track 3, panel C track 3, Figure C4.8 panel A track 2), including the a sequence from which HSV-1 SmaI G comigrated with SmaI I. The major L terminus is type 2, including the a sequence (Figure C4.9 panel A track 2, Figure C4.10 panel A track 6). The minor type 1 L terminus is barely detectable in the SstI profile (Figure C4.9 panel A track 3), as expected since L is fixed. The S terminus is type 1 (Figure C4.11 panel A track 3, Figure C4.12 panel A track 5). Therefore subclone is heterotypic for TR_L and IR_L, including the a sequences, whereas TR_S and IR_S are identical.

Subclone 5 is also fixed in L and similar in structure to subclone 1 except that the crossovers in TR_S/IR_S are in HSV-1 BamHI q SmaI F rather than in HSV-1 BamHI m' (e.g. Figure C4.7 panel A track 4, Figure C4.12 panel A track 6).

Subclone 9 is free in L and has a more complex structure. HSV-2 BamHI v, the normal type 2 L terminus, was not detected in the gross analysis (Figure C4.5), suggesting that both L termini are type 1 as indicated in Figure C4.6. Indeed, this was supported by the finding that the major joint of normal size is type 1 throughout (Figure C4.7 panel A track 5, panel C track 4, Figure C4.8 panel A track 3), and that the L terminus which comigrated with HSV-1 BamHI s is type 1 (Figure C4.9 panel A track 6, Figure C4.10 panel A track 2). It is noted that HSV-1 SmaI P is a few bp smaller in this subclone than in tsB. Bands representative of the type 2 L terminus were detected in the SstI digest of the S terminus (Figure C4.11 panel A track 5). Therefore, although the normal type 2 L terminus is not present, this sequence is

represented in a fragment which migrated at a position 0.3×10^6 daltons larger. In order to clarify these results, the L termini were isolated separately via the unique HpaI fragments and cleaved with SstI (Figure C4.9 panel B tracks 3 and 4). The BamHI L terminus from HSV-1 HpaI m is type 1 as expected, but that from HSV-2 HpaI g consists of a mixture of two species. The first is a normal type 1 BamHI s fragment, and the second is a type 2 terminus with an additional 0.3×10^6 daltons of DNA at the terminus. Indeed, HSV-2 HpaI g of subclone 9 consists of a fragment of normal size and one a little larger (Figure C4.5). The precise nature of the larger fragment was not determined, but it seems from SmaI analysis not to terminate in a type 2 a sequence. The most likely explanation of these results is that subclone 9 consists of a mixture of two structures differing at one L terminus, as indicated in Figure C4.17. The type 1 a sequence is a likely candidate for the additional DNA at the type 2 terminus of subclone 9b, but this was not proven. The actual explanation may be more complex, but it is clear that further recombination has occurred at the L termini of the subclone 9 genome, generating regions of type-specific homology between TR_L and IR_L.

Subclone 29 has two types of L termini. One is the normal type 2 terminus and the other contains HSV-1 SstI B but a smaller terminal fragment SstI X (Figure C4.9 panel A tracks 7-9, Figure C4.10 panel A track 3). This is consistent with the observation of HSV-2 BamHI v and a slightly smaller fragment in the gross BamHI profile (Figure C4.5). Figure C4.10 panel A track 4 shows that the smaller terminus consists of HSV-1 DNA except that the HSV-1 a sequence has been replaced by an HSV-2 a sequence (see also Figure C4.9 panel B tracks 5-6 and Figure C4.13 panel D tracks 3-4). The smaller

size is explained by the fact that the HSV-2 a sequence is approximately 150 bp smaller than that of HSV-1 (see Section 3). The crossover is located in HSV-1 SmaI P, which spans the b-a junction, generating a new fragment of 28±2 bp as shown in Figure C4.18. Both the normal type 2 terminus and the novel one are reflected in profiles of joint fragments (Figure C4.7 panel A track 6, panel C track 5, Figure C4.8 panel B tracks 2-3). The S terminus is type 2 (Figure C4.11 panel A track 6, Figure C4.12 panel A track 8). Subclone 29, which is free in L, has type-specific homology between TR_L and IR_L which is confined essentially to the type 2 a sequences.

Figure C4.8 panel C shows that the joint region of subclone 14 has HSV-1 a and c sequences, but fragments totalling only 600 bp of HSV-1 HpaI m adjacent to the HpaI site in the b sequence. Intervening b sequence fragments are absent. These results confirm that 3×10^6 daltons of DNA have been deleted from IR_L adjacent to the a sequence. The predominant L terminus is type 2 (Figure C4.9 panel C track 3, Figure C4.10 panel B track 4), and the S terminus is type 1 (Figure C4.11 panel B track 4, Figure C4.12 panel B track 4).

Subclone 22 has a HpaI L terminus of approximately 1.4×10^6 daltons (2400 bp), which is about 5×10^6 daltons smaller than the equivalent fragment in tsl. Cleavage of this fragment with SmaI produced four detected fragments of 1000, 850, 300 and 55 bp. The order of these fragments from the L terminus is 300-1000-55-850 bp, and was deduced as follows. The 300 bp fragment corresponds to HSV-1 SmaI G from the normal HSV-1 L terminus of subclone 22 (Figure C4.10 panel C); therefore the novel terminus has a type 1 and not a type 2 a sequence. The 850 bp fragment is similar in size to the only

SmaI fragment from HSV-2 HpaI g which was not produced from HSV-2 HpaI f (Figure C4.10 panel D tracks 5-6), and therefore spans the TR_L-U_L junction. It is not exactly the same size because TR_L-U_L junction fragments are somewhat variable in size in intertypic recombinants. The 55 bp fragment is common to HSV-1 HpaI f and g and therefore is contained within TR_L/IR_L. The 1000 bp fragment must span the deleted region. It is concluded that subclone 22 contains no more than 1.2×10^6 daltons of HSV-2 TR_L, the deletion extending over approximately 5×10^6 daltons within TR_L. Hybridisation data shown in Figure C4.12 confirmed the identity of novel joint and terminal fragments in restriction profiles of subclones 14 and 22, and demonstrated their freedom from contamination by non-deleted genomes.

Structures of the joints and termini of other recombinants

Previously determined genome structures are shown in Figure C4.3 of several recombinants which apparently are heterotypic for all or part of TR_L and IR_L or TR_S and IR_S, and which yet invert normally. These were analysed for undetected crossovers.

The BamHI profile of Bxl(31-2) is shown in Figure C4.14. The joint fragment is larger than those of the parental viruses, and when cleaved with SstI produced HSV-2 IR_L and HSV-1 IR_S fragments (Figure C4.13 panel A track 2). The type 2 L terminus corresponding to this joint fragment is 0.3×10^6 daltons larger than HSV-2 BamHI v, and did not produce the usual type 2 terminal SmaI X fragment (Figure C4.13 panel A track 3, panel B track 3). The other L terminus (track 5) is type 1, with a larger SmaI G fragment than tsB which is also present at the type 2 L terminus and at the joint (panel B tracks 2,3,5). These results suggest that one

L terminus of Bxl(31-2) is type 1, and that the other is type 2 with the addition of a type 1 a sequence at the terminus adjacent to a type 2 a sequence. Isolation of the two L termini separately and digestion with SstI showed a normal HSV-1 L terminus (Figure C4.13 panel C track 4), and a fragment containing, among contaminating fragments, HSV-2 SstI 3 and 4 but a larger SstI terminal fragment (track 3). Isolation of the two SstI L terminal fragments and digestion with SmaI showed the normal HSV-1 terminus and one consisting of the HSV-2 terminus with the addition of an HSV-1 a sequence adjacent to the HSV-2 a sequence at the terminus (Figure C4.13 panel D tracks 3-4). The joint fragment corresponding to the normal type 1 L terminus was not analysed, so it is shown with a single a sequence in Figure C4.17 although it probably contains more than one, in view of the absence of HSV-1 BamHI k of normal size in Figure C4.14 panel A. It is concluded from these results that Bxl(31-2), which inverts normally, has type-specific homology between HSV-1 a sequences at the joint and L terminus.

Bx6(17-6), RS6 and R13-2 were analysed similarly, and data are shown in Figures C4.14 and C4.15. The restriction site between HSV-2 SstI 3 and 4 is absent in Bx6(17-6), but SstI 7 is present. The crossover in IR_L of R13-2 is located within HSV-1 SmaI N.

Dxl(2) was initially thought to be heterotypic for TR_L/IR_L and TR_S/IR_S. The BamHI joint fragment is 0.3 x 10^6 daltons larger than that of HSV-1 or HSV-2 DNA, and SmaI digestion shows it to consist of HSV-1 IR_L sequences adjacent to HSV-2 IR_S sequences with one intervening a sequence of each type (Figure C4.16 panel A track 3, Figures C4.17 and C4.18). Therefore a sequence homology between joint and termini is

maintained for both L and S. A similar structure with the a sequences reversed in order was identified in a subclone of subclone 2 of Bxl(28-1) (described below; Figure C4.16 panel A track 4), and is also illustrated in Figure C4.18. The basic structure of Dxl(2) shown in Figure C4.17 is further complicated by the presence of a proportion of L termini with an additional type 2 a sequence at the terminus (Figure C4.16 panel C track 3, panel D track 3). This is even further complicated by segregation of the sequences in TR_S/IR_S. For instance in Figure C4.16 panel B HSV-1 SstI C and D and HSV-2 SstI 5 and 6 are present in TR_S and IR_S, which were isolated separately. These features of the L and S termini are also apparent in the data of Figure C4.13 panel D tracks 7-10, but have not been included in Figure C4.17.

Expression of IE 110/118 by Bxl(28-1) subclones

To investigate the expression of IE 110/118 by subclones 14 and 22, the genomes of which are deleted in TR_L or IR_L, infected cell polypeptides were prepared from these, and other recombinants, and subjected to polyacrylamide gel electrophoresis by Dr H.S. Marsden and Mr G. Hope. Immediate early polypeptides were induced in HFL cells (a line of human foetal lung cells established by Dr B. Carritt in the Institute of Genetics, Glasgow) as described by Preston et al. (1978). The recombinants used produced equivalent plaque numbers on both BHK C13 and HFL cells. The method involved infection of cells at high multiplicity and incubation for 5 hr at 37^0 in the presence of cycloheximide. The drug was removed and polypeptides labelled with ^{35}S-methionine in the presence of actinomycin D. Whole

infected cell polypeptides were prepared by lysis *in situ* of the cells (Marsden *et al.*, 1978). Since IE 110 has been shown to partition with the nucleus (Pereira *et al.*, 1977), in some experiments nuclei were prepared by treatment of cells with 0.65% NP40 in ice-cold isotonic buffer, before boiling in denaturing buffer.

Figure C4.20, which is a composite of SDS-polyacrylamide gels from several experiments, shows immediate early polypeptides induced by HSV-1 strain 17, HSV-2 strain HG52 and several recombinants, the genome structures of which are shown in Figure C4.19. Lanes 1-10 show whole infected cell polypeptides, and lanes 11-21 show nuclear polypeptides. Six HSV-1 coded (175, 136'(143), 110, 87, 68, 63) and six HSV-2 coded (182, 138, 134-132, 118, 67, 64) IE polypeptides can be identified in these experiments.

As observed previously (Preston *et al.*, 1978), recombinants Bxl(31-2) and Bxl(28-1), which are heterotypic for the repeats bounding U_L, induced both IE 110 (HSV-1) and the equivalent HSV-2 polypeptide IE 118. Similarly, subclones 1 and 29, which are heterotypic for TR_L and IR_L, induced both IE 110 and IE 118. The latter inverts normally in L and S but subclone 1 is fixed in L, and therefore whether or not the L segment inverts normally seems not to affect expression of the IE 110/118 genes. Subclone 14, which lacks HSV-1 sequence from IR_L (including a region from which the 5' end of the mRNA for IE 110 is transcribed; F.J. Rixon, personal communication), induced only the HSV-2 IE 118. Conversely, subclone 22, which lacks HSV-2 sequence from TR_L (including the whole of the gene for IE 118; A.J. Easton, personal communication), induced only the HSV-1 IE 110. Subclone 11 induced IE 118 and a polypeptide which migrated faster than IE 110. Figure C4.19 shows that a

crossover is located within the DNA sequences coding for the HSV-1 IE 110 mRNA. The altered polypeptide probably arose as a consequence of the crossover position in IR_L. The apparently smaller size may result from non-colinearity of initiation or termination codons for the two polypeptides, a deletion of some coding sequence as a result of the crossover, or from altered processing of the hybrid polypeptide.

Structures of subclones of Bxl(28-1) subclones

Seventeen subclones each were isolated by three cycles of plaque purification at 31° from Bxl(28-1) subclones 2 and 29, in order to determine whether a fixed subclone would give rise to free progeny, and whether a free subclone would give rise to fixed progeny. The gross structures of the progeny subclones were analysed using XbaI, HindIII, EcoRI, BglII, HpaI, KpnI and BamHI, and are shown in Figure C4.21.

Structure of the genome of RE4

The restriction sites in RE4 DNA were analysed in the usual way. HindIII and BamHI digests are shown in Figure C4.22 in tracks labelled R. The results of these digests and others suggested that the RE4 genome is deleted in IR_L and that a sequence from elsewhere has been inserted in place of IR_L. A likely explanation was that the inserted DNA contains restriction sites for HindIII and BamHI, thus generating novel fragments from one L terminus (i.e. the faint band slightly larger than HSV-2 HindIII o, and the band intermediate in size between HSV-1 BamHI t and uv). The fragment slightly smaller than HSV-1 HindIII o and the fragment intermediate in size between HSV-1 BamHI p and r may contain the junction between normal U_L sequence and the inserted sequence (fragment TJ). These fragments could not be explained as

resulting from normal crossovers in the RE4 genome, and neither could other novel fragments thought to be joint fragments resulting from the novel L terminus, such as the fragment which is slightly smaller than HSV-1 BamHI k. The proposed genome structure of RE4 is illustrated in Figure C4.26, and experiments are described below in support of this unusual arrangement.

The results of experiments involving hybridisation of HSV-2 HindIII n and o to nitrocellulose sheets containing HSV-1, HSV-2 and RE4 HindIII or BamHI digests are shown in Figure C4.23. HSV-2 HindIII n hybridised not only as expected to HSV-1 HindIII a present in RE4 DNA, but also to the novel joint and terminal fragments. HSV-2 HindIII o hybridised in each case to fragment TJ, thought to represent the junction between normal and inserted sequences. The isolated RE4 HindIII TJ fragment hybridised to HSV-1 HindIII abcd and BamHI b and f', and to HSV-2 HindIII b and o and BamHI f, i and r. The hybridisation results certainly cannot be explained in terms of legitimate crossovers, because RE4 HindIII TJ contains sequences from HSV-2 HindIII o (HSV-2 BamHI f) and HSV-2 HindIII b (HSV-2 BamHI ir), which are not contiguous in the HSV-2 genome. These experiments support the hypothesis that RE4 DNA is deleted in at least the majority of IR_L, with the insertion of an HSV-2 sequence from U_L as shown in Figure C4.26.

To locate more precisely the junction between normal and inserted DNA the BamHI and BamHI/HindIII TJ fragments were isolated and cleaved with a number of restriction endonucleases (Figure C4.24). The results show that HindIII TJ contains almost all of HSV-2 HindIII o, with the exception of not more than 25 bp at the right hand end (see HhaI digests). Results with HhaI and AluI show that not more than 260 bp of HSV-2

BamHI i are adjacent to HSV-2 HindIII o sequences. The results of this experiment have been included in Figure C4.26.

Cleavage of the RE4 BamHI novel joint fragment demonstrates the presence of HSV-2 IR_S fragments but the absence of IR_L fragments of either HSV-1 or HSV-2 (Figure C4.25 panel A track 2, panel C track 2, panel D track 2). Instead, SmaI fragments characteristic of HSV-2 BamHI r were produced (panel C track 2). HSV-2 SmaI 5 and 12 are present, suggesting a type 2 a sequence at the joint; the absence of HSV-2 SmaI 10 shows that not more than approximately 45 bp of the HSV-2 b sequence are adjacent to the a sequence. These results and those from the novel L terminus (panel C tracks 5-6) show that the novel L terminus comprises inserted HSV-2 sequence with a terminal type 2 a sequence, and that the novel joint comprises the inserted sequence joined to the HSV-2 c sequence via a type 2 a sequence. The two L termini of RE4 are heterotypic, including the a sequence (compare novel terminus with panel E track 1). This is true also of the S termini, the type 1 terminus being shown in panel F track 2. A further complexity in the genome structure of RE4 is that the BamHI type 1 S terminus, isolated from unique fragments, also contains type 2 S terminal fragments (panel B tracks 2-3). This suggests that, like Bxl(28-1), at least the type 1 S terminus of RE4 DNA consists of a mixture of structures presumably generated by secondary recombination involving the repetitive regions.

Restriction profiles of RE4 and the recleavage experiments described above suggest that the RE4 genome is fixed in L and S in the I_S arrangement (Figure C4.26). For example, autodensitometric scanning of Figure C4.22 tracks R revealed that HSV-1 HindIII m, containing the type 1 S terminus, is in

three- to four-fold excess over HSV-1 HindIII g, which contains the type 2 S terminus. Two discernible joint fragments, the second and third bands in the HindIII profile, were under-represented. The type 1 L terminus HSV-1 BamHI s was predominant over the faint novel terminus which migrated below HSV-1 BamHI t, and the type 1 S terminus HSV-1 BamHI q was predominant over the faint type 2 S terminus which migrated below it. The normal type 1 joint HSV-1 BamHI k was faint compared with the novel joint of faster mobility.

The RE4 genome lacks 10.5 kbp of DNA including IR_L and at least 300 bp of adjacent U_L sequence, and has an insertion of 2.8 kbp of HSV-2 DNA at this location from the BamHI i-r (HindIII b-n) region. Moreover there is complete type-specific heterology between IR_S and TR_S and between TR_L and the joint region, and the genome is fixed in L and S in the I_S arrangement.

Seventeen subclones of RE4 were isolated by three cycles of plaque-purification at 31°, and their genome structures analysed. HindIII and BamHI profiles of several subclones are shown in Figure C4.22. All subclones retain the deletion and insertion, and the crossovers in U_L and U_S present in RE4. Eight of the subclones had restriction profiles identical to that of RE4, but some more clearly showed the fixed nature of L and S (e.g. subclone 6), implying that RE4 is not a pure structure. The other nine subclones were plaque purified twice more because several seemed still to comprise mixtures of genome structures after the first three plaque purifications. The profiles of most of these subclones are included in Figure C4.22. They are free in S, with the possible exception of subclone 3, and most are free also in L with the exceptions of 17 and perhaps 3, 4 and 5. The distinction between fixed and free, however, is not clear in every case and it is likely that

some subclones may not be pure even after five rounds of plaque purification. The nine subclones have secondary crossovers in repetitive regions which were not analysed further. For example, subclones 1, 5, 7, 15, 16 and 17 each have a crossover in HSV-2 BamHI g', resulting in a smaller fragment, which suggests that both S termini in these subclones are type 1. Subclone 1 possibly has a crossover in type 1 TR_L, since it lacks HSV-1 BamHI s and instead has a terminal fragment larger than HSV-1 BamHI o, as well as two other fragments resulting from the larger L terminus. Subclones 7, 12, 15 and 16 have a BamHI joint fragment 0.3 x 10^6 larger in m.wt. than that of RE4, probably resulting from an extra a sequence in the joint fragment containing inserted DNA. It is concluded from these initial studies that in RE4, as in Bxl(28-1), the conversion of the fixed phenotype to the free occurs by additional recombination events in repetitive regions which generate type-specific homology between these regions. Detailed restriction analysis of the genomes of RE4 subclones is necessary to more clearly define the secondary recombination events.

DISCUSSION

Fixed and free recombinants

Bxl(28-1) contains a mixture of at least two genome structures differing in TR_S/IR_S. These structures, and the fact that the recombinant was initially isolated by six cycles of plaque purification, suggest that the original recombination event produced the recombinant genome structure shown in Figure C4.18. Further intermolecular recombination probably then produced the structures present in Bxl(28-1). Intra- or inter-molecular recombination events could then have given rise to the subclones. The structures present in subclone 27 presumably arose by survival of the initial recombinant Bxl(28) during plaque purification, in similar fashion to the isolation of Bxl(28-1).

The subclones of Bxl(28-1) fall into two classes, those which are fixed in L and those in which L inverts normally. Each fixed subclone had a fourfold excess of one orientation of L over the other, and a similar ratio was observed for L and S in RE4. It is important to note that in no case was inversion of a segment entirely absent, and it seems from the analysis that residual inversion is a property of the fixed genome rather than a result of the presence of contaminants with secondary crossovers. The fixed phenotype may represent either preferential replication or preferential packaging into virions of DNA with one orientation of L. Analysis of nuclear DNA did not support the latter (data not shown). Alternatively, fixed recombinants could be the result of a biased mechanism for maturation of unit length DNA from concatemers.

The structures of Bxl(28-1) subclones and other recombinants show that normal inversion of L depends on a region of type-specific homology between TR_L and IR_L. All

normally inverting subclones possess secondary crossovers in TR_L or IR_L which give rise to such homology. Subclone 29, Bxl(31-2) and perhaps subclone 9b show that homology between the a sequences is sufficient to allow normal inversion. It has not been demonstrated, however, that a sequence homology is necessary for normal inversion, as a recombinant with homologous regions of TR_L and IR_L but heterologous a sequences was not observed. Analysis of subclones of the subclones supports the hypothesis of homology requirement: a fixed form (heterologous) gave rise to some normally inverting progeny (homologous), but the reverse conversion was not observed. This is explicable in that crossovers in the fixed form can generate homology between TR_L and IR_L, and these were indeed detected, whereas a normally inverting form cannot generate heterology since it does not contain the necessary sequences.

RE4, its unusual genome structure aside, is analogous to Bxl(28-1) because it is fixed in L and S in the I_S orientation. Initial studies of the structures of RE4 and its subclones suggest that the homology requirement applies to both L and S, since normally inverting progeny possess secondary crossovers in TR_S/IR_S. The structures of recombinants with two adjacent heterotypic a sequences at the joint indicate either that the a sequence is crucial in segment inversion or that such an arrangement is fortuitously maintained. It is interesting to note that the L terminus of Dxl(2) had a single type 1 a sequence in about half the molecules and an additional type 2 a sequence in the other half (only the former is shown in Figure C4.17).

It is proposed from these results that inversion of the L and S segments of the HSV genome is caused by a site-specific recombination event in, or near, the a sequence. This

hypothesis has been strengthened by the observation that insertion of a 4.2 kbp fragment containing the HSV-1 a sequence into the HSV-1 dPyK gene caused additional inversions about the novel joint (Mocarski et al., 1980; J. Smiley, personal communication). Insertion of a fragment of similar size from elsewhere in the genome caused no additional inversions (Mocarski et al., 1980).

Models for segment inversion

Models previously proposed for the mechanism of inversion involve inter- or intra-typic recombination, and are illustrated in Figure C4.27. Model 1, which proceeds via physical separation of L and S and their subsequent rejoining to give the four arrangements, has not been supported by electron microscopic or genetic studies (Jacob et al., 1979), although the rejoining would be specific for the a sequence. Similarly model 2, which proposes that inversion occurs by intermolecular recombination, could involve a site-specific event, but has not the support of genetic studies (Honess et al., 1980). In model 3A inversion occurs by DNA synthesis with concurrent intramolecular recombination, but does not fit in with the currently accepted model for DNA replication. Intramolecular recombination between inverted repeats in linear or circular form (model 3B) was originally proposed as a non-specific event, but in the light of the above results this is unlikely to be the case. However, the model can equally well accommodate site-specific recombination at the a sequences.

Model 3C is explained in the legend to Figure C4.27. It has the strengths that it accommodates the current scheme for DNA replication, and that inversion can be interpreted in terms of a site-specific event at the a sequence. One

prediction of the model is that all the a sequences, and perhaps adjacent regions of the b and c sequences, are obligatorily identical within a replicated DNA molecule. This feature arose because of the rather overrated hypothesis that not all four genome arrangements participate in recombination or replication, and therefore that generation of the four arrangements must involve an obligatory event. This hypothesis is discussed in Section 2. Ben-Porat et al. (1980) have recently shown that both arrangements of the PRV genome enter the nucleus, circularise, replicate, invert and are matured. Knipe et al. (1978) attempted to make a case for obligatory identity from the observation that intertypic recombinants possess homotypic sequences in the a, and perhaps adjacent regions of the c, sequences bounding U_S. This feature was common in the recombinants described in Section 2 and in this Section. A less assumptive conclusion is that the tendency for identity of these regions is the result of facile recombination within repeated regions, rather than the result of an obligatory event in DNA synthesis. A second piece of evidence stems from the claimed mapping of an HSV-1 ts mutation (tsc75) in each a sequence. Parenthetically, this does not dictate that a sequences must be obligatorily identical, since the initial mutation in one a sequence could have been transferred to the other a sequences by facile recombination at an early stage. Regardless of this, Preston (in press) has shown that this lesion maps within the V_{mw} IE 175 gene some 2 kbp away from the a sequence in the c sequence. There is therefore no conclusive evidence for obligatory identity of the a sequences. The observation that the size distribution pattern of the HSV-1 a sequences is the same at the joint and both termini (Section 3 of Results) could again be explained by facultative identity of

these regions. It is clear from the work presented here that some recombinants are able to replicate without a requirement for obligatory identity of a sequences (e.g. subclone 1 of Bxl(28-1) and RE4). Therefore either this is not the mechanism of inversion, or these recombinants replicate by an alternative mechanism. The number of modes of HSV DNA synthesis is unknown.

The stage at which inversion occurs during virus growth has not been identified, although isomerisation proceeds to equilibrium at an early stage in plaque formation (Ben-Porat et al., 1980). The event could take place essentially before the production of concatemers, or could be post-replicative as in model 3C. It is possible that inversion is not only a result of recombination, but also of the mode of cleaving unit-length molecules from concatemers. If HSV concatemers are produced from a circular intermediate, their structure in the joint regions would be determined by the structure of the joint regions of the circular molecule. Therefore the mechanism by which linear input DNA circularises would have a direct effect upon concatemer joint structure.

Mechanisms for genome circularisation and maturation

There is strong evidence from electron microscopy that HSV and PRV DNA replication involves concatemeric, and perhaps circular, intermediates. Jean and Ben-Porat (1976) observed linear PRV DNA molecules with single stranded termini and unit-length circles in DNA from infected cell nuclei in the absence of de novo protein synthesis, and proposed that exposure of complementary single stranded termini by an exonuclease precedes circularisation. Despite a strong belief in the terminal redundancy of PRV DNA, Ben-Porat et al. (1979) were unable to demonstrate a terminal repetition by exonuclease treatment and

annealing *in vitro*. Nevertheless, it is probable that PRV DNA is circular at least during the initial stages of DNA replication (Ben-Porat and Veach, 1980). On the other hand, although HSV-1 DNA is without doubt terminally redundant, it has not been shown that circularisation *in vivo* occurs by annealing of exposed single stranded termini. The majority of Bx1(28-1-1) DNA has a type 1 *a* sequence at the S terminus and a type 2 *a* sequence at the L terminus, and would be unlikely to circularise by this mechanism since the HSV-1 and HSV-2 *a* sequences are not complementary (Section 3 of Results). Therefore, if replication proceeds via a circular intermediate, either circularisation occurs by an alternative mechanism, such as blunt-end ligation, or Bx1(28-1-1) DNA contains a minor population of molecules with homotypic termini (e.g. an extra type 1 *a* sequence at the type 2 L terminus) which alone replicate to produce for unknown reasons a large proportion of non-replicating progeny with heterotypic termini (i.e. the major form of Bx1(28-1-1) genome). The biological properties of this recombinant have not been elucidated, and only the major genome structure was analysed, so it is not at present possible to distinguish between these alternatives.

The two forms of circular HSV-1 DNA which would be produced by complementary-end and blunt-end ligation, and the concatemers generated therefrom, are shown in Figure C4.28. It is noted that blunt-end ligation produces concatemers in which alternate joints may be distinguished by a cleavage process to produce unit-length linear molecules. It is necessary for both segments to invert independently to produce the four arrangements. This feature is included in model 3C in Figure C4.27, on the incorrect assumption that the HSV-1 genome has two *a* sequences and would therefore display two dissimilar

joint structures after complementary-end ligation. As shown in Section 3 of Results, the majority of HSV-1 DNA molecules contain a single a sequence at the joint and termini, and by complementary-end ligation would produce concatemers with identical joints at all locations (Figure C4.28). Cleavage of unit-length molecules from such concatemers would then necessitate a second criterion, such as an approximate length measurement. The initial choice of cleavage site in this case may be a factor in inversion, as explained in Figure C4.28. Jacob et al. (1979) commented that cleavage must be separate from encapsidation in order to allow progeny DNA to enter the replicative pool: this would not be the case if circular monomers are readily produced from concatemers by recombination. Ladin et al. (1980) have shown that maturation of PRV DNA is dependent upon capsid assembly. The nature of the cleavage event, whether it is a double stranded cut or a single stranded nick at each end of the a sequence followed by repair of single stranded DNA, is unknown.

Fixed recombinants are rare. Indeed, the marker rescue experiments described in Section 2 were aimed at generating such recombinants and yet failed to do so. The fixed recombinants studied raise a number of questions regarding HSV DNA replication, such as the mechanism by which subclone 1 of Bxl(28-1), that is Bxl(28-1-1), circularises. More problematical is the way in which the DNA of this recombinant is matured. With respect to a sequences, circular or concatemeric Bxl(28-1-1) DNA is structurally identical to Bxl(31-2) DNA, and yet the former has a fixed phenotype and the latter inverts normally. Further analyses of minor DNA populations and of the circular structures of these recombinants is necessary to answer such questions.

Expression of Bxl(28-1) subclones deleted in TR_L/IR_L

The results of experiments conducted with subclones of Bxl(28-1) which are deleted in either TR_L or IR_L showed that expression of only one of the two copies of V_{mw} IE 110/118 is sufficient for a productive infection in vitro. Even extensive deletion of TR_L resulted in viable virus, as evidenced by subclone 22 which lacks a considerable sequence beyond the V_{mw} IE 118 gene. Clearly, any other genetic information in TR_L/IR_L is not required in diploid amounts for continued virus replication in tissue culture. RE4 has a deletion extending from the joint, through IR_L, and at least 300 bp into U_L, but the polypeptides induced by this recombinant have not yet been examined. The observation that all recombinants examined, including those with deleted sequences, have retained a sequences at the joint and termini may be a result of a requirement for, or regeneration of, the a sequences in DNA replication. Deletions in TR_S/IR_S analogous to those in TR_L/IR_L have not been observed, perhaps because of a requirement for or greater selective advantage of functional diploidy of TR_S/IR_S in productive infection, or because of the mechanism of deletion which is at present unknown.

There are several possible explanations of the diploidy for TR_L/IR_L and TR_S/IR_S displayed by all field isolates examined to date. Two out of twenty Bxl(28-1) subclones were deleted in TR_L or IR_L, and yet no equivalent deletions have been detected in over a hundred intertypic recombinants isolated so far in Glasgow. As with natural isolates, the frequency with which deletions occur or are isolated in the latter case must be lower. The deleted genomes resulted from recombinants which are completely heterologous for one or both sets of repeats. No other recombinant or field isolate has been shown to possess

completely heterotypic diploid regions; thus, if deletions are naturally being generated, their frequency must be so low as to have escaped isolation in the relatively small number of field isolates examined to date. It is not known why heterotypic recombinants with deletions in TR_L/IR_L were so readily isolated, and neither is it known whether this is primarily a result of the frequent generation of deletions or of their subsequent selective advantage. It should be noted that deleted TR_L or IR_L sequences can be regenerated in homotypic genomes by recombination, whereas this is not possible in heterotypic recombinants.

If viruses with deletions in the genome were at a selective disadvantage for growth, naturally occurring deletion mutants would not survive. The detailed growth characteristics of deletion mutants have yet to be investigated in vitro and in vivo. It is possible that increased gene dosage resulting from diploid sequences confers an advantage in establishing an infection at low multiplicities, or in cell types naturally resistant to virus infection or replication, and this might be particularly important in the natural host.

It is also possible that the presence of inverted diploid regions, or the expression of genes contained in them, is essential for an unidentified function of the virus, for example in the processes of latency or cellular transformation. An unlikely alternative is that the presence of diploid regions is of no crucial consequence to HSV, but merely reflects a propensity of DNA to replicate wherever possible.

Genome structure of RE4

Finally, it is worthwhile commenting on the extraordinary genome structure of RE4. This viable recombinant lacks 10.5 kbp of DNA, including IR_L and a short adjacent region of U_L.

2.8 kbp of HSV-2 DNA have been inserted into the position of the deletion, an event which presumably occurred in the same cell as the initial recombination event, rendering the genome heterotypically "diploid" for this region of U_L. It is not yet known whether the inserted sequences are functional in RE4, even to the point of fulfilling a function of deleted U_L sequences. It is of interest to note that the inserted sequence almost certainly contains upstream sequences and perhaps a part of the transcribed sequence for the gene coding for HSV-2 MCP, a major late polypeptide. This interesting deleted recombinant which is also fixed in both segments furnishes opportunity for further study.

Figure C4.1

Structures of the joints and long termini of Bxl(28-1).

A
1 tsB BamH I k
2 Bxl(28-1) BamH I major joint
3 Bxl(28-1) BamH I minor joint
4 tsl BamH I g

Digested with Sst I; 2% agarose gel.

B
1 tsB BamH I k
2 Bxl(28-1) BamH I major joint
3 tsl-BamH I g and g

Digested with Sma I; 7.5% polyacrylamide gel.

C
1 tsB BamH I st
2 tsl BamH I vw
3 Bxl(28-1) BamH I major type 2 long terminus
4 Bxl(28-1) BamH I minor type 1 long terminus, with HSV-2 BamH I k

Digested with Sst I; 2% agarose gel.

D
1 tsB BamH I st
2 tsl BamH I vw
3 Bxl(28-1) BamH I major type 2 long terminus

Digested with Sma I; 7.5% polyacrylamide gel.

Sst I or Sma I restriction fragments of joint or long terminal fragments are indicated to the left (HSV-1) and right (HSV-2) of each panel.
X denotes a genome terminal fragment, and J the Sma I fragment spanning the junction between the two tandem a sequences in HSV-2 BamH I g.

With respect to the text, the terms long terminus and L terminus, and short terminus and S terminus, are equivalent.

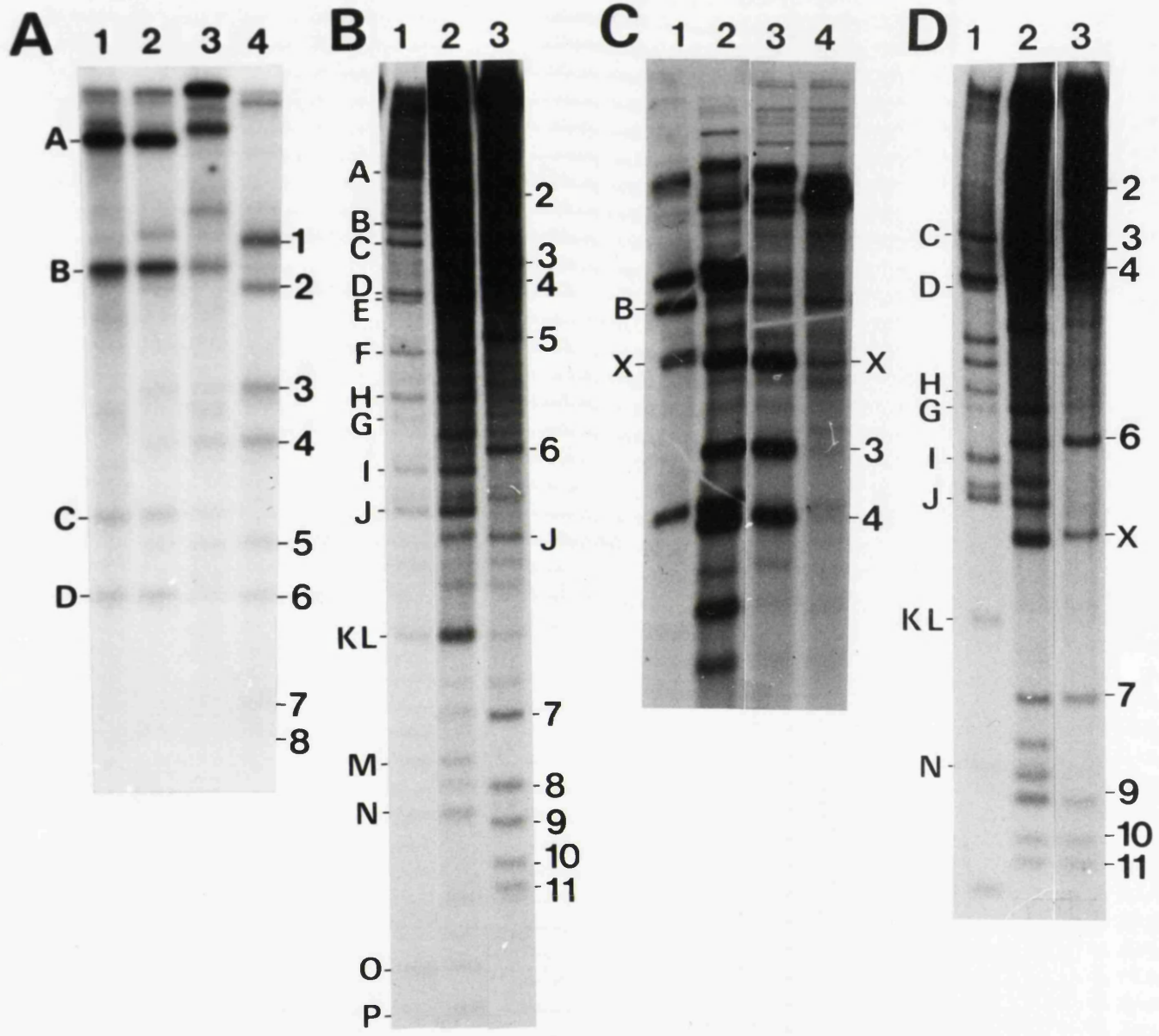

A
1 2 3 4
A
B
C
D
1
2
3
4
5
6
7
8
B
1 2 3
A
B
C
D
E
F
H
G
I
J
KL
M
N
O
P
2
3
4
5
6
J
7
8
9
10
11
C
1 2 3 4
B
X
X
3
4
D
1 2 3
C
D
H
G
I
J
KL
N
2
3
4
6
X
7
9
10
11

Figure C4.2

Structures of the short termini of Bxl(28-1).

A
1 tsB EcoR I k
2 Bxl(28-1) EcoR I right hand S terminus
3 tsl EcoR I m

Digested with Sst I; 1.5% agarose gel.

B As panel A but digested with Kpn I.

C
1 tsB Hind III m
2 Bxl(28-1) Bgl II left hand S terminus
3 tsl Bgl II m

Digested with Sst I; 1.5% agarose gel.

D As panel C but digested with Kpn I.

E
1 tsB BamH I pq
2 tsl BamH I r
3 tsl BamH I st
4 Bxl(28-1) BamH I type 1 short terminus, with HSV-2 BamH I rs
5 Bxl(28-1) BamH I type 2 short terminus
6 tsl BamH I u

Digested with Sma I; 7.5% polyacrylamide gel.

Sst I, Kpn I or Sma I restriction fragments of short terminal fragments are indicated to the left (HSV-1) and right(HSV-2) of each panel. In panels A and C only those Sst I fragments mapping in the BamH I terminal fragment are indicated.
X denotes a genome terminal fragment.

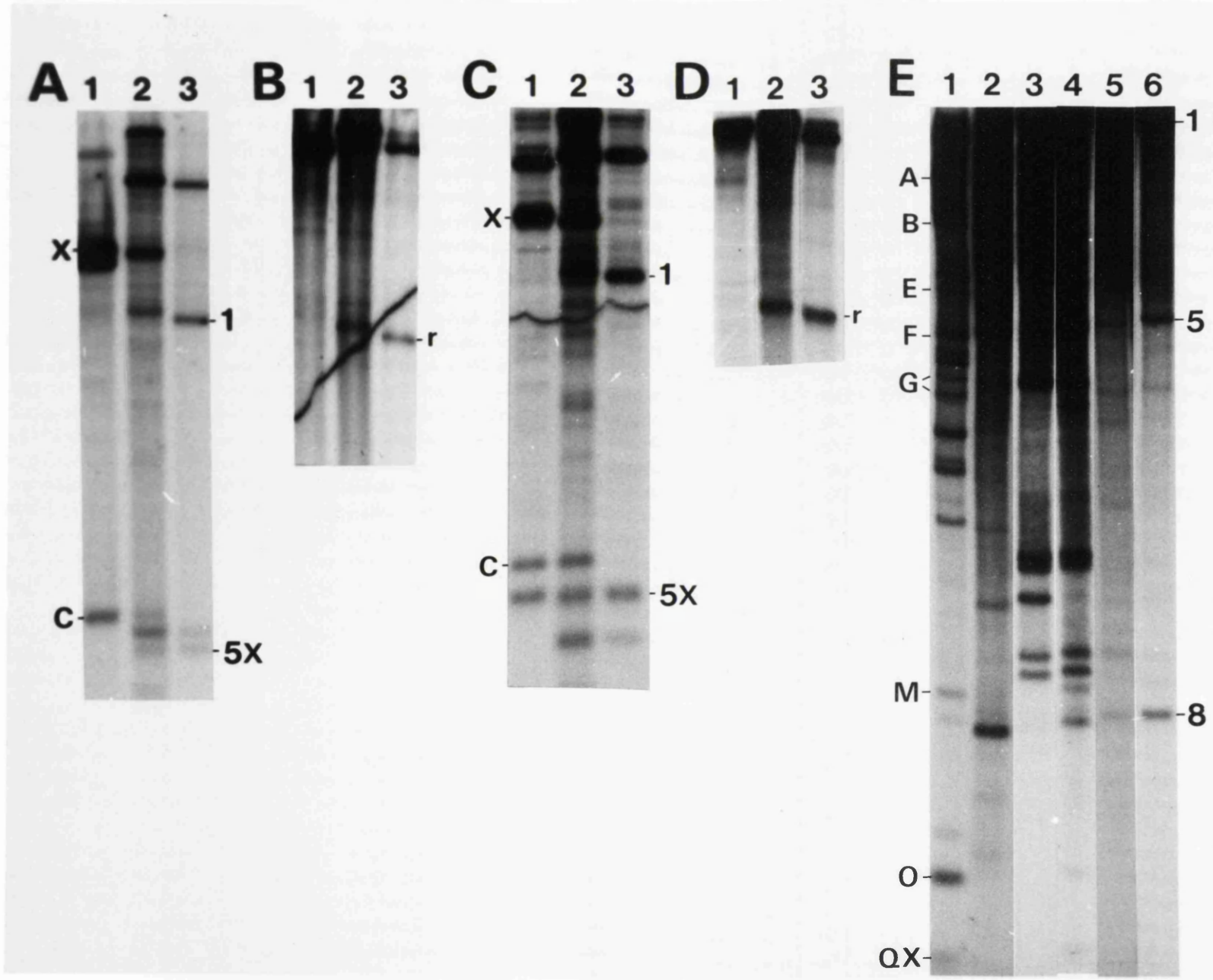

A
1 2 3
X
1
C
5X
B
1 2 3
r
C
1 2 3
X
1
C
5X
D
1 2 3
r
E
1 2 3 4 5 6
1
A
B
E
F
G
5
M
8
O
QX

Figure C4.2

Structures of the short termini of Bxl(28-1).

A
1 tsB EcoR I k
2 Bxl(28-1) EcoR I right hand S terminus
3 tsl EcoR I m

Digested with Sst I; 1.5% agarose gel.

B As panel A but digested with Kpn I.

C
1 tsB Hind III m
2 Bxl(28-1) Bgl II left hand S terminus
3 tsl Bgl II m

Digested with Sst I; 1.5% agarose gel.

D As panel C but digested with Kpn I.

E
1 tsB BamH I pq
2 tsl BamH I r
3 tsl BamH I st
4 Bxl(28-1) BamH I type 1 short terminus, with HSV-2 BamH I rs
5 Bxl(28-1) BamH I type 2 short terminus
6 tsl BamH I u

Digested with Sma I; 7.5% polyacrylamide gel.

Sst I, Kpn I or Sma I restriction fragments of short terminal fragments are indicated to the left (HSV-1) and right(HSV-2) of each panel. In panels A and C only those Sst I fragments mapping in the BamH I terminal fragment are indicated.
X denotes a genome terminal fragment.

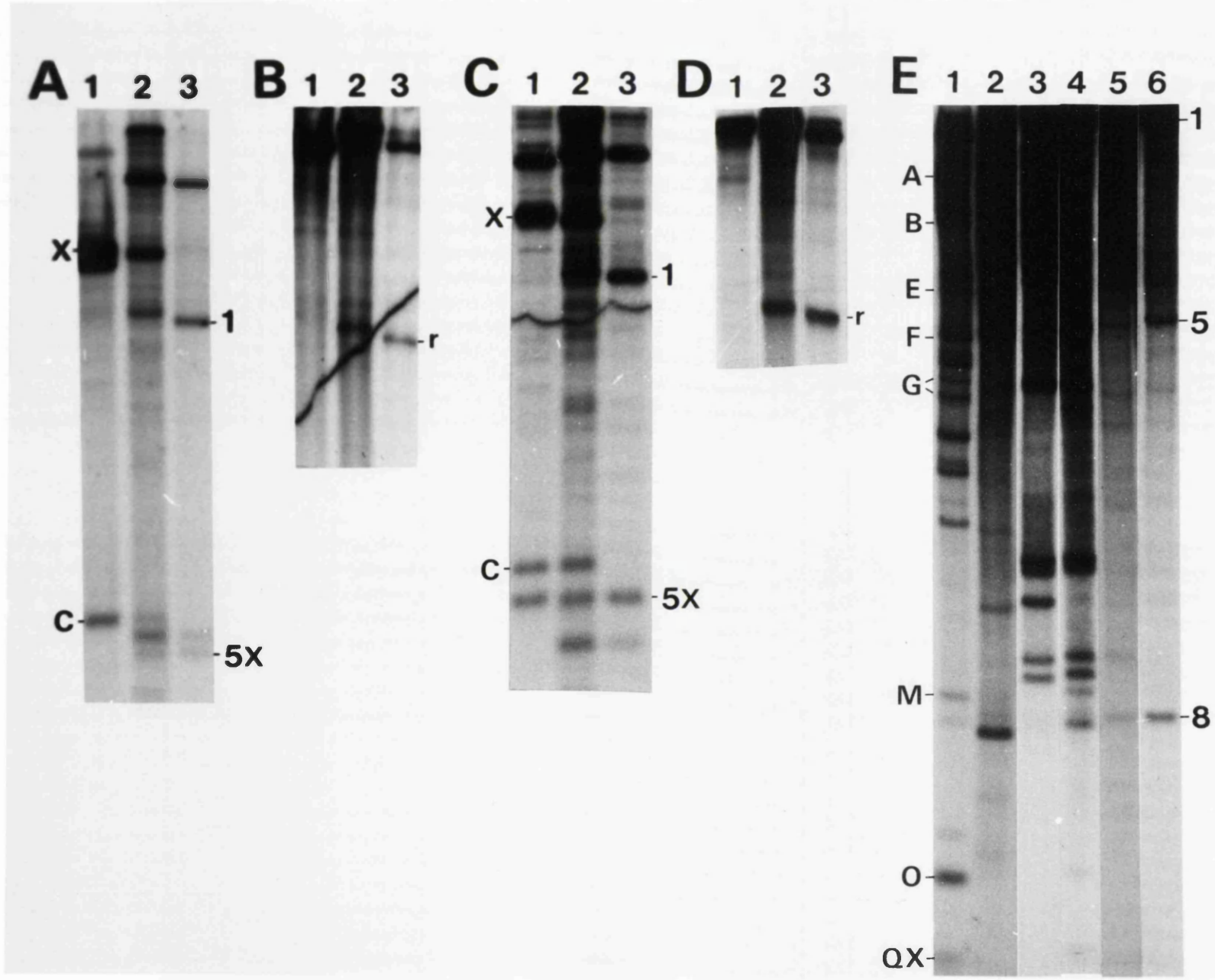

A
1
2
3
X
1
C
5X
B
1
2
3
r
C
1
2
3
X
1
C
5X
D
1
2
3
r
E
1
2
3
4
5
6
1
A
B
E
F
G
5
M
8
O
QX

Figure C4.3

Previously published recombinant genome structures. HSV-1 sequences are indicated above and HSV-2 sequences below the representative genome in each case. Cross-hatching denotes the region of uncertainty in crossover position.

References:

Bxl(28-1), Bxl(31-2) and Dxl(2) - Preston et al. (1978),

RS6 - Stow and Wilkie (1978),

Bx6(17-6) - Marsden et al. (1978),

R13-2 - Chartrand et al. (1981).

Each recombinant inverts normally in L and S except Bxl(28-1), in which L is fixed in the orientation present in the P and I_S genome arrangements.

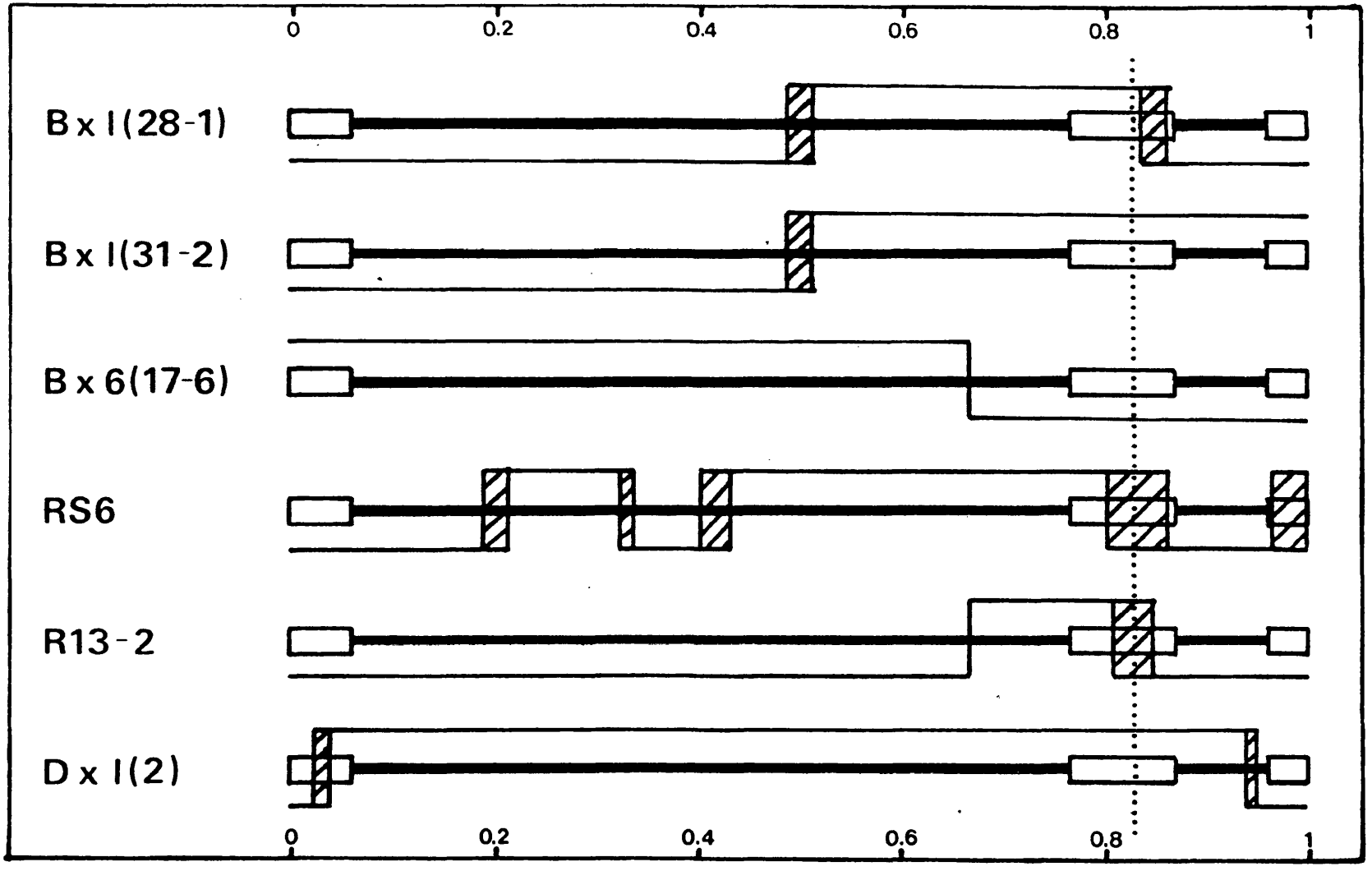

0
0.2
0.4
0.6
0.8
1
B x I(28-1)
B x I(31-2)
B x 6(17-6)
RS6
R13-2
D x I(2)
0
0.2
0.4
0.6
0.8
1

Figure C4.4

Restriction profiles of *in vivo* ^{32}P-labeled DNA from subclones of Bxl(28-1) for *Eco*R I (A,B) and *Hpa* I (C,D). The subclone numbers are given at the top of the tracks, and also included are HSV-1 *ts*B (T1), HSV-2 *ts*1 (T2) and Bxl(28-1) (R). Restriction fragments are indicated to the left (HSV-1) and right (HSV-2) of each panel. 0.4% agarose gels were used.

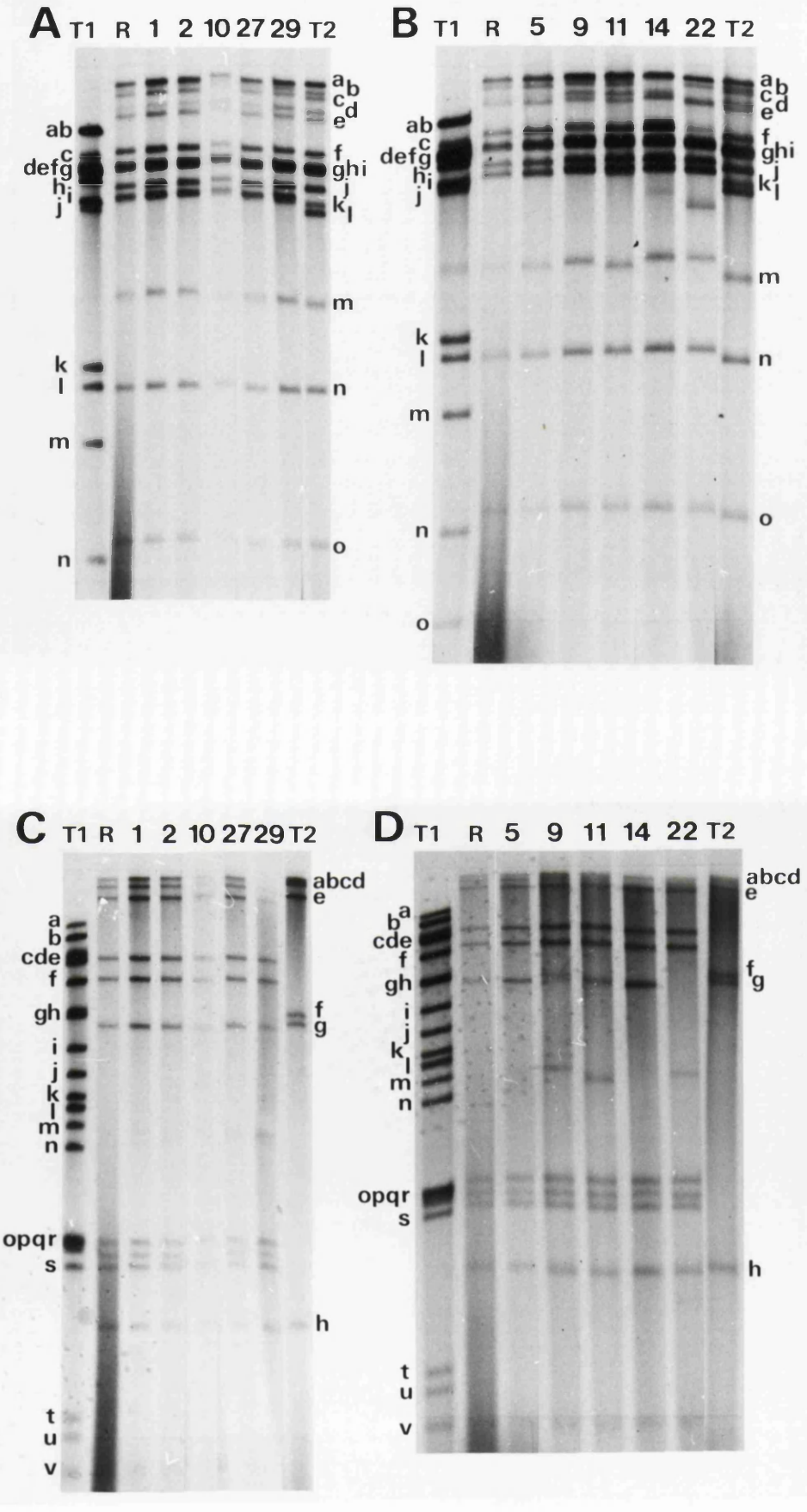

A
T1 R 1 2 10 27 29 T2
ab
c
defg
h i
j
k
l
m
n
a b
c d
e
f
g h i
j
k l
m
n
o
B
T1 R 5 9 11 14 22 T2
ab
c
defg
h i
j
k
l
m
n
o
a b
c d
e
f
g h i
j
k l
m
n
o
C
T1 R 1 2 10 27 29 T2
a
b
cde
f
gh
i
j
k
l
m
n
opqr
s
t
u
v
abcd
e
f
g
h
D
T1 R 5 9 11 14 22 T2
b a
cde
f
gh
i
j
k
l
m
n
opqr
s
t
u
v
abcd
e
f g
h

Figure C4.5

Restriction profiles of *in vivo* ^{32}P-labeled DNA from subclones of Bxl(28-1) for *Kpn* I (A,B) and *Bam*H I (C,D). The subclone numbers are given at the top of the tracks, and also included are HSV-1 *ts*B (T1), HSV-2 *ts*1 (T2) and Bxl(28-1) (R). Restriction fragments are indicated to the left (HSV-1) and right (HSV-2) of each panel. 0.7% and 1.0% agarose gels were used.

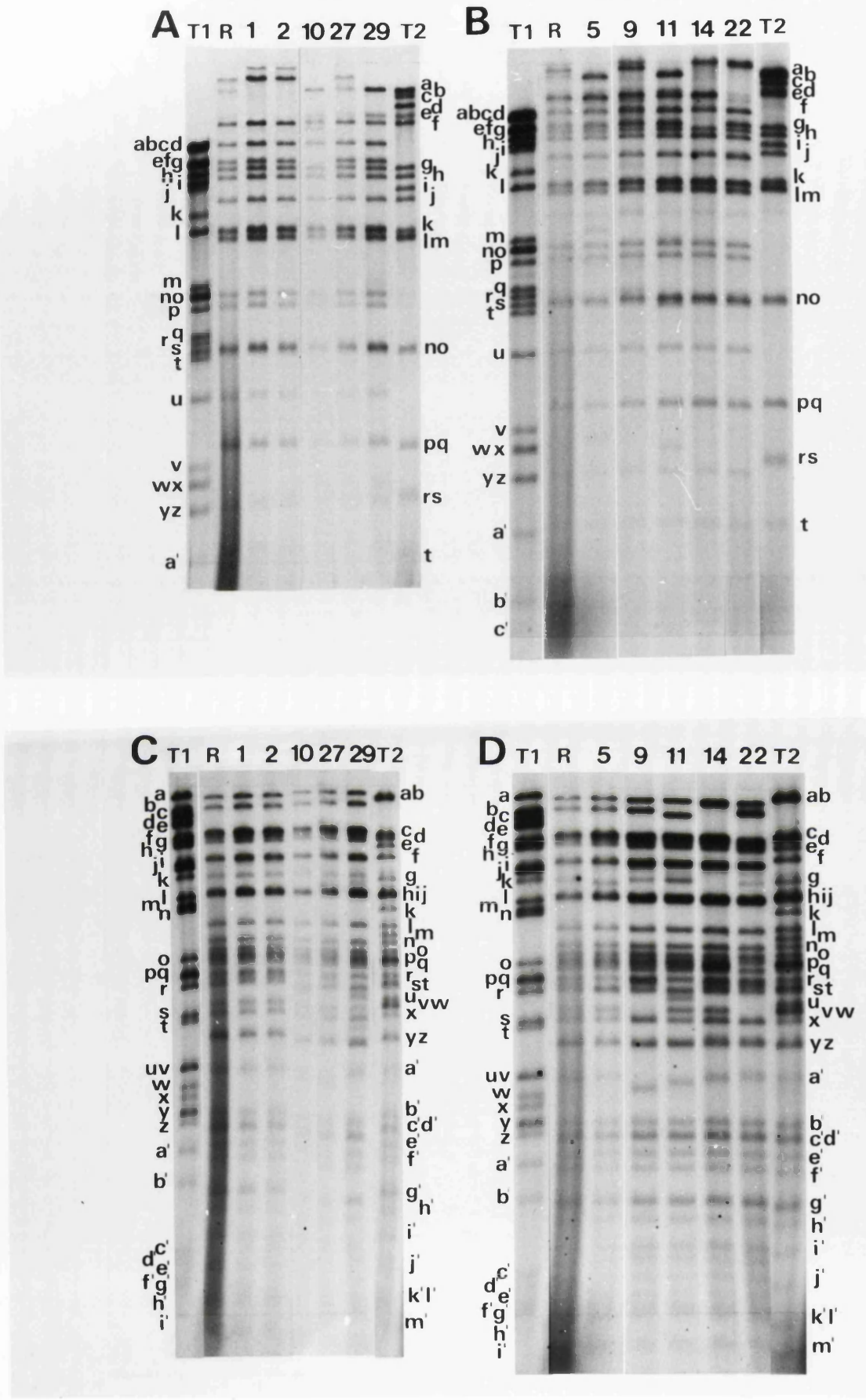
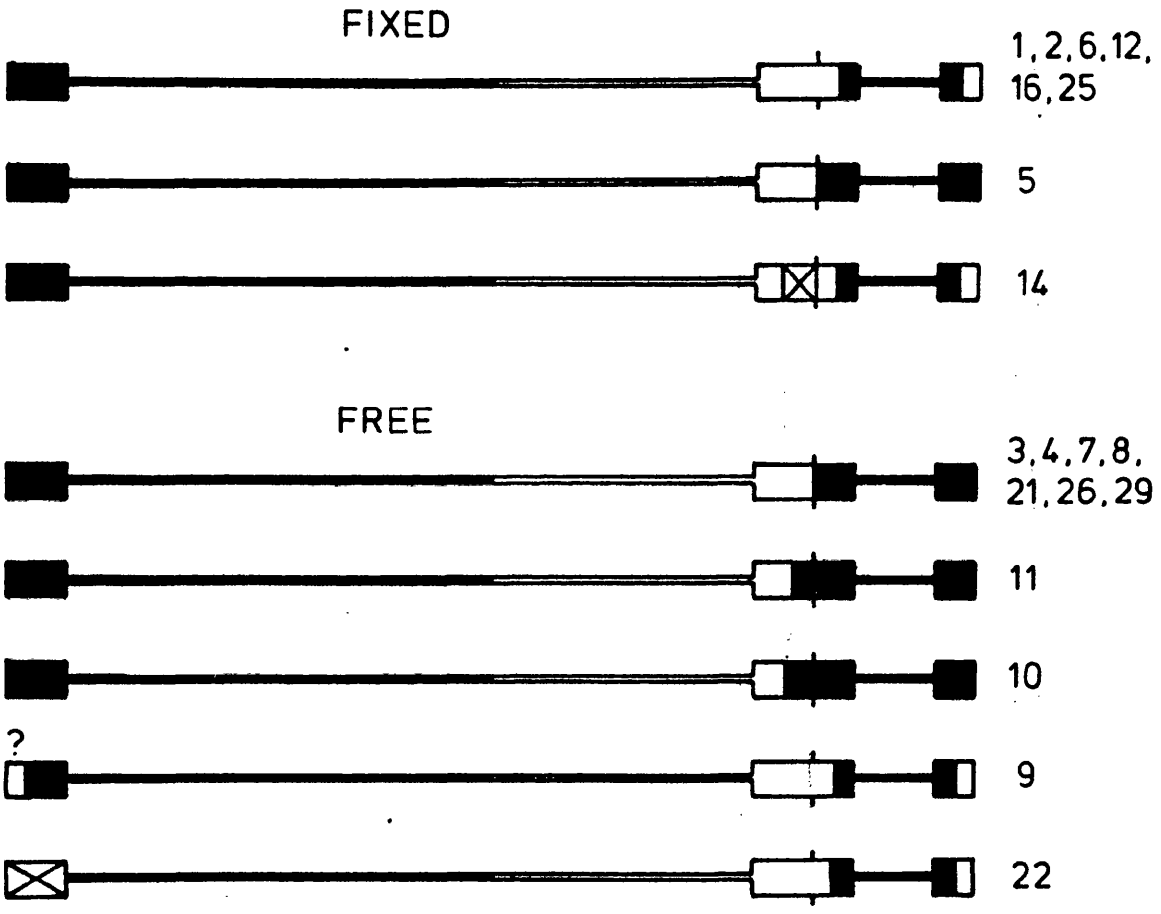

A
T1 R 1 2 10 27 29 T2
B
T1 R 5 9 11 14 22 T2
C
T1 R 1 2 10 27 29 T2
D
T1 R 5 9 11 14 22 T2

Figure C4.6

Gross structures of Bx1(28-1) subclones. Black represents HSV-2 DNA, and white HSV-1 DNA, using the conventional representation of the HSV genome. Regions of uncertainty of crossover have been omitted since precise details are given in Figure C4.17. A cross indicates deleted sequences. The inversion property of L is noted, and the subclone number is given on the right.



27 as B x 1 (28-1)

Figure C4.7

Structures of the joints of Bxl(28-1) subclones.

A
1 tsB BamH I k
2 tsl BamH I g
3 subclone 1 BamH I joint
4 subclone 5 BamH I joint
5 subclone 9 BamH I joint
6 subclone 29 BamH I joint

Digested with Sst I; 2% agarose gel.

B
1 tsB BamH I k
2 subclone 10 BamH I joint
3 tsl BamH I g
4 tsl BamH I g

Digested with Sst I; 2% agarose gel.

C
1 tsB BamH I k
2 tsl BamH I g
3 subclone 1 BamH I joint
4 subclone 9 BamH I joint
5 subclone 29 BamH I joint

In vivo ^{32}P-labeled DNA cleaved with Sma I; 7.5% polyacrylamide gel.

Sst I or Sma I restriction fragments of joint fragments are indicated to the left (HSV-1) and right (HSV-2) of each panel.

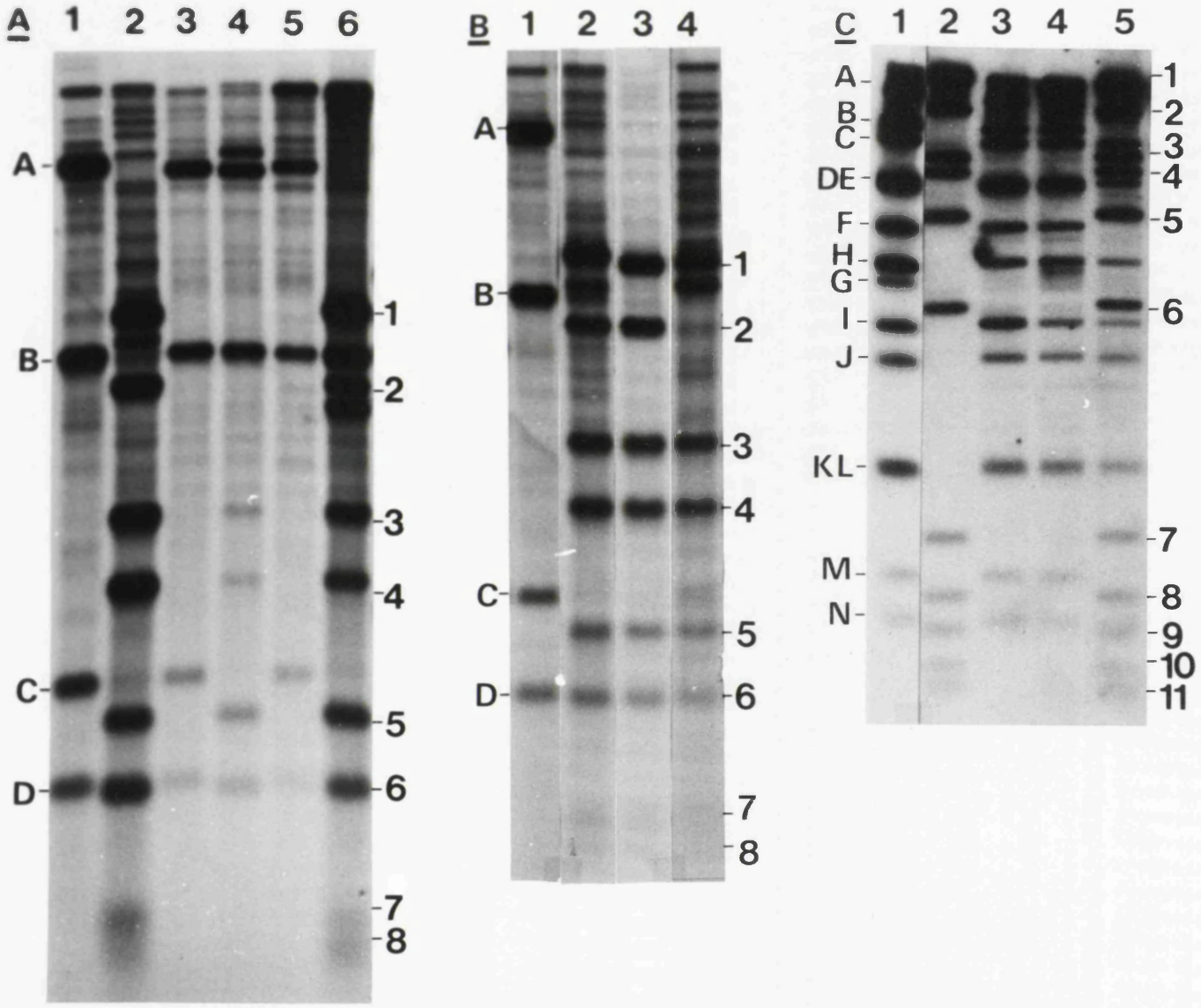

A 1 2 3 4 5 6
A
B
C
D
1
2
3
4
5
6
7
8
B 1 2 3 4
A
B
C
D
1
2
3
4
5
6
7
8
C 1 2 3 4 5
A
B
C
DE
F
H
G
I
J
KL
M
N
1
2
3
4
5
6
7
8
9
10
11

Figure C4.8

Structures of the joints of Bxl(28-1) subclones.

A
1 tsB BamH I k
2 subclone 1 BamH I joint
3 subclone 9 BamH I joint
4 tsl BamH I g

Digested with Sma I; 7.5% (upper) and 15% (lower) polyacrylamide gels.

B
1 tsB BamH I k
2 subclone 29 BamH I larger joint
3 subclone 29 BamH I smaller joint
4 tsl BamH I g

Digested with Sma I; 7.5% (upper) and 15% (lower) polyacrylamide gels.

C
1 tsB BamH I k
2 subclone 14 BamH I/Hpa I joint
3 tsB Hpa I m

Digested with Sma I; 7.5% polyacrylamide gel.

D
1 tsB BamH I k
2 subclone 10 BamH I joint
3 tsl BamH I g

Digested with Sma I; 7.5% polyacrylamide gel.

Sma I restriction fragments of joint fragments are indicated to the left (HSV-1) and right (HSV-2) of each panel.

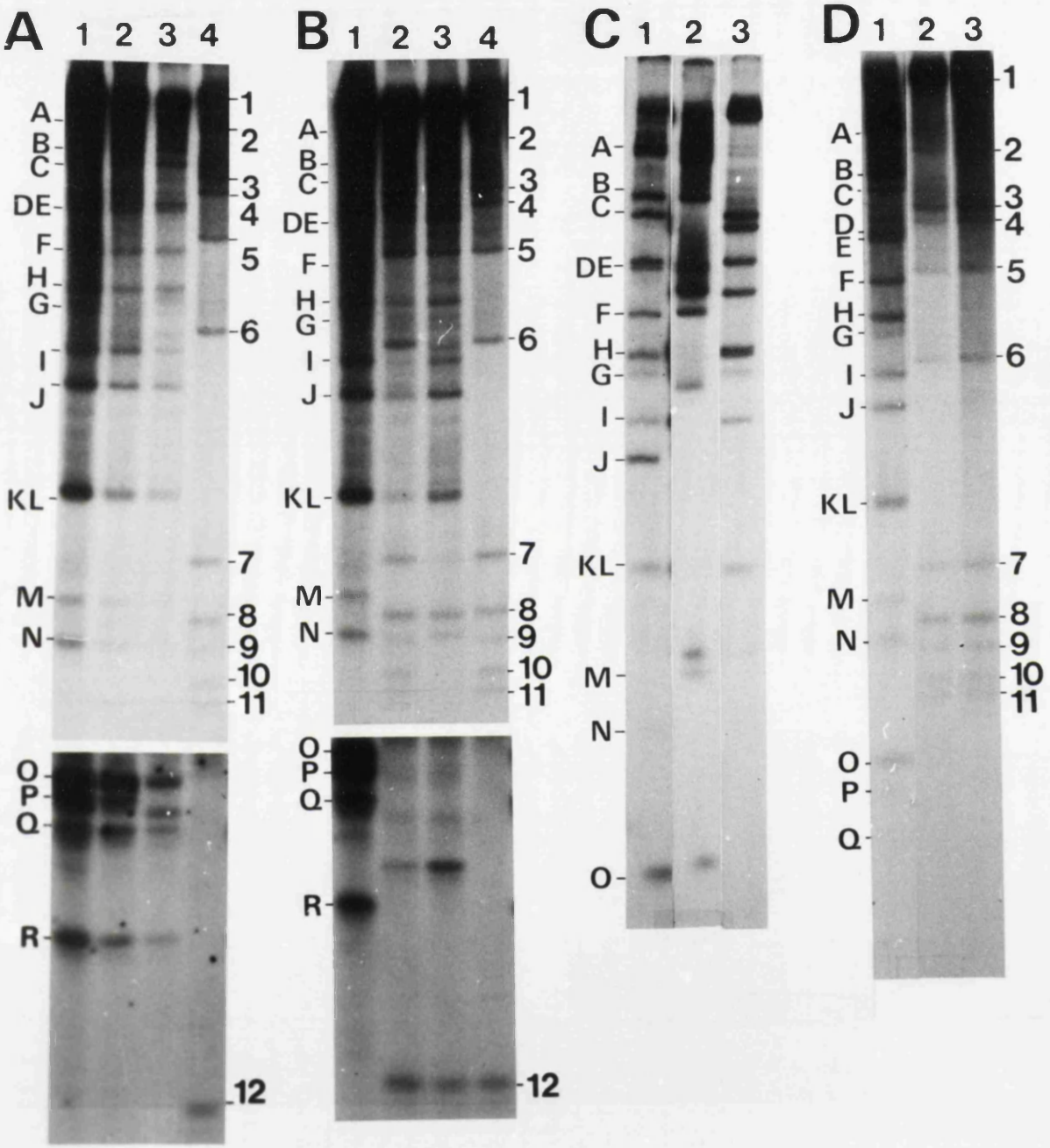

A
1 2 3 4
A
B
C
DE
F
H
G
I
J
KL
M
N
O
P
Q
R
1
2
3
4
5
6
7
8
9
10
11
12
B
1 2 3 4
A
B
C
DE
F
H
G
I
J
KL
M
N
O
P
Q
R
1
2
3
4
5
6
7
8
9
10
11
12
C
1 2 3
A
B
C
DE
F
H
G
I
J
KL
M
N
O
D
1 2 3
A
B
C
D
E
F
H
G
I
J
KL
M
N
O
P
Q
1
2
3
4
5
6
7
8
9
10
11

Figure C4.9

Structures of the long termini of Bxl(28-1) subclones.

A
1 tsB BamH I s
2 subclone 1 BamH I type 2 long terminus
3 subclone 1 BamH I type 1 long terminus, with HSV-2 BamH I x
4 subclone 5 BamH I type 2 long terminus
5 subclone 5 BamH I type 1 long terminus, with HSV-2 BamH I x
6 subclone 9 BamH I type 1 long terminus, with HSV-2 BamH I x
7 subclone 29 BamH I type 2 long terminus, with HSV-2 BamH I u
8 subclone 29 HSV-2 BamH I x
9 subclone 29 BamH I type 1 long terminus, with HSV-2 BamH I x
10 Bxl(31-2) BamH I type 1 long terminus, with HSV-2 BamH I x
11 tsl BamH I uvw
12 tsl BamH I wx

Digested with Sst I; 2% agarose gel.

B
1 tsB BamH I st
2 tsl BamH I vw
3 subclone 9 BamH I type 2 long terminus
4 subclone 9 BamH I type 1 long terminus
5 subclone 29 BamH I type 2 long terminus
6 subclone 29 BamH I type 1 long terminus

Digested with Sst I; 2% agarose gel. Termini in tracks 3-6 were isolated via the unique Hpa I long terminal fragments.

C
1 tsl BamH I uvw
2 subclone 10 BamH I type 2 long terminus
3 subclone 14 BamH I type 2 long terminus

Digested with Sst I; 2% agarose gel.

Sst I restriction fragments of long terminal fragments are indicated to the left (HSV-1) and right (HSV-2) of each panel.
* denotes a genome terminal fragment.

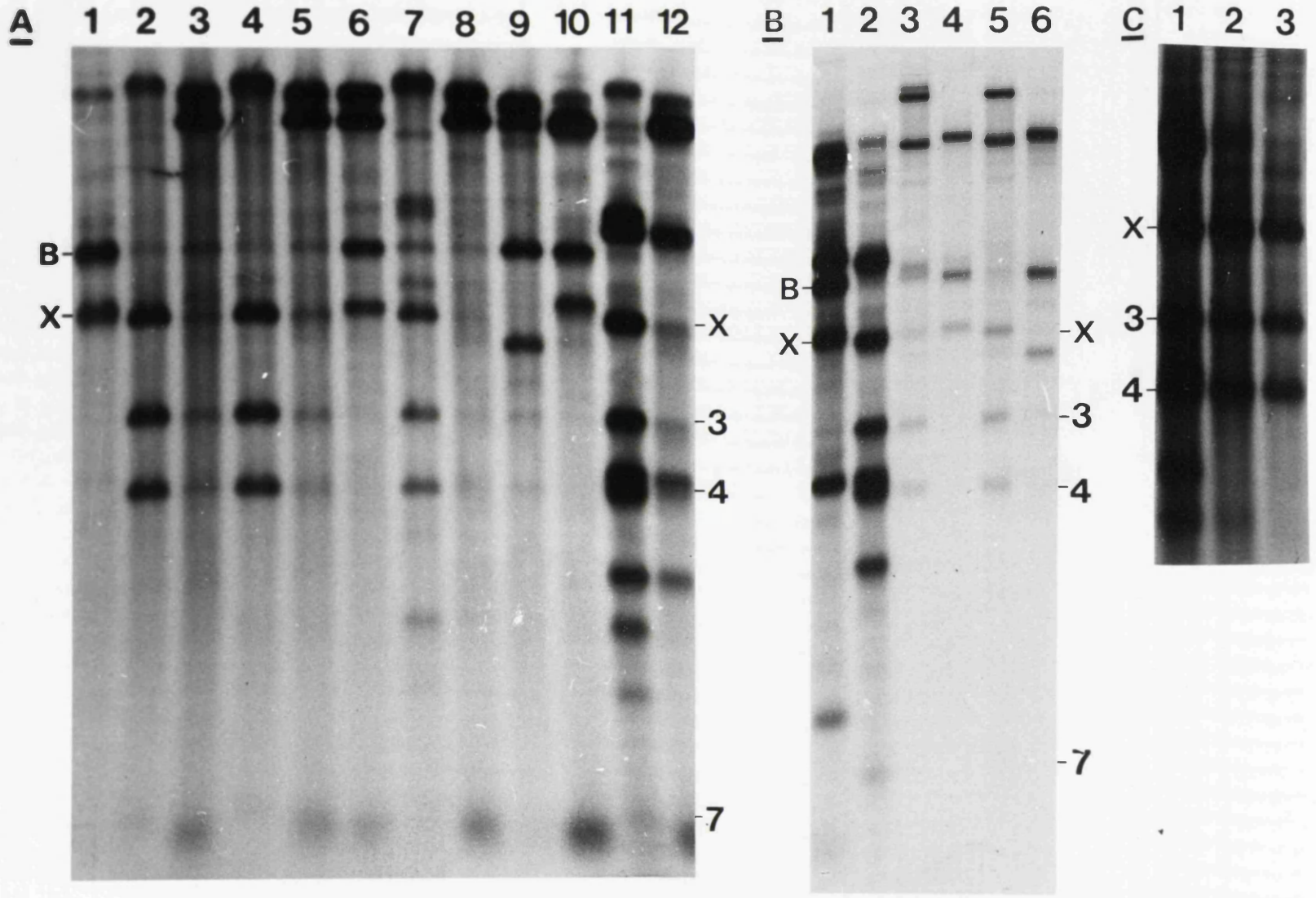

A
1 2 3 4 5 6 7 8 9 10 11 12
B
X
X
3
4
7
B
1 2 3 4 5 6
B
X
X
3
4
7
C
1 2 3
X
3
4

Figure C4.10

Structures of the long termini of Bxl(28-1) subclones.

A
1 tsB BamH I s
2 subclone 9 BamH I type 1 long terminus, with HSV-2 BamH I x
3 subclone 29 BamH I type 2 long terminus
4 subclone 29 BamH I type 1 long terminus
5 tsl BamH I vw
6 subclone 1 BamH I type 2 long terminus

Digested with Sma I; 7.5% (upper) and 15% (lower) polyacrylamide gels.

B
1 tsB BamH I k
2 tsl BamH I vw
3 subclone 10 BamH I type 2 long terminus
4 subclone 14 BamH I type 2 long terminus

Digested with Sma I; 7.5% polyacrylamide gel.

C
1 tsB Hpa I m
2 subclone 22 type 2 Hpa I deleted long terminus

Digested with BamH I/Sma I; 7.5% polyacrylamide gel.

D
1 tsB BamH I s
2 tsB Hpa I m
3 subclone 22 type 1 Hpa I long terminus
4 subclone 22 type 2 Hpa I deleted long terminus
5 tsl Hpa I g
6 tsl Hpa I f
7 tsl BamH I vw

Digested with Sma I; 2% agarose (upper) and 7.5% polyacrylamide (lower) gels.

Sma I restriction fragments of long terminal fragments are indicated to the left (HSV-1) and right (HSV-2) of each panel.
X denotes a genome terminal fragment.

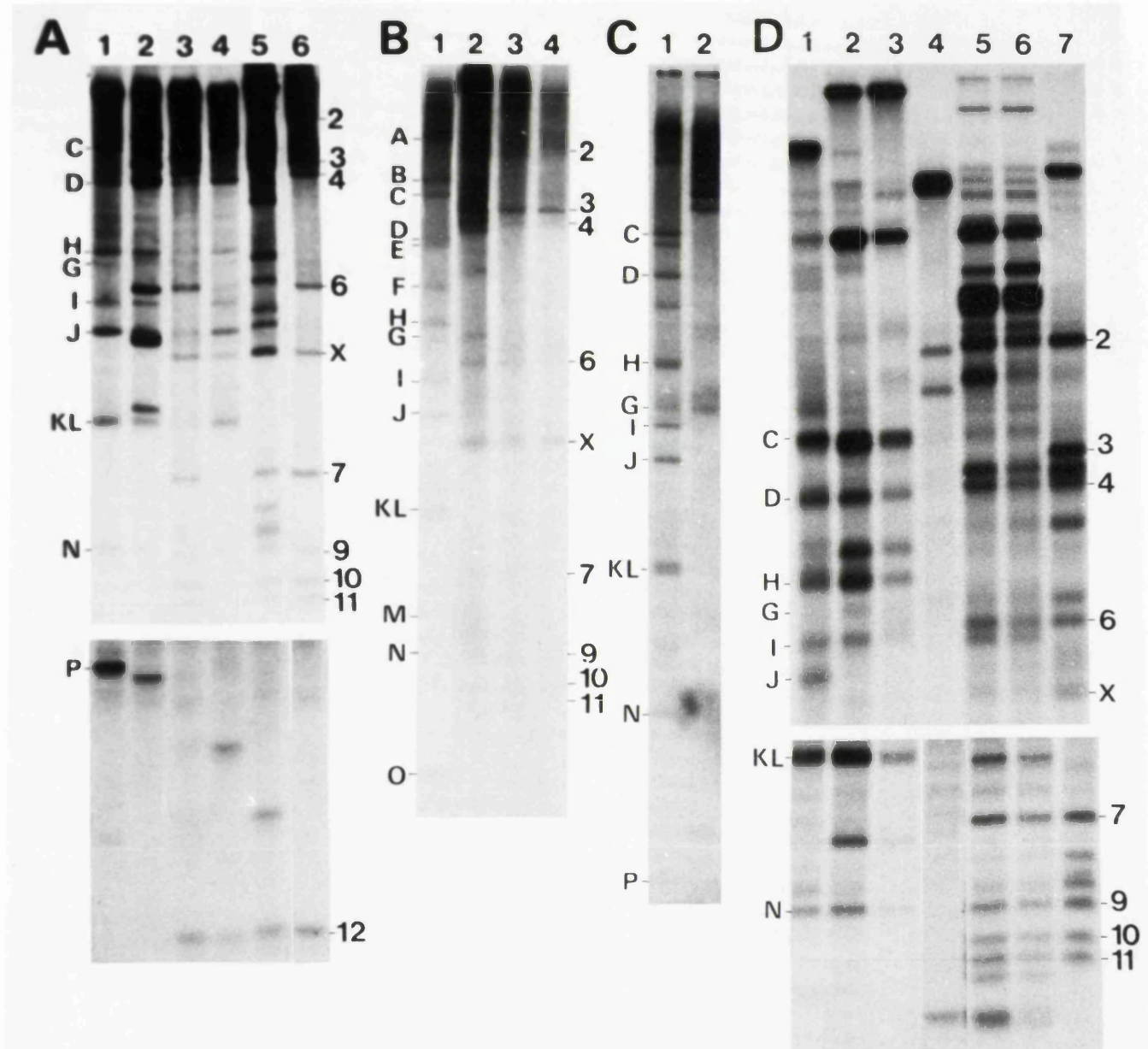

A
1 2 3 4 5 6
C
D
H
G
I
J
KL
N
P
2
3
4
6
X
7
9
10
11
12
B
1 2 3 4
A
B
C
D
E
F
H
G
I
J
KL
M
N
O
2
3
4
6
X
7
9
10
11
C
1 2
C
D
H
G
I
J
KL
N
P
D
1 2 3 4 5 6 7
C
D
H
G
I
J
KL
N
2
3
4
6
X
7
9
10
11

Figure C4.11

Structures of the short termini of Bxl(28-1) subclones.

A
1 tsB BamH I pq
2 tsl BamH I u
3 subclone 1 BamH I short terminus, with HSV-2 BamH I r
4 subclone 5 BamH I short terminus, with HSV-2 BamH I s
5 subclone 9 BamH I short terminus, with HSV-2 BamH I r
6 subclone 29 BamH I short terminus
7 Bxl(31-2) BamH I short terminus, with HSV-2 BamH I r
8 tsl BamH I pq
9 tsl BamH I r

Digested with Sst I; 2% agarose gel.

B
1 tsl BamH I r
2 tsB BamH I pq
3 subclone 10 BamH I short terminus
4 subclone 14 BamH I short terminus, with HSV-2 BamH I r
5 tsl BamH I u

Digested with Sst I; 2% agarose gel.

C Restriction profiles of in vivo ^{32}P-labeled DNA from subclones of Bxl(28-1) for BamH I, in the region of the very small fragments. The subclone numbers are given at the top of the tracks, and also included are HSV-1 tsB (T1) and HSV-2 tsl (T2). Restriction fragments are indicated to the left (HSV-1) and right (HSV-2). A 7.5% polyacrylamide gel was used.

Sst I restriction fragments of short terminal fragments are indicated to the left (HSV-1) and right (HSV-2) of panels A and B.
X denotes a genome terminal fragment. Also marked in parentheses in panel A are Sst I 3 and 4 of HSV-2 BamH I y, the long terminus.

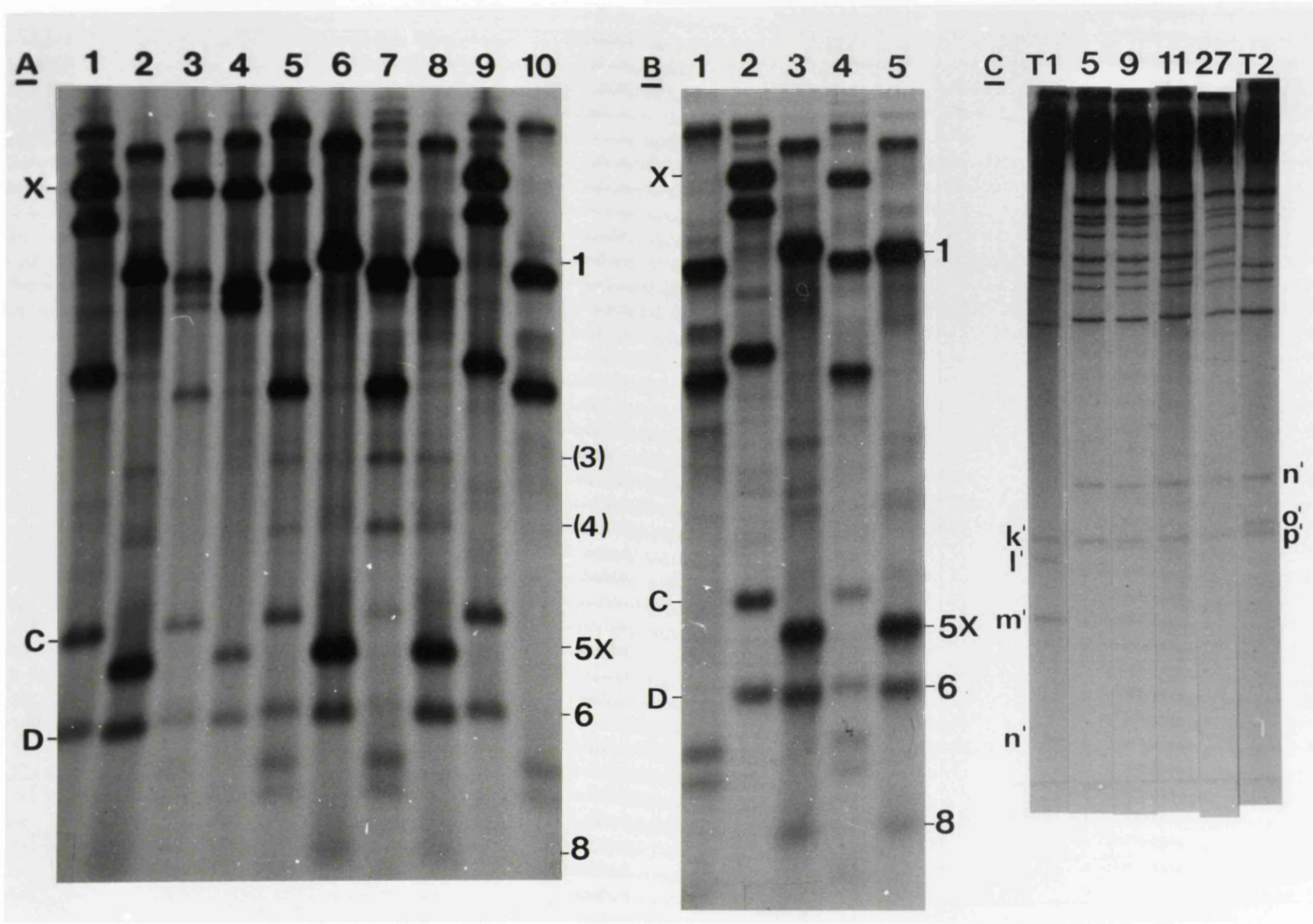

A
1
2
3
4
5
6
7
8
9
10
X
C
D
1
(3)
(4)
5X
6
8
B
1
2
3
4
5
X
C
D
1
5X
6
8
C
T1
5
9
11
27
T2
k'
l'
m'
n'
n'
o'
p'

Figure C4.12

LEFT HAND TWO PANELS

Structures of the short termini of Bxl(28-1) subclones.

A
1 tsB BamH I pq
2 tsl BamH I r
3 tsl BamH I st
4 tsl BamH I u
5 subclone 1 BamH I short terminus, with HSV-2 BamH I rs
6 subclone 5 BamH I short terminus, with HSV-2 BamH I s
7 subclone 9 BamH I short terminus, with HSV-2 BamH I r
8 subclone 29 BamH I short terminus

Digested with Sma I; 7.5% polyacrylamide gel.

B
1 tsl BamH I r
2 tsB BamH I pq
3 subclone 10 BamH I short terminus
4 subclone 14 BamH I short terminus
5 tsl BamH I u

Digested with Sma I; 7.5% polyacrylamide gel.

Sma I restriction fragments of short terminal fragments are indicated to the left (HSV-1) and right (HSV-2) of the panels.
X denotes a genome terminal fragment.

RIGHT HAND TWO PANELS

Autoradiograph of nitrocellulose blot strips containing KpnI or BamHI restriction fragments of subclone 14 (S14) or 22 (S22) DNA, to which nick translated DNA probes had been hybridised.

The probes were: HSV-1 DNA (H), and a mixture of plasmids containing HSV-1 BamHI k and HSV-2 BamHI g (J). HSV-1 DNA hybridised strongly to HSV-1 fragments in the recombinant genomes, and to a lesser extent to HSV-2 fragments. An explanation of the bands to which the J probe hybridised is given (NLT - novel L terminus; H1 - HSV-1 fragment; H2 - HSV-2 fragment; S - S segment, since the subclone genomes have no KpnI sites in S).

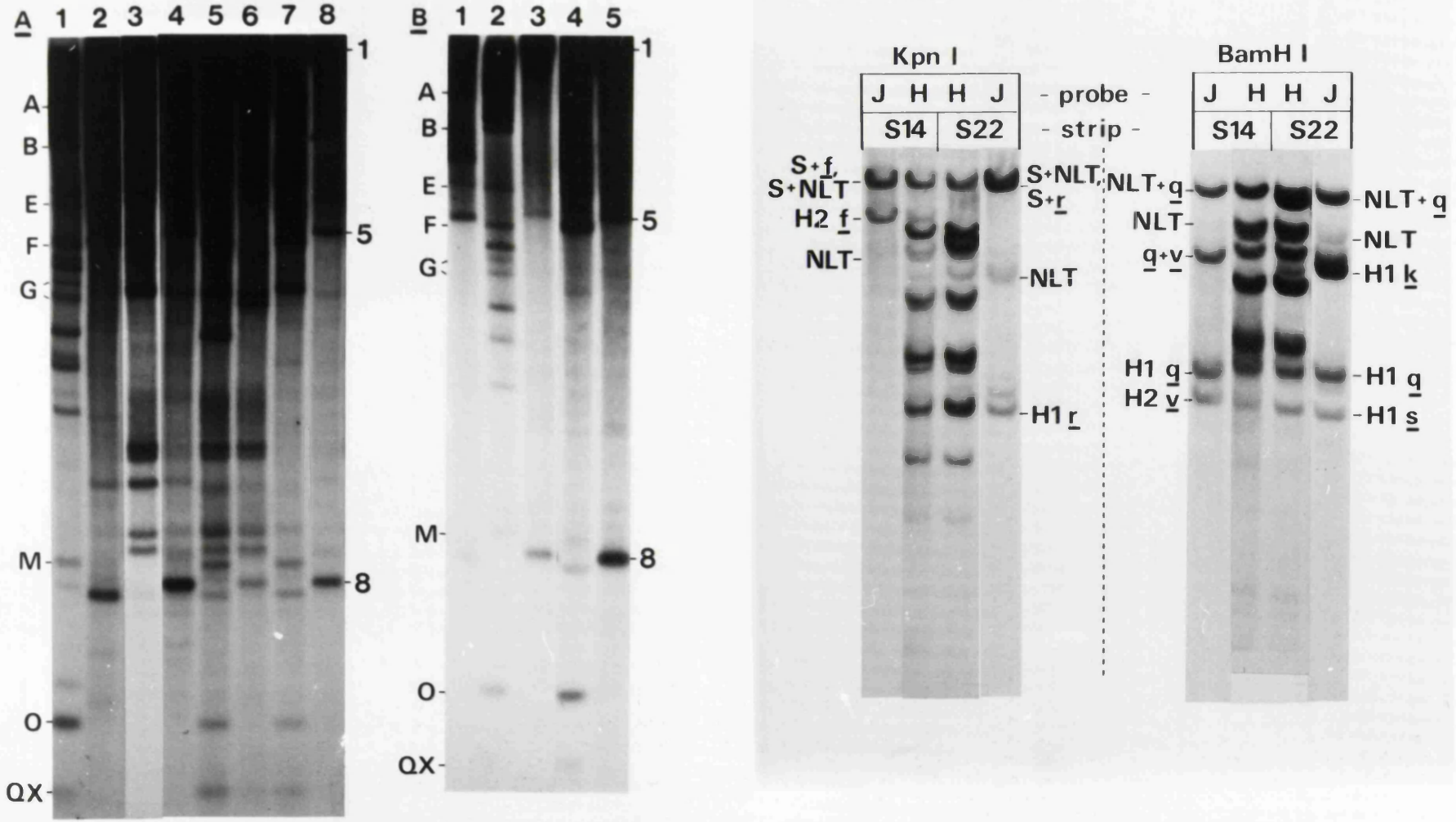

A 1 2 3 4 5 6 7 8
A
B
E
F
G
M
O
QX
1
5
8
B 1 2 3 4 5
A
B
E
F
G
M
O
QX
1
5
8
Kpn I
BamH I
J H H J
S14 S22
- probe -
- strip -
S+f
S+NLT
H2 f
NLT
S+NLT
S+r
NLT
H1 r
NLT+q
NLT
q+v
H1 q
H2 v
NLT+q
NLT
H1 k
H1 q
H1 s

Figure C4.13

Structures of the joints and termini of Bxl(31-2).

A
1 tsB BamH I st
2 Bxl(31-2) BamH I joint, with other fragments.
3 Bxl(31-2) BamH I short terminus and type 2 larger long terminus, with HSV-2 BamH I r
4 Bxl(31-2) HSV-2 BamH I s
5 Bxl(31-2) BamH I type 1 long terminus, with HSV-2 BamH I x
6 ts1 BamH I vw

Digested with Sst I; 2% agarose gel.

B As for panel A but digested with Sma I; 7.5% polyacrylamide gel.

C
1 tsB BamH I st
2 ts1 BamH I vw
3 Bxl(31-2) BamH I type 2 long terminus
4 Bxl(31-2) BamH I type 1 long terminus
5 Dxl(2) BamH I long terminus from the right hand end of L
6 Dxl(2) BamH I long terminus from the left hand end of L
7 Dxl(2) BamH I short terminus from the right hand end of S
8 Dxl(2) BamH I short terminus from the left hand end of S
9 tsB BamH I pg
10 ts1 BamH I u

Fragments for tracks 3-8 isolated from larger unique fragments. Digested with Sst I; 2% agarose gel.

D
1 HSV-1 Sst I long terminus
2 ts6 Sst I long terminus
3 Bxl(31-2) Sst I type 2 larger long terminus
4 Bxl(31-2) Sst I type 1 long terminus
5 Dxl(2) Sst I long terminus from the right hand end of L
6 Dxl(2) Sst I long terminus from the left hand end of L
7 ts6 Sst I long terminus

Sst I fragments isolated from larger fragments, which were unique for tracks 3-6. Digested with Sma I; 7.5% polyacrylamide gel.

Restriction fragments of BamH I terminal fragments are indicated to the left (HSV-1) and right (HSV-2) of the panels except in panel C where long terminal fragments are indicated on the left and short terminal fragments on the right, with HSV-2 fragments in parentheses. A denotes a genome terminal fragment.

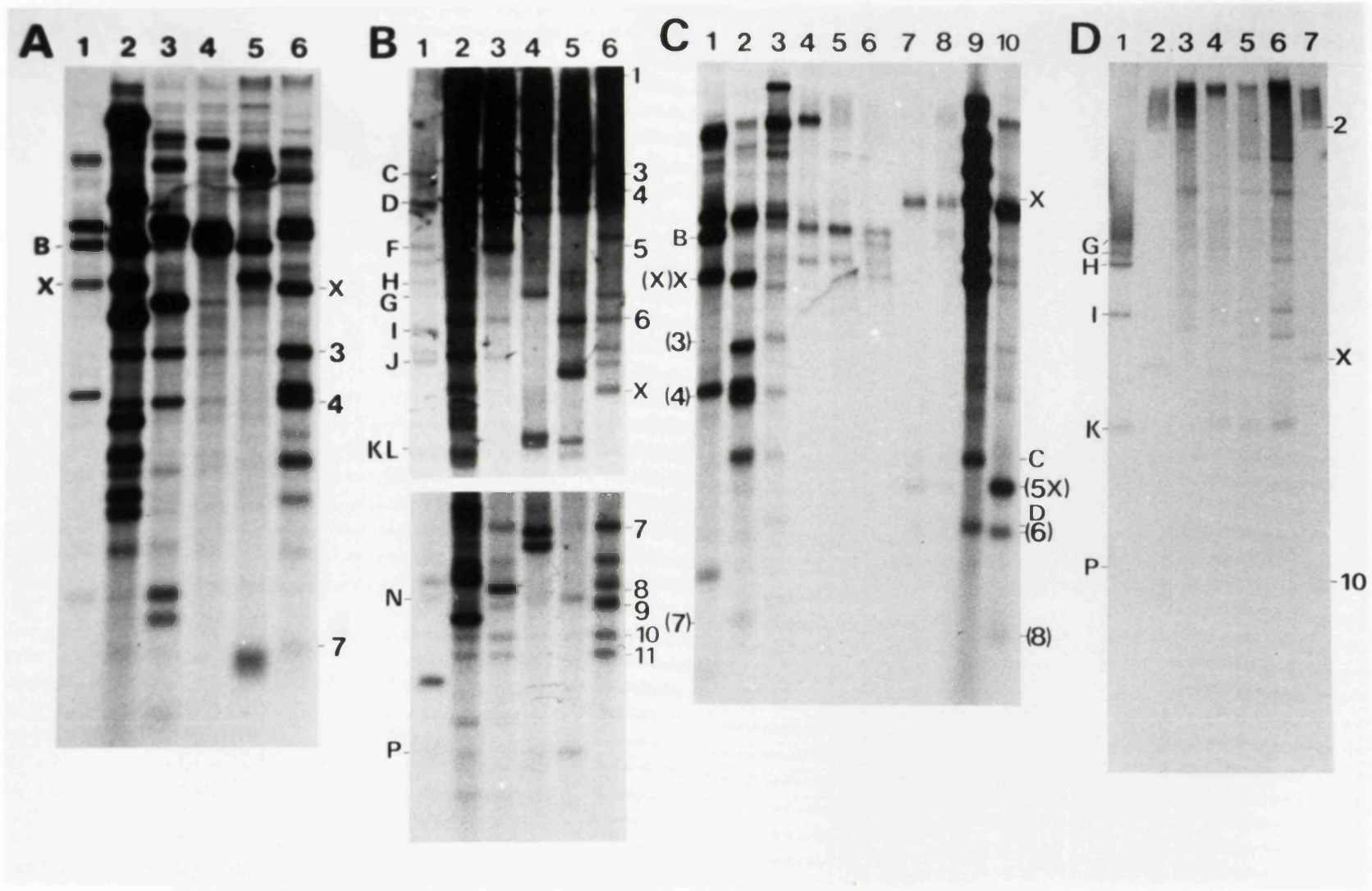

A
1 2 3 4 5 6
B
X
X
3
4
7
B
1 2 3 4 5 6
C
D
F
H
G
I
J
KL
1
3
4
5
6
X
N
P
7
8
9
10
11
C
1 2 3 4 5 6 7 8 9 10
B
(X)X
(3)
(4)
(7)
X
C
(5X)
D
(6)
(8)
D
1 2 3 4 5 6 7
G
H
I
K
P
2
X
10

Figure C4.14

Structures of the joints and termini of Bx6(17-6).

A
1 tsB
2 Bx1(31-2)
3 Bx6(17-6)
4 tsl

In vivo ^{32}P-labeled DNA digested with BamH I; 1% agarose gel.

B
1 tsB BamH I st
2 tsB BamH I s
3 tsB BamH I k
4 Bx6(17-6) BamH I joint
5 Bx6(17-6) BamH I short terminus
6 Bx6(17-6) BamH I long terminus, with HSV-2 BamH I w
7 tsl BamH I g
8 tsl BamH I u
9 tsl BamH I vw
10 tsl BamH I x

Digested with Sst I; 2% agarose gel

C
1 tsB BamH I k
2 Bx6(17-6) BamH I joint
3 tsl BamH I g

Digested with Sma I; 7.5% polyacrylamide gel.

D
1 tsB BamH I s
2 tsB BamH I k
3 Bx6(17-6) BamH I short terminus
4 Bx6(17-6) BamH I long terminus, with HSV-2 BamH I w
5 tsl BamH I u
6 tsl BamH I vw
7 tsl BamH I x

Digested with Sma I; 7.5% polyacrylamide gel.

HSV-1 and HSV-2 restriction fragments are indicated to the left and right, respectively, of each panel. In panel A they refer to the BamH I digest of HSV-1 and HSV-2 DNA, and in panels B-D to the Sst I or Sma I digest of BamH I joint fragments.

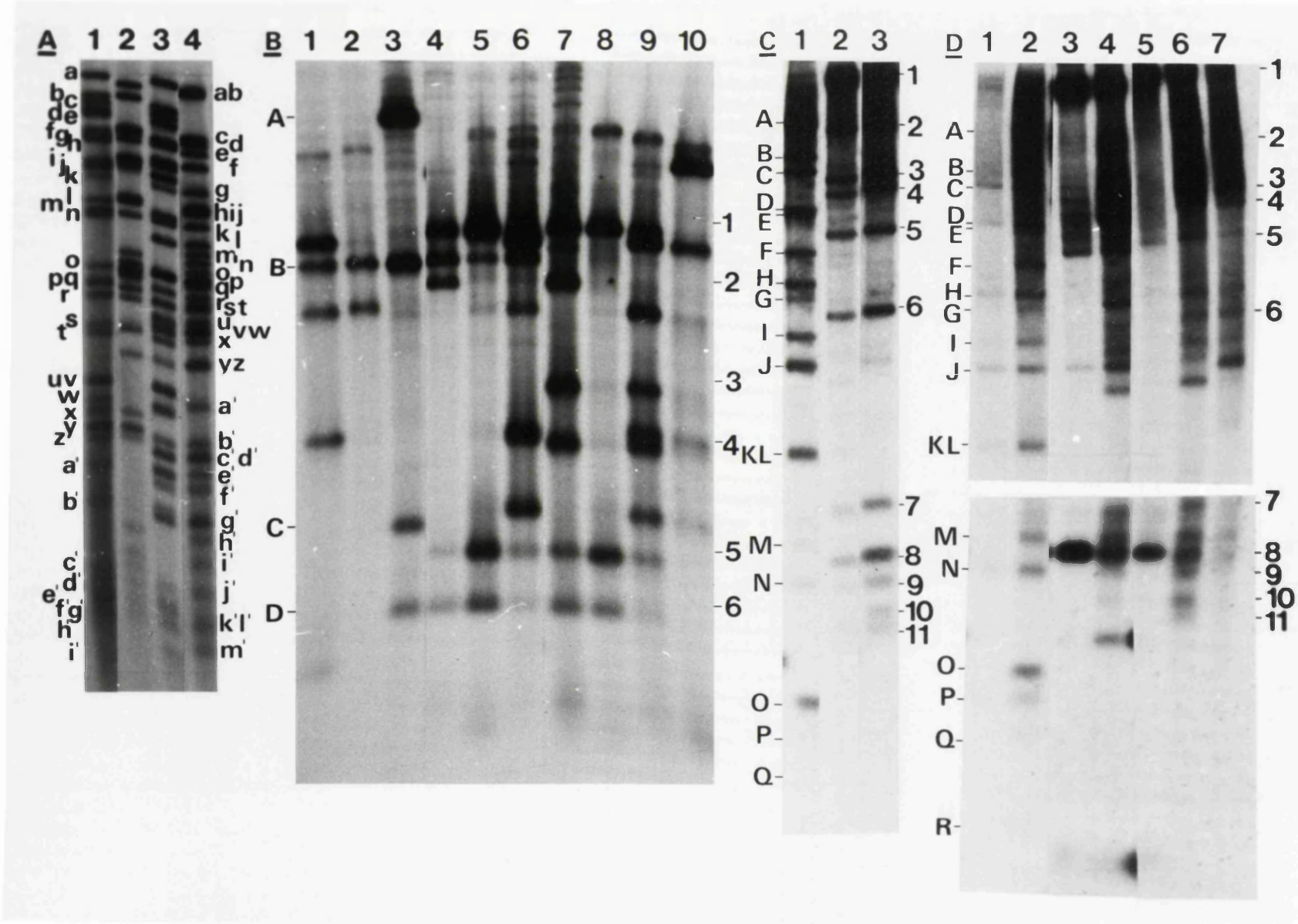

A
1 2 3 4
B
1 2 3 4 5 6 7 8 9 10
C
1 2 3
D
1 2 3 4 5 6 7

Figure C4.15

Structures of the joints and termini of RS6 and R13-2.

A
1 tsB BamH I k
2 tsB BamH I s
3 RS6 BamH I joint
4 RS6 BamH I long terminus
5 tsl BamH I g
6 tsl BamH I u
7 tsl BamH I vw

Digested with Sst I; 2% agarose gel.

B As for panel A but digested with Sma I; 7.5% polyacrylamide gel.

C
1 tsB BamH I k
2 tsl BamH I g
3 R13-2 BamH I larger joint
4 R13-2 BamH I smaller joint
5 R13-2 BamH I type 2 long terminus
6 R13-2 BamH I type 1 long terminus, with HSV-2 BamH I x
7 tsB BamH I s
8 tsl BamH I vw

Digested with Sma I; 7.5% (upper) and 15% (lower) polyacrylamide gels.

Sst I or Sma I restriction fragments of BamH I joint fragments are indicated to the left (HSV-1) and right (HSV-2) of each panel.

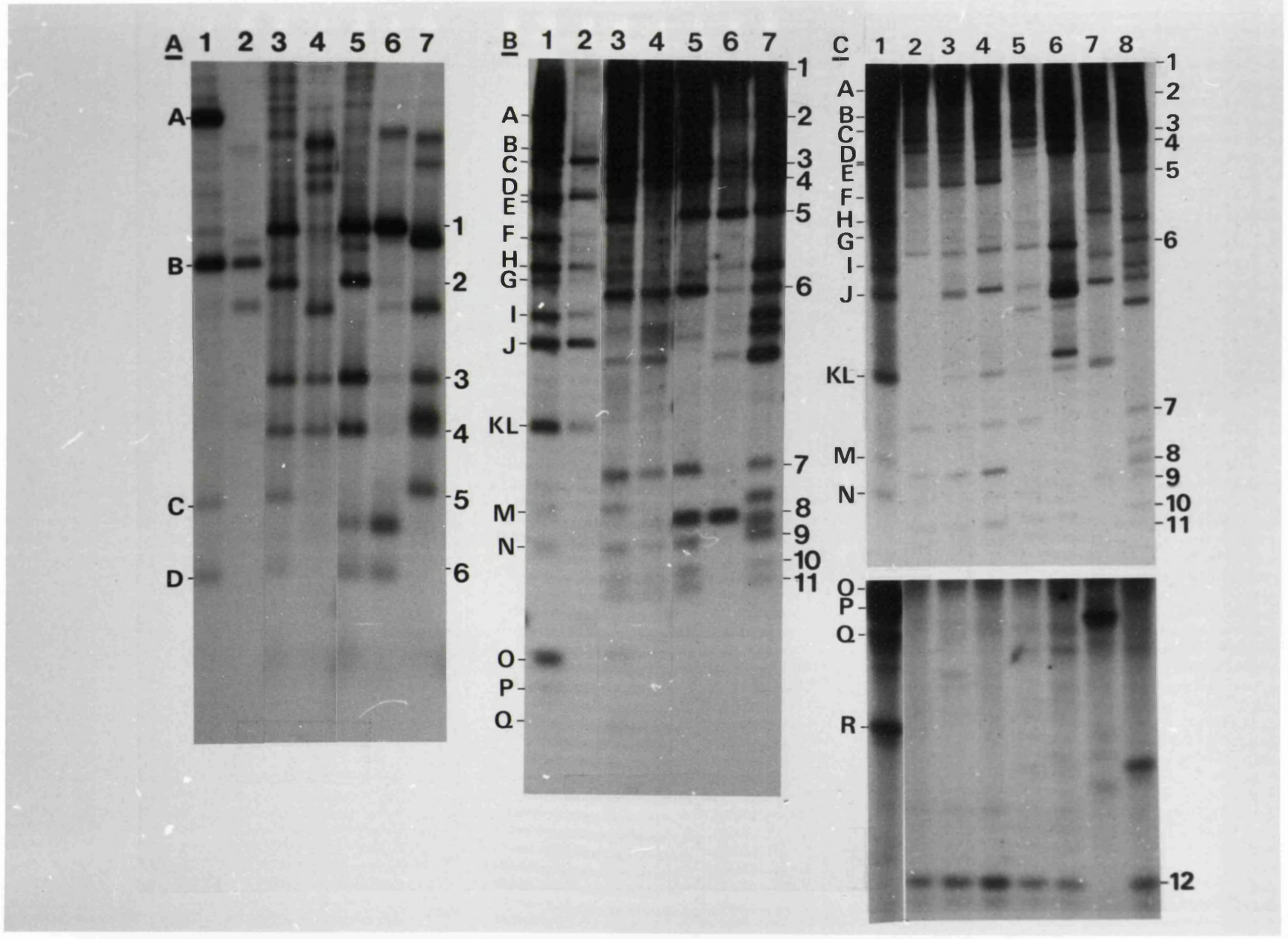

A
1 2 3 4 5 6 7
A
B
C
D
1
2
3
4
5
6
B
1 2 3 4 5 6 7
A
B
C
D
E
F
H
G
I
J
KL
M
N
O
P
Q
1
2
3
4
5
6
7
8
9
10
11
C
1 2 3 4 5 6 7 8
A
B
C
D
E
F
H
G
I
J
KL
M
N
O
P
Q
R
1
2
3
4
5
6
7
8
9
10
11
12

Figure C4.16

Structures of the joints and termini of Dxl(2).

A
1 tsB BamH I k
2 tsD BamH I k
3 Dxl(2) BamH I joint
4 subclone of subclone 2 of Bxl(28-1) BamH I joint
5 tsl BamH I g

Digested with Sma I; 7.5% (upper) and 15% (lower) polyacrylamide gels.

B
1 tsB Sst I C and D (isolated)
2 Dxl(2) BamH I short terminus from the right hand end of S
3 Dxl(2) BamH I short terminus from the left hand end of S
4 tsl BamH I u

Digested with Sst I, except for track 1; 7.5% polyacrylamide gel.

C
1 tsB BamH I pq
2 tsB BamH I s
3 Dxl(2) BamH I short terminus and larger long terminus
4 Dxl(2) BamH I long terminus
5 ts6 BamH I u
6 ts6 BamH I vw

Digested with Sst I; 2% agarose gel.

D
1 tsB BamH I pq
2 tsB BamH I s
3 Dxl(2) BamH I short terminus and larger long terminus
4 Dxl(2) BamH I long terminus
5 ts6 BamH I u
6 ts6 BamH I vw

Digested with Sma I; 7.5% (upper) and 15% (lower) polyacrylamide gels.

Restriction fragments of BamH I joint (panel A) and long and short terminal fragments (panels B-D) are indicated to the left (HSV-1) and right (HSV-2) of each panel. X_L denotes the terminal Sst I or Sma I fragment of L, and X_S the terminal fragment of S.

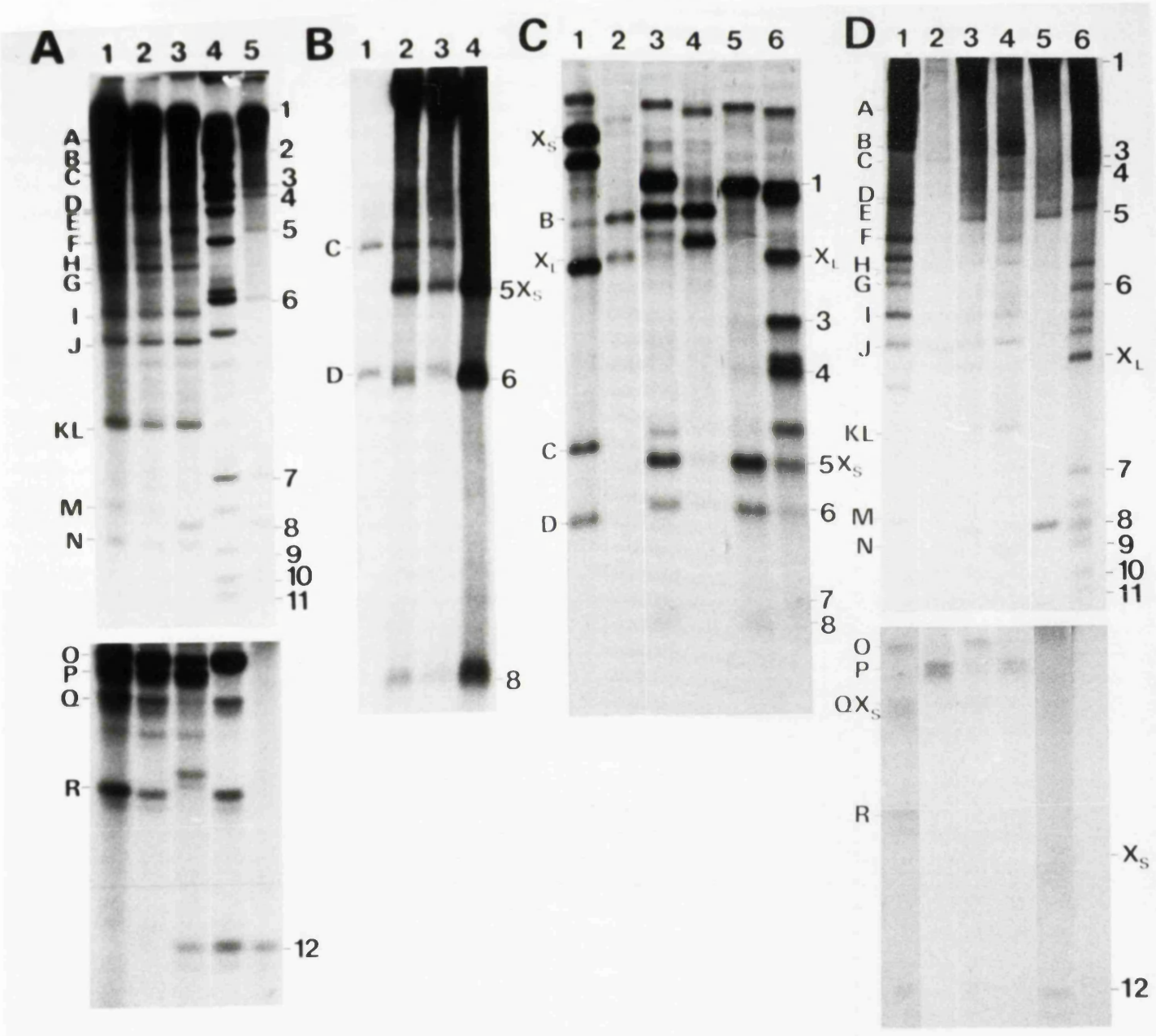

A
1 2 3 4 5
A
B
C
D
E
F
H
G
I
J
KL
M
N
O
P
Q
R
1
2
3
4
5
6
7
8
9
10
11
12
B
1 2 3 4
C
D
5XS
6
8
C
1 2 3 4 5 6
XS
B
XL
C
D
1
XL
3
4
5XS
6
7
8
D
1 2 3 4 5 6
A
B
C
D
E
F
H
G
I
J
KL
M
N
O
P
QXS
R
1
3
4
5
6
XL
7
8
9
10
11
XS
12

Figure C4.17

Detailed genome structures of Bxl(28-1) subclones and other recombinants: (a) fixed in L, (b) free in L. U_L and U_S have been shortened, and the crossover in U_L for Bxl(28-1) subclones is in the region 0.490-0.515. Rectangles represent repetitive regions in the conventional manner, black indicating type 2 and white type 1 DNA. The a sequences are shown on a larger scale for clarity. Restriction sites defining crossovers are shown at the top and bottom of the diagram, and also above (HSV-1) and below (HSV-2) each representative genome (B BamH I, K Kpn I, R EcoR I, H Hpa I). Crosses indicate deleted sequences and cross-hatching shows regions of uncertainty for crossovers where greater than 300 bp. In some cases crossovers were determined by fine restriction mapping in the joint and terminal regions. Recombinant Dxl(2) has two heterotypic a sequences at the joint and so IR_S has been shifted rightwards.

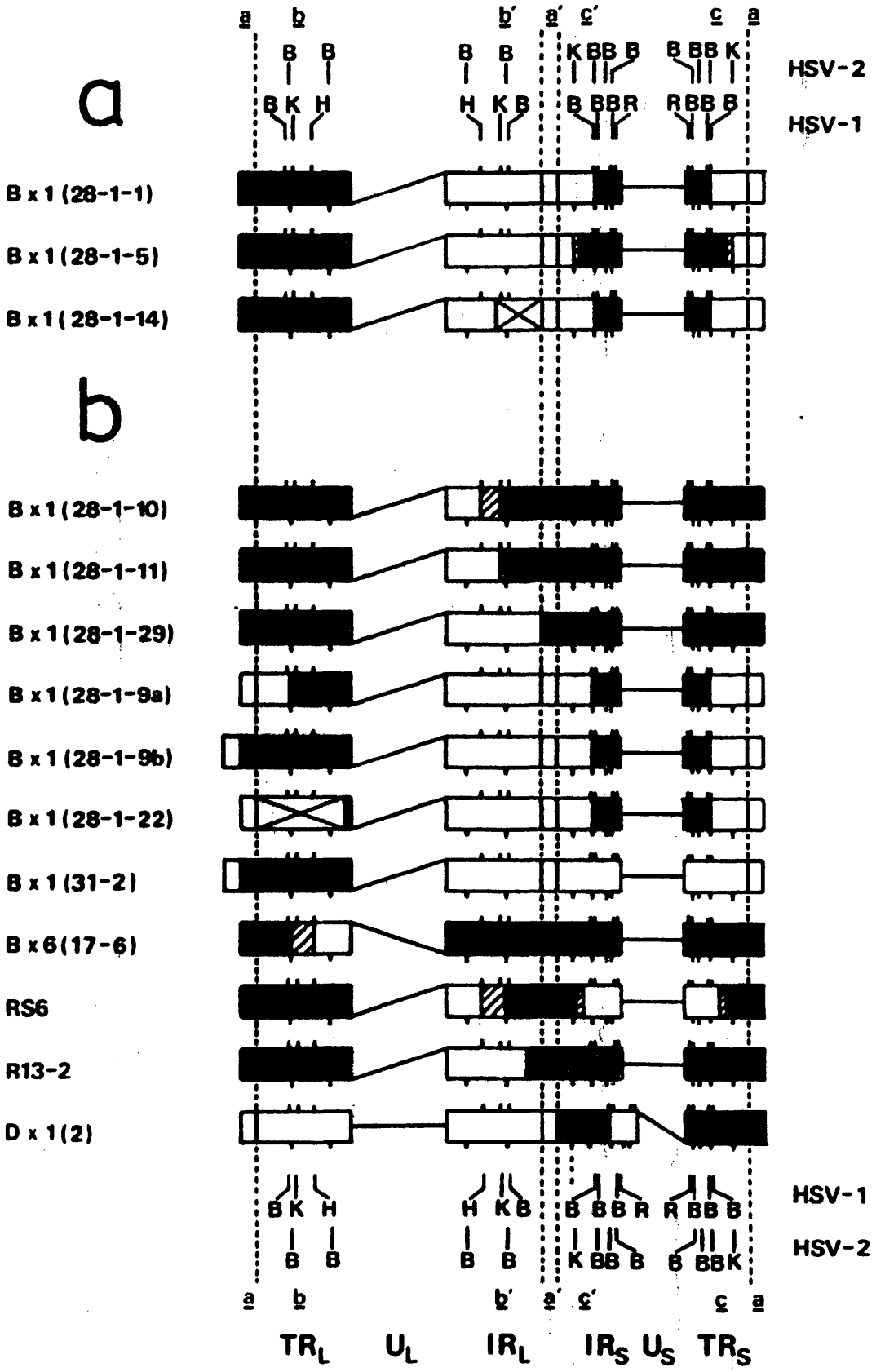

a
b
B x 1 (28-1-1)
B x 1 (28-1-5)
B x 1 (28-1-14)
B x 1 (28-1-10)
B x 1 (28-1-11)
B x 1 (28-1-29)
B x 1 (28-1-9a)
B x 1 (28-1-9b)
B x 1 (28-1-22)
B x 1 (31-2)
B x 6 (17-6)
RS6
R13-2
D x 1 (2)
HSV-2
HSV-1
HSV-1
HSV-2
a b b' a' c' c a
TRL UL IRL IRS US TRS

Figure C4.18

Upper panel

Genome structures possibly present in Bxl(28-1), and that of the putative original recombinant Bxl(28) from which they arose. Black indicates HSV-2 and white HSV-1 sequences.

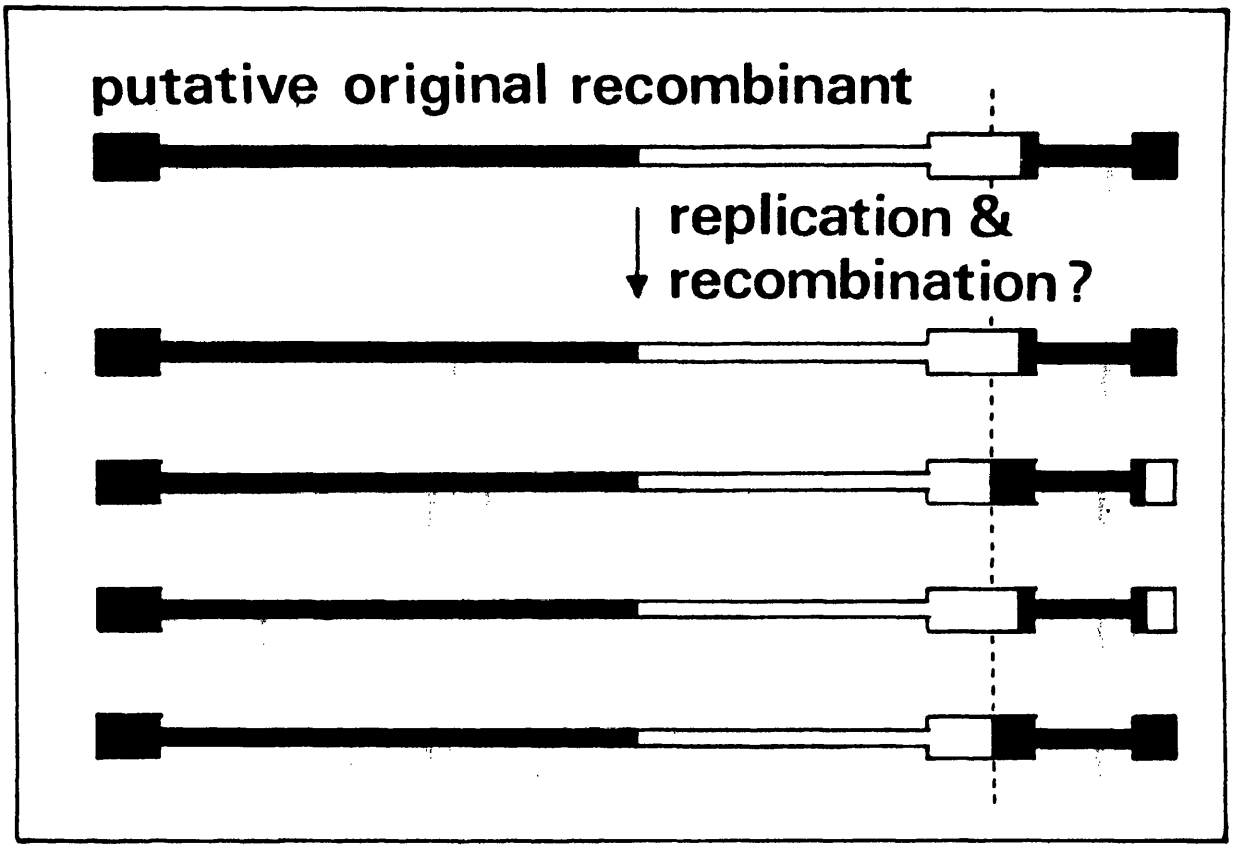
Lower panel

Structures of the joint regions of three recombinant genomes. Black indicates HSV-2 and white HSV-1 sequences, and cross-hatching denotes the region of uncertainty of crossover position. The a sequences are shown as broader rectangles and the junction between two a sequences by vertical lines extending above and below them. Sizes of novel SmaI fragments containing the crossovers are given.

a Subclone 29 of Bxl(28-1) (Figure C4.10 panel A track 4; entire genome structure in Figure C4.17).

b Dxl(2) (Figure C4.16 panel A track 3; entire genome structure in Figure C4.17).

c Subclone of subclone 2 of Bxl(28-1) (Figure C4.16 panel A track 4; entire genome structure in Figure C4.21, second type of progeny).


putative original recombinant
replication &
recombination?

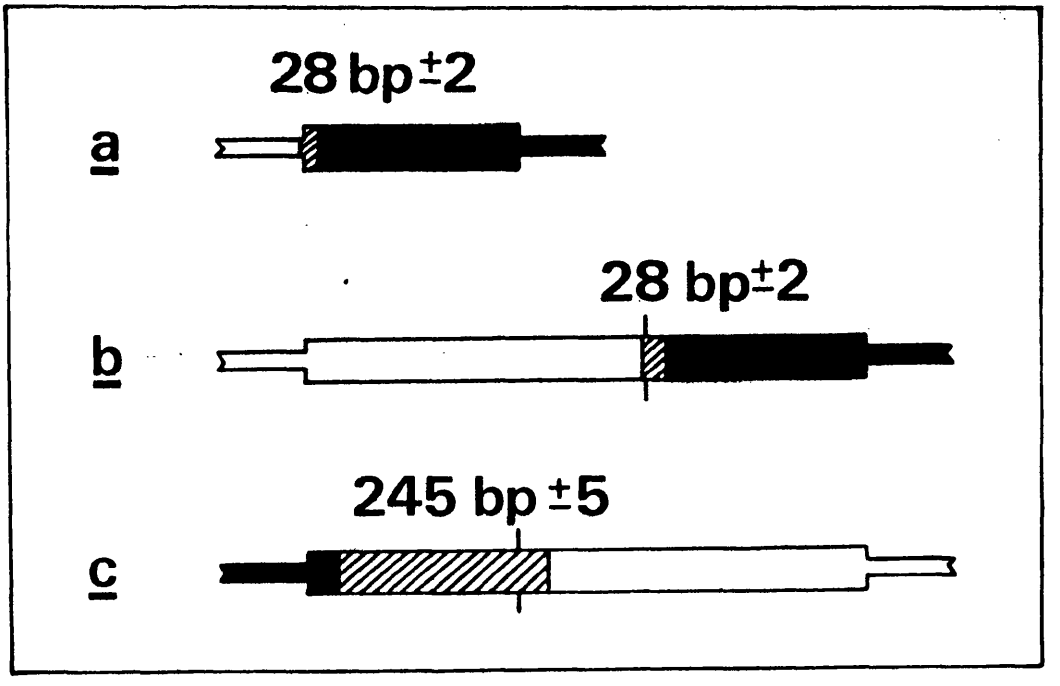

28 bp±2
a
28 bp±2
b
245 bp±5
c

Figure C4.19

Genome structures of subclones of Bxl(28-1) used to investigate expression of IE 110/118.
U_L and U_S have been shortened to emphasise details of the repetitive regions. Restriction sites within repetitive regions are shown above the genome for HSV-1 and below for HSV-2 (B = BamH I, K = Kpn I, H = Hpa I, R = EcoR I). Black denotes HSV-2 DNA and white HSV-1 DNA, cross-hatching indicates uncertainty of crossover position where greater than 300 bp, and a cross signifies deleted sequences. The crossover position in U_L is between 0.490 and 0.515 fractional genome units for each subclone. The positions and direction of transcription of the genes coding for IE 110 and IE 118 are shown (A. Easton, F. Rixon and J.B. Clements, personal communication).

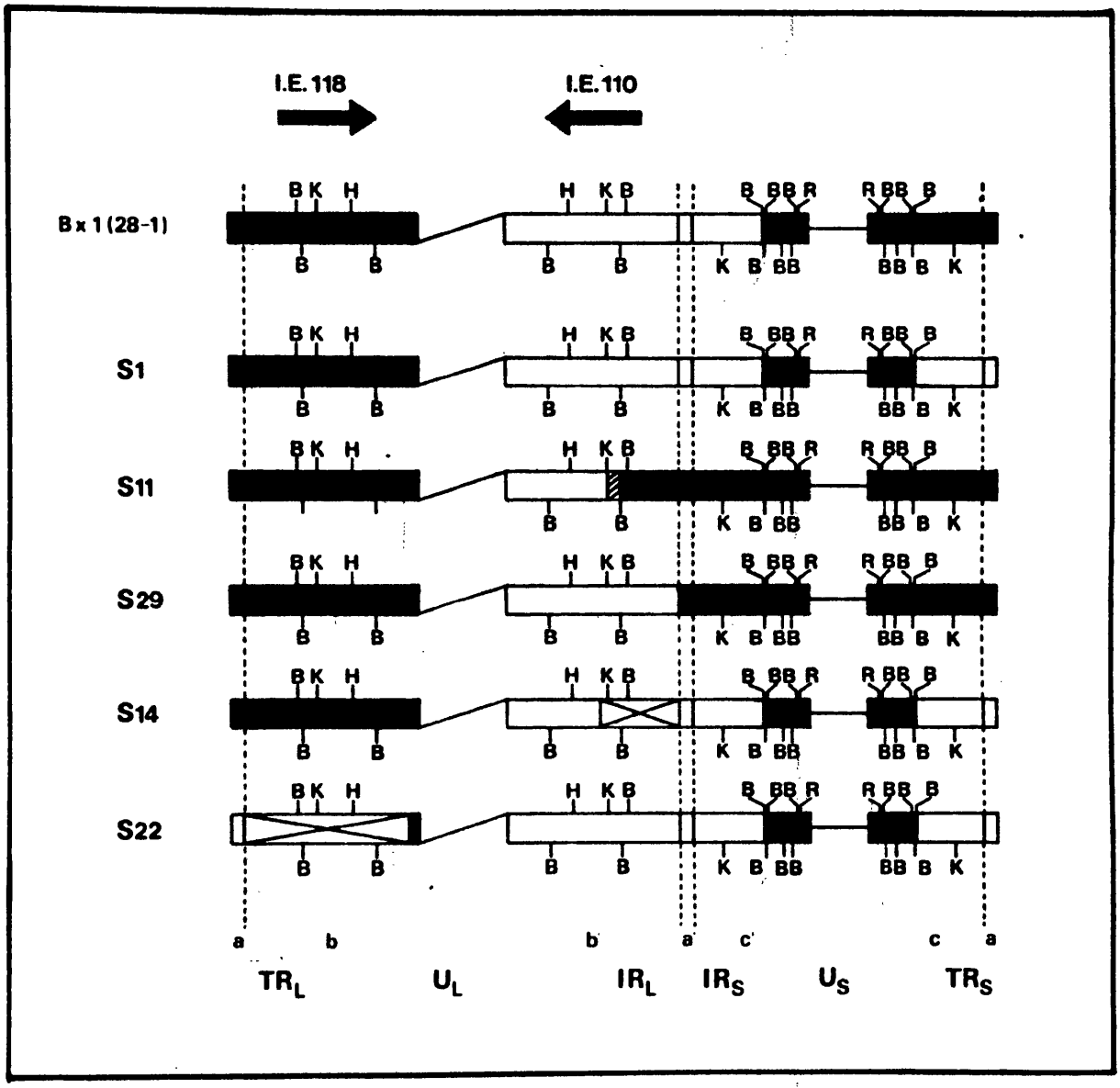

I.E. 118
I.E. 110
B x 1 (28-1)
S1
S11
S29
S14
S22
a
b
b'
a'
c'
c
a
TR$_L$
U$_L$
IR$_L$
IR$_S$
U$_S$
TR$_S$

Figure C4.20

Autoradiograph of IE polypeptides induced in HFL cells by HSV-1 tsB (T1), HSV-2 tsl (T2), recombinants Bxl(31-2) and Bxl(28-1) and subclones (S) derived from Bxl(28-1). Also included are mock-infected (MI) samples. IE polypeptides from whole infected cells were separated on 5-12% polyacrylamide gradient gels (lanes 1-10) or nuclear extracts were made and separated on 7.5% polyacrylamide gels (lanes 11-21). Numbers to the left of lane 1 and to the right of lane 21 show the apparent molecular weights (x 10^{-3}) of HSV-1 IE polypeptides. Numbers between lanes 10 and 11 show the apparent molecular weights (x 10^{-3}) of HSV-2 IE polypeptides.
Symbols are placed to the left of the lane to which they refer.

■ HSV-1 polypeptide

o HSV-2 polypeptide

\+ altered polypeptide

This experiment was performed by Dr H.S. Marsden.

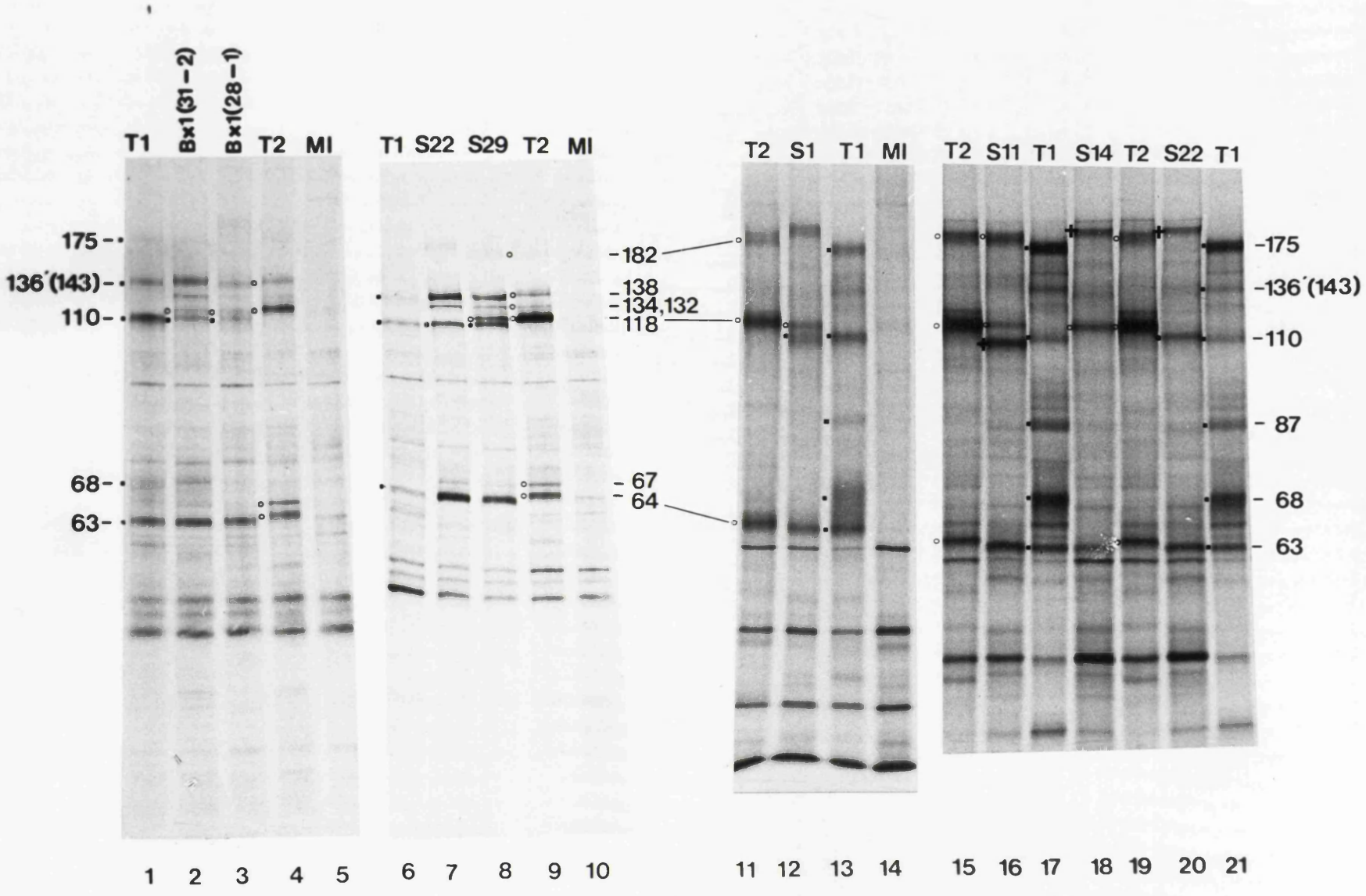

T1
Bx1(31–2)
Bx1(28–1)
T2
MI
T1 S22 S29 T2 MI
T2 S1 T1 MI
T2 S11 T1 S14 T2 S22 T1
175
136´(143)
110
68
63
182
138
134,132
118
67
64
87
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21

Figure C4.21

Genome structures of the progeny of two subclones of Bxl(28-1): (a) subclone 29, (b) subclone 2. Schematic details are the same as in Figure C4.17, except that a sequences are not emphasised. The parental structures are shown, and the number of progeny with each resulting genome structure. F indicates fixed in L and N indicates normally inverting (free) in L.

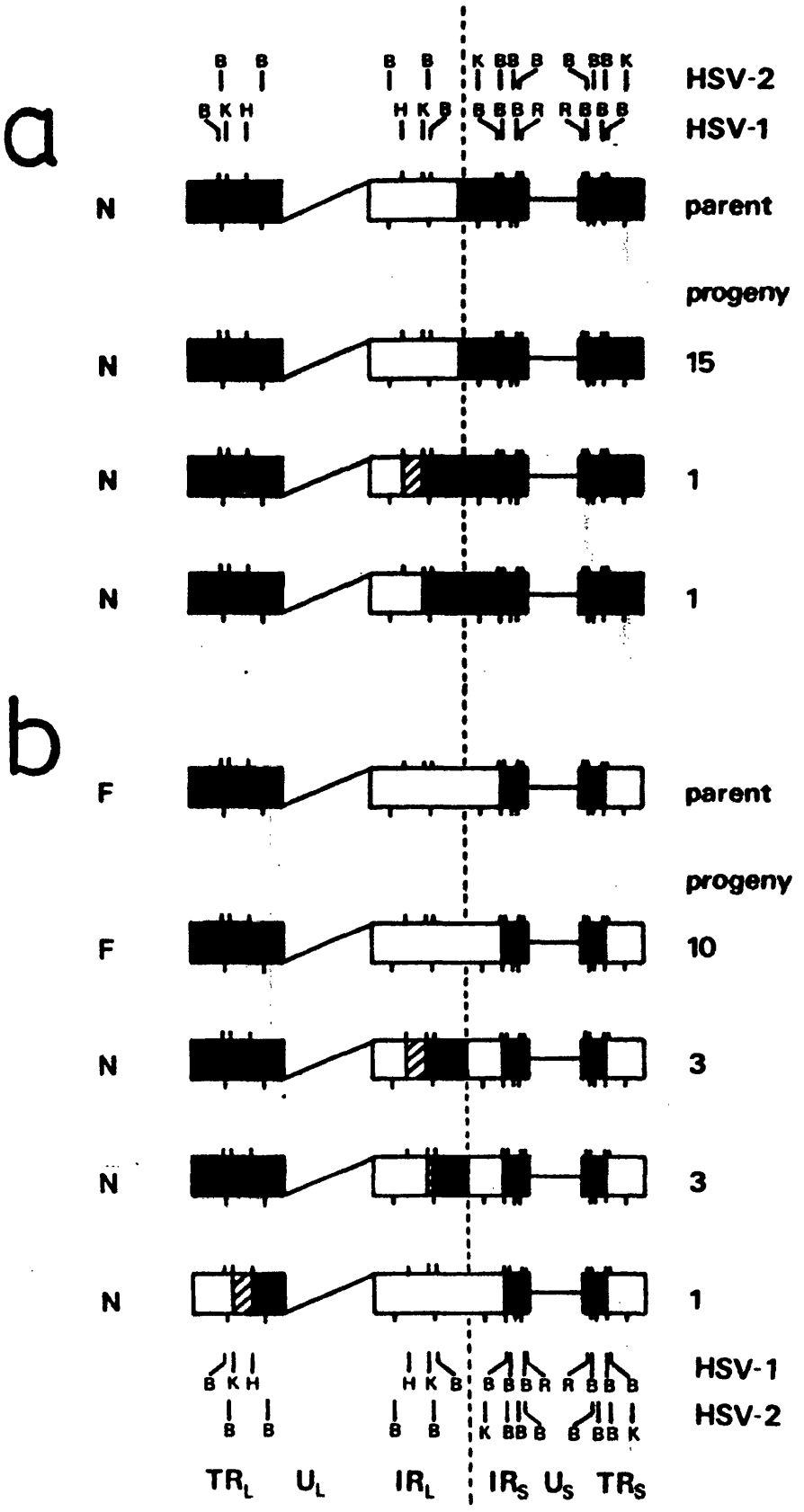

a
b
HSV-2
HSV-1
parent
progeny
15
1
1
parent
progeny
10
3
3
1
HSV-1
HSV-2
N
F
TRL UL IRL IRS US TRS

Figure C4.22

Restriction profiles of *in vivo* ^{32}P-labeled DNA from RE4 (R) and some of its subclones (numbered) compared with HSV-1 (T1) and HSV-2 (T2) for *Hind* III (left; 0.4% agarose gel) and *BamH* I (right; 1% agarose gel). HSV-1 restriction fragments are indicated to the left of each panel, and HSV-2 restriction fragments to the left of the vertical line.

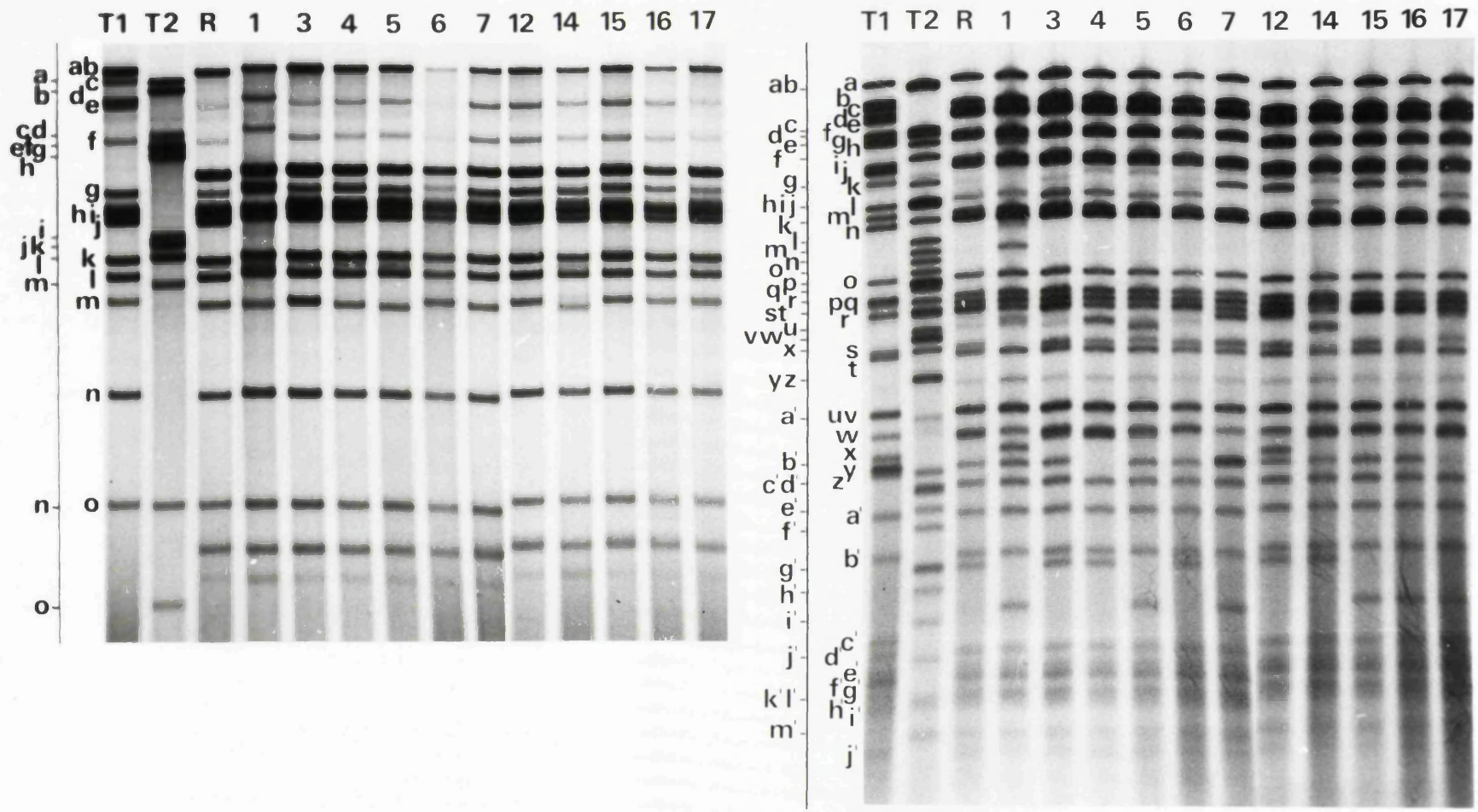

T1 T2 R 1 3 4 5 6 7 12 14 15 16 17
T1 T2 R 1 3 4 5 6 7 12 14 15 16 17

Figure C4.23

Hybridisation of specific probes to Southern blots of RE4 restriction digests.

The tracks are Hind III (upper half of Figure) or BamH I (lower half) digests of HSV-1 (T1), RE4 (R) or HSV-2 (T2) DNA transferred to nitrocellulose from agarose gels. A-C were transferred simultaneously from the same gel, and likewise D-E. The nitrocellulose filters were cut into portions containing three tracks and hybridised with nick translated DNA as follows.

A RE4 DNA
B recombinant plasmid containing HSV-2 Hind III n
C recombinant plasmid containing HSV-2 Hind III o
D RE4 DNA
E Hind III TJ fragment isolated from RE4 DNA (slightly contaminated with RE4 Hind III $\bar{x}$)

Those fragments which hybridised to probes B, C and E are indicated: HSV-1 fragments on the left of the track, HSV-2 on the right. Fragments novel to RE4 are indicated below the band. The interpretation of the novel fragments is as follows (see Figure C4.26).

TJ Hind III or BamH I fragment containing the junction between HSV-2 Hind III o (HSV-2 BamH I f) and HSV-2 Hind III b (HSV-2 BamH I i)

x Hind III or BamH I terminal fragment of L generated by the transposition

$\bar{x}$ x with an additional a sequence

j Hind III or BamH I joint fragments containing x, generated by the transposition

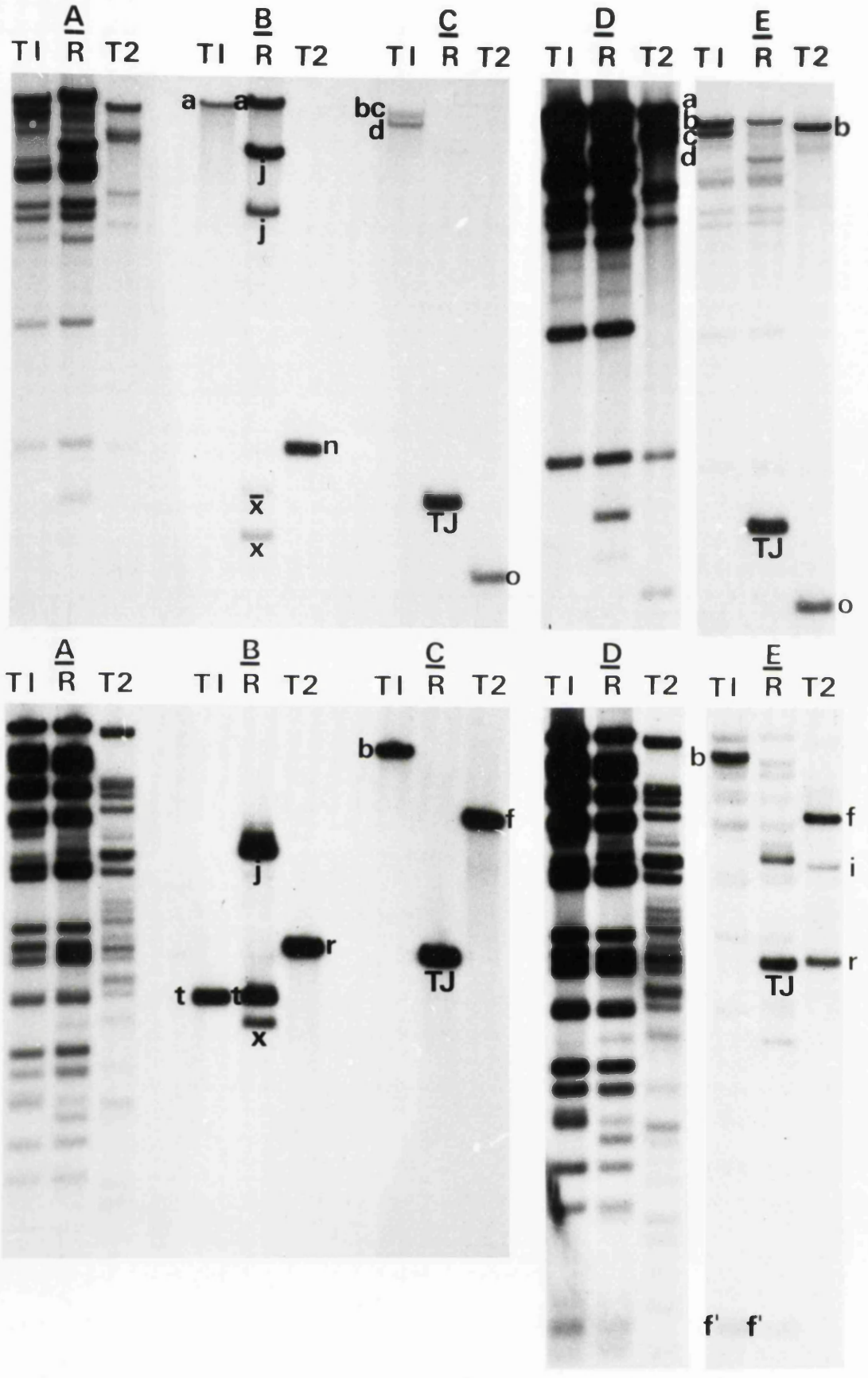

A
B
C
D
E
T1 R T2
a
bc
d
j
n
x̄
x
TJ
o
b
f
i
r
t
f'

Figure C4.24

Location of the inserted DNA in RE4.

A

M	pBR322 HaeIII markers
1	HSV-2 BamHI i digested with HhaI
2	RE4 BamHI TJ digested with HhaI
3	RE4 HindIII/BamHI TJ digested with HhaI
4	HSV-2 HindIII o digested with HhaI
5	HSV-2 BamHI i digested with AluI
6	RE4 BamHI TJ digested with AluI
7	RE4 HindIII/BamHI TJ digested with AluI
8	HSV-2 HindIII o digested with AluI

7.5% (upper) and 15% (lower) polyacrylamide gels.

B

1	HSV-2 BamHI i digested with SmaI
2	RE4 BamHI TJ digested with SmaI
3	RE4 HindIII/BamHI TJ digested with SmaI
4	HSV-2 HindIII o digested with SmaI
M	pBR322 HinfI markers

2% agarose gel.

Marker sizes are indicated in bp.

TJ denotes a restriction fragment containing the junction between normal HSV-2 and inserted HSV-2 sequences. The locations of BamHI TJ and the double digest fragment HindIII/BamHI TJ are shown in Figure C4.26.

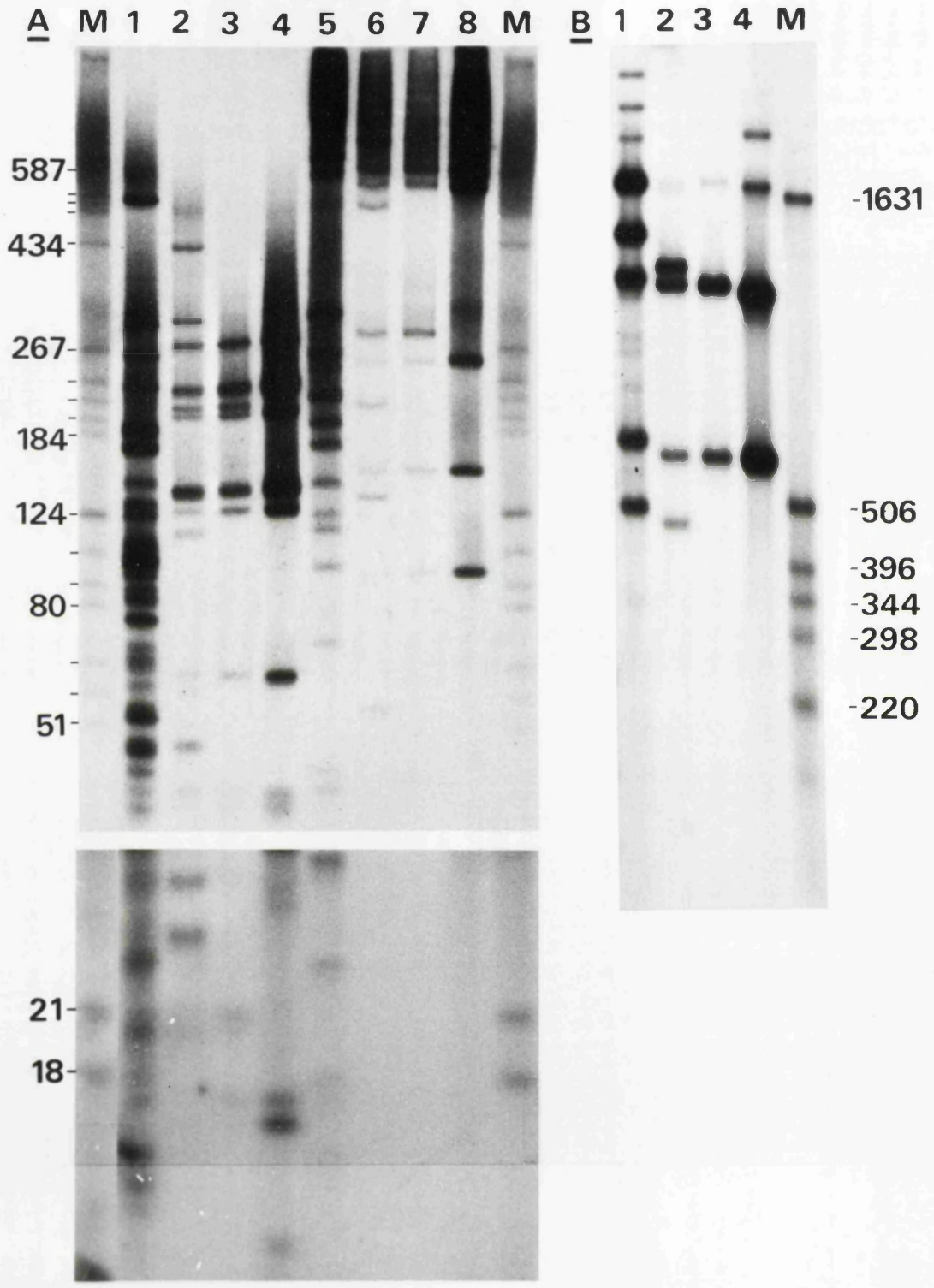

A
M
1
2
3
4
5
6
7
8
M
587
434
267
184
124
80
51
21
18
B
1
2
3
4
M
1631
506
396
344
298
220

Figure C4.25

Structures of the joints and termini of RE4.

A
1 tsB BamH I k
2 RE4 BamH I major joint
3 tsl BamH I g

Digested with Sst I; 2% agarose gel.

B
1 tsB BamH I q
2 RE4 BamH I major short terminus, isolated from type 1 EcoR I k
3 RE4 BamH I major short terminus, isolated from type 1 Hind III m

Digested with Sst I; 2% agarose gel.

C
1 tsB BamH I k
2 RE4 BamH I major joint
3 tsl BamH I g
4 tsl BamH I r
5 RE4 BamH I novel long terminus
6 RE4 Hind III novel long terminus

Digested with Sma I; 7.5% polyacrylamide gel.

D
1 tsB BamH I k
2 RE4 BamH I major joint
3 tsl BamH I g

Digested with Sma I; 15% polyacrylamide gel.

E
1 RE4 BamH I type 1 long terminus
2 tsB BamH I s

Digested with Sma I; 7.5% polyacrylamide gel.

F
1 tsB BamH I q
2 RE4 BamH I major short terminus

Digested with Sma I; 7.5% polyacrylamide gel.

Sst I or Sma I fragments of the joint or terminal fragments of HSV-1 (left) and HSV-2 (right) are indicated. X denotes a genome terminal fragment.

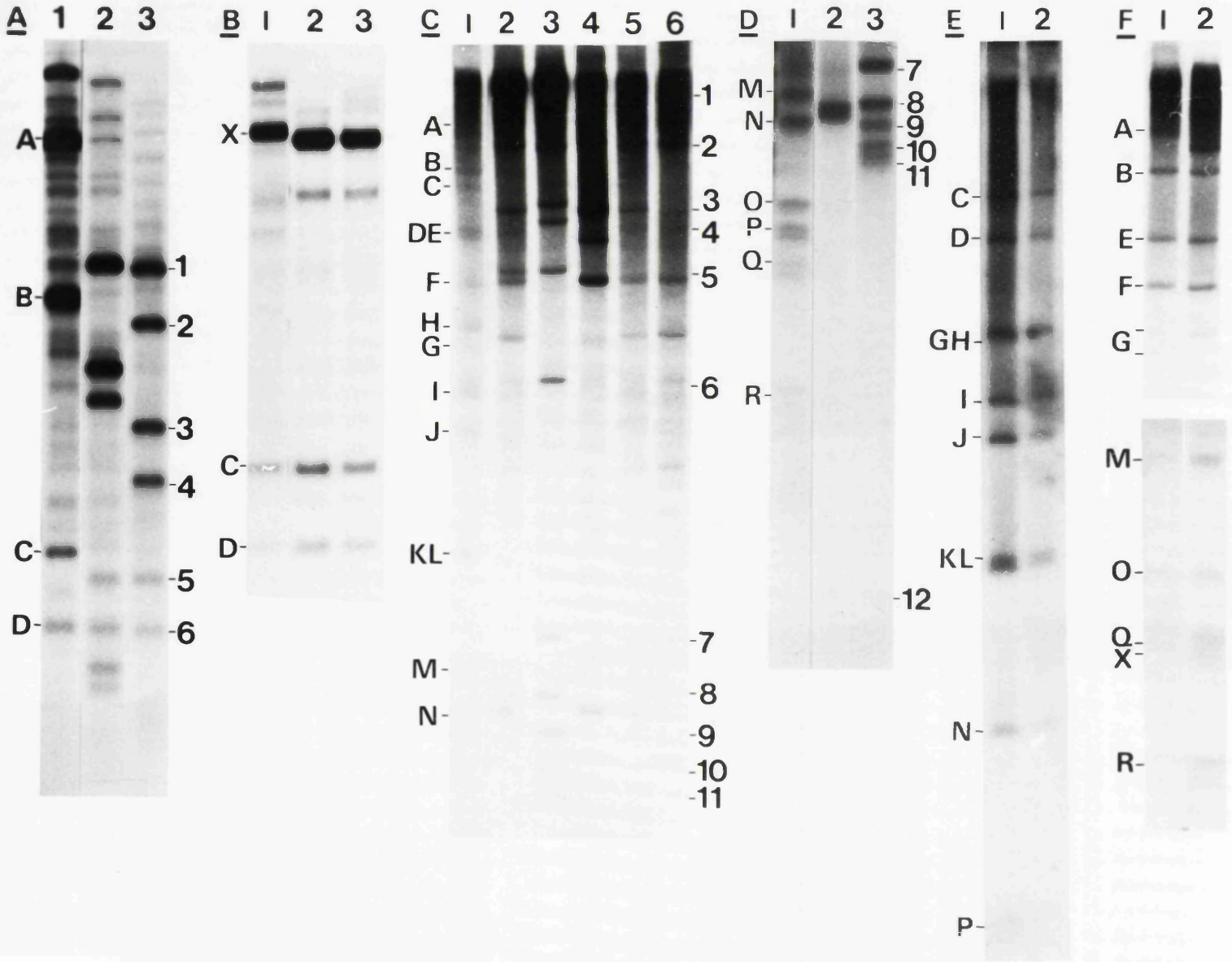

A 1 2 3
A
B
C
D
1
2
3
4
5
6
B I 2 3
X
C
D
C I 2 3 4 5 6
A
B
C
DE
F
H
G
I
J
KL
M
N
1
2
3
4
5
6
7
8
9
10
11
D I 2 3
M
N
O
P
Q
R
7
8
9
10
11
12
E I 2
C
D
GH
I
J
KL
N
P
F I 2
A
B
E
F
G
M
O
Q
X
R

Figure C4.26

Genome structure of RE4.

BamHI fragments present in RE4 DNA are shown at the top of the Figure. Black indicates HSV-2 and white HSV-1 sequence. Regions of uncertainty of crossover location are shown by horizontal hatching and the inserted HSV-2 sequence from U_L is denoted by cross-hatching. The nature of the a sequences (HSV-1 or HSV-2: 1 or 2) is shown inside the stars. RE4 is fixed in L and S in the I_S arrangement as illustrated.

The lower part of the Figure shows HSV-2 HindIII and BamHI fragments which are present in the region of the insertion, vertical arrows indicating restriction sites. Sizes in bp of SmaI, AluI and HhaI fragments containing the junction between normal and inserted ("transposed") HSV-2 sequences are given. To the right in parentheses are given sizes in bp of fragments produced by digestion of the HindIII/BamHI fragment containing the junction which were also produced from HSV-2 BamHI i.

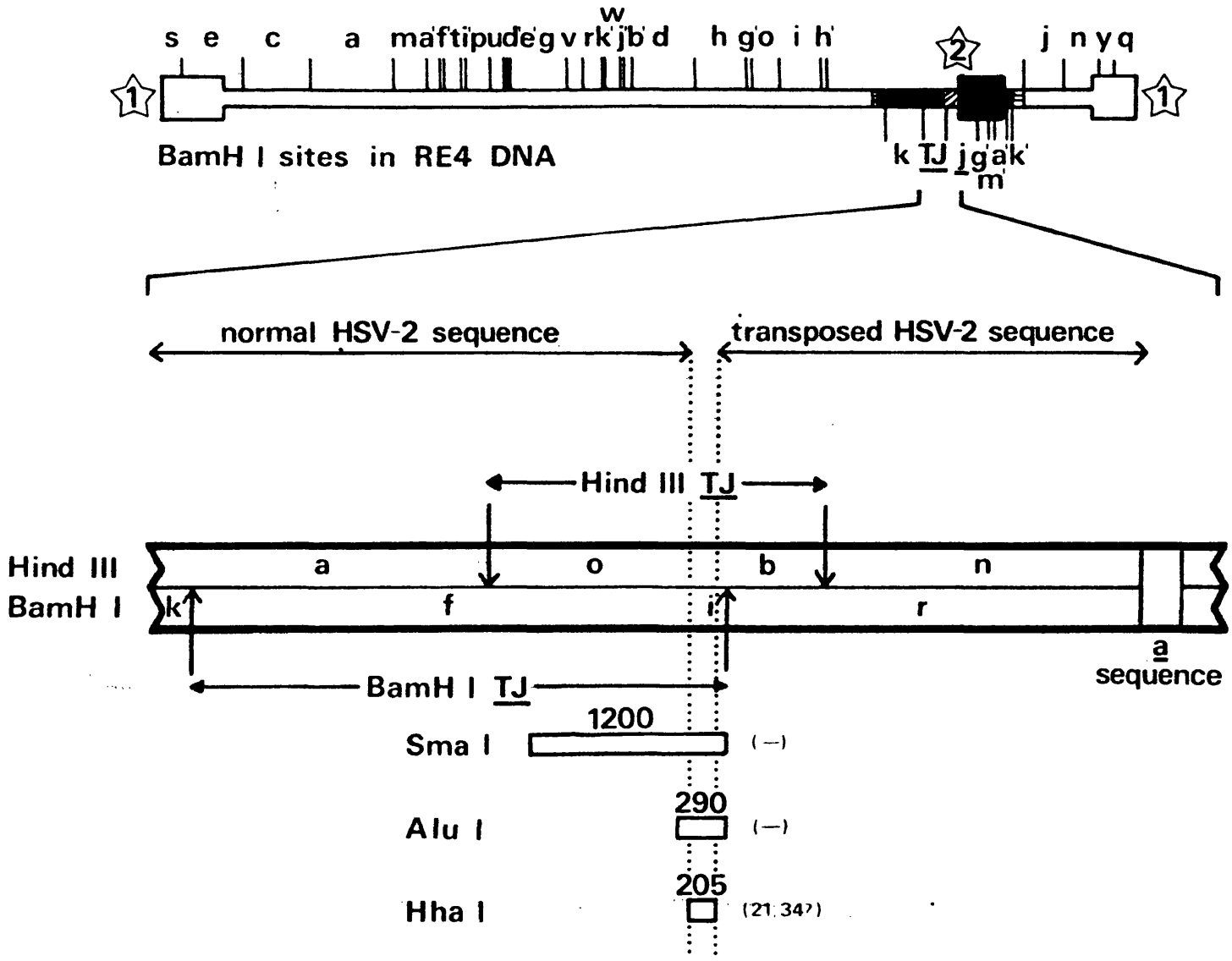

s e c a m a f t i p u d e g v r k w j b d h g o i h
1
2
1
j n y q
BamH I sites in RE4 DNA
k TJ j g a k m
normal HSV-2 sequence
transposed HSV-2 sequence
Hind III TJ
Hind III
BamH I
a o b n
k f i r
a sequence
BamH I TJ
1200
Sma I (—)
290
Alu I (—)
205
Hha I (21, 34?)

Figure C4.27 (4 pages)

Models for the inversion of L and S in HSV DNA.

Model 1 shows separation of L and S, in linear or circular form, and their subsequent recombination in the a sequences (Hayward et al., 1975b; Skare and Summers, 1977).

Model 2 shows intermolecular recombination of linear or circular molecules in a repeat sequence to produce the other arrangements after appropriate cleavage events (Skare and Summers, 1977).

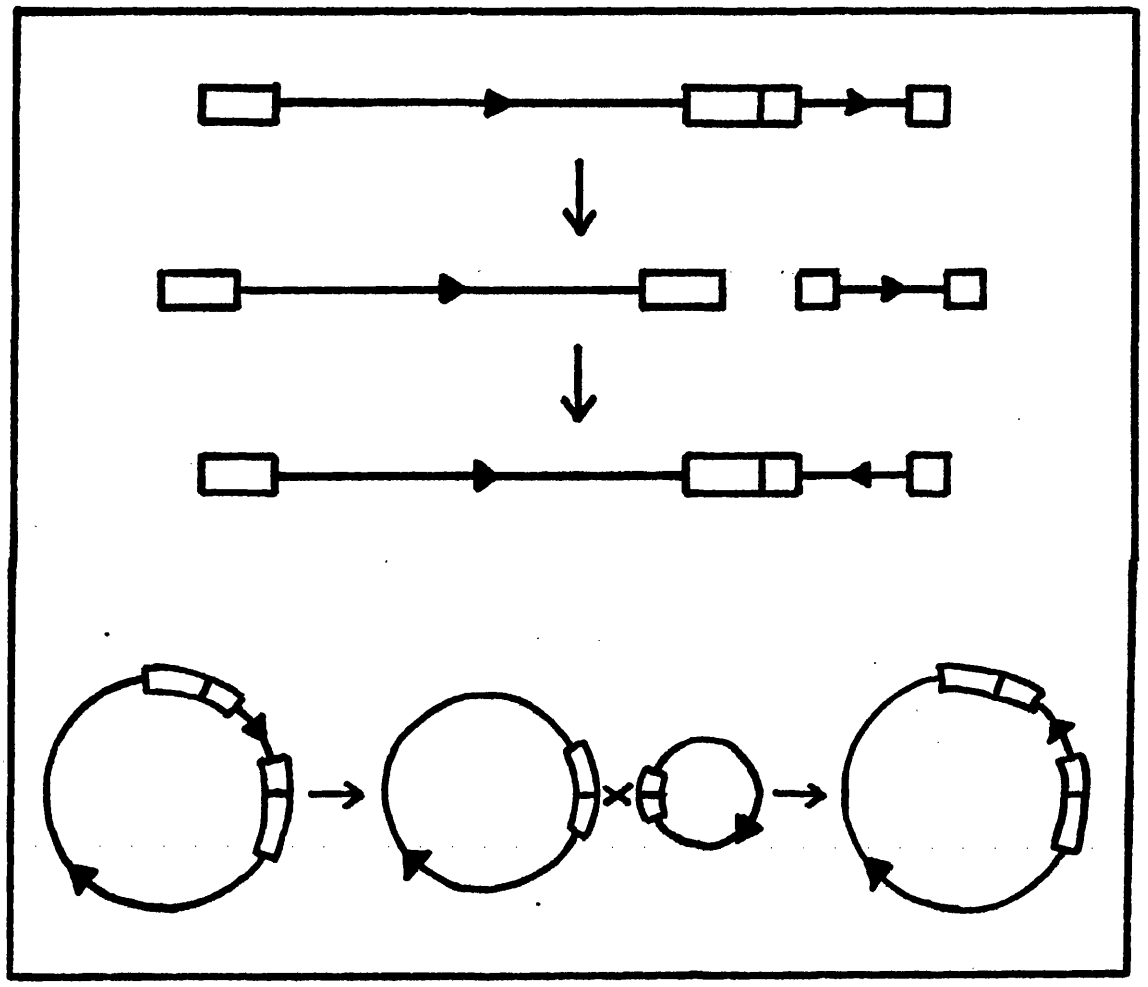
Model 3A shows intramolecular recombination after DNA synthesis using a single strand of the genome as template (Skare and Summers, 1977).

Model 3B shows intramolecular recombination in linear or circular form in a repeat sequence (Sheldrick and Berthelot, 1974; Skare and Summers, 1977; Smiley et al., 1980).

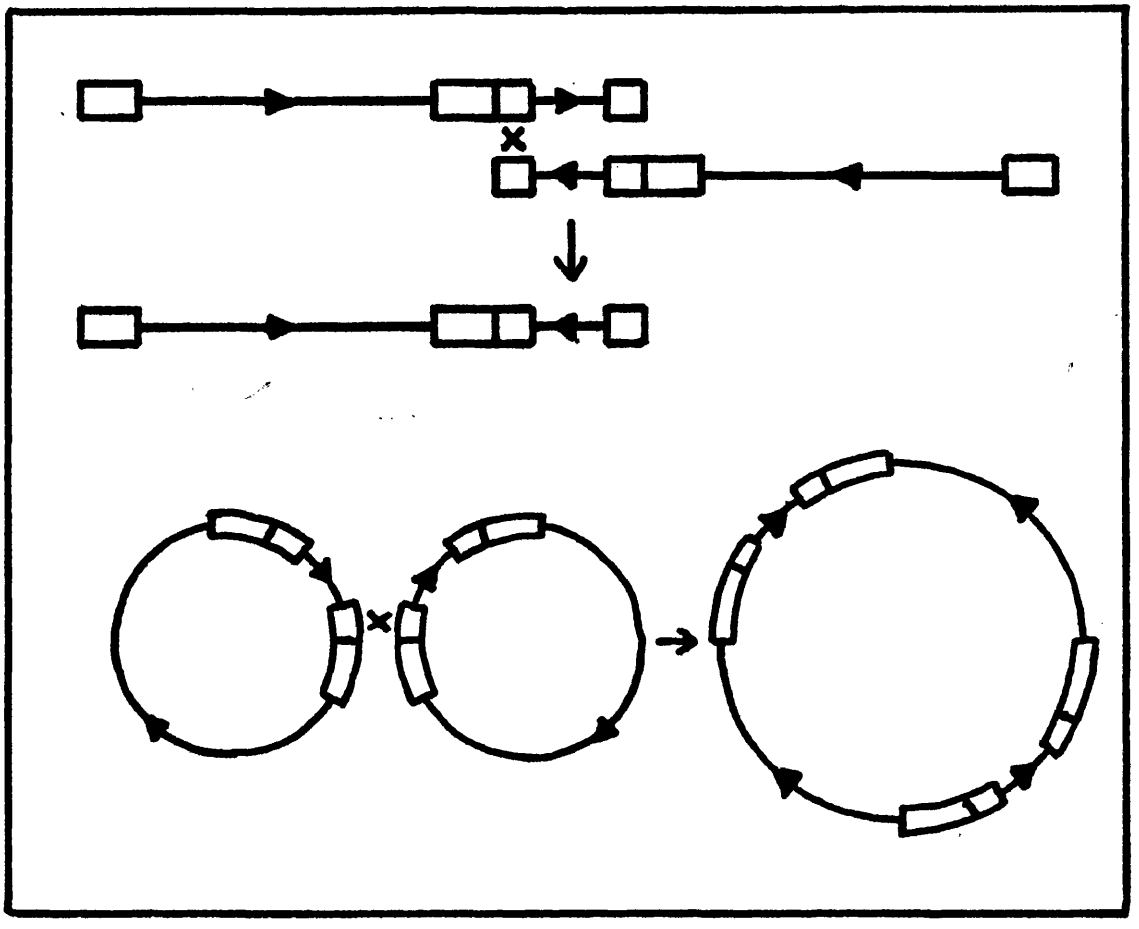
Model 3C shows intramolecular recombination involving repair of a sequences (Jacob and Roizman, 1979; Roizman, 1979). Briefly, the model involves circularisation of the linear DNA molecule by the action of an exonuclease followed by ligation of complementary single stranded termini. DNA is replicated by a rolling circle mechanism to generate concatemers, which are cleaved in alternate joint regions to produce unit-length molecules lacking

an a sequence at one terminus. The missing a sequence is regenerated by "copying" from the joint region. At this point, nicking at the joint a sequence and branch migration causes inversion of the segment to produce the P, I_S and I_L arrangements. The I_{SL} arrangement is produced by a second round of replication of I_S or I_L, with concurrent inversion.

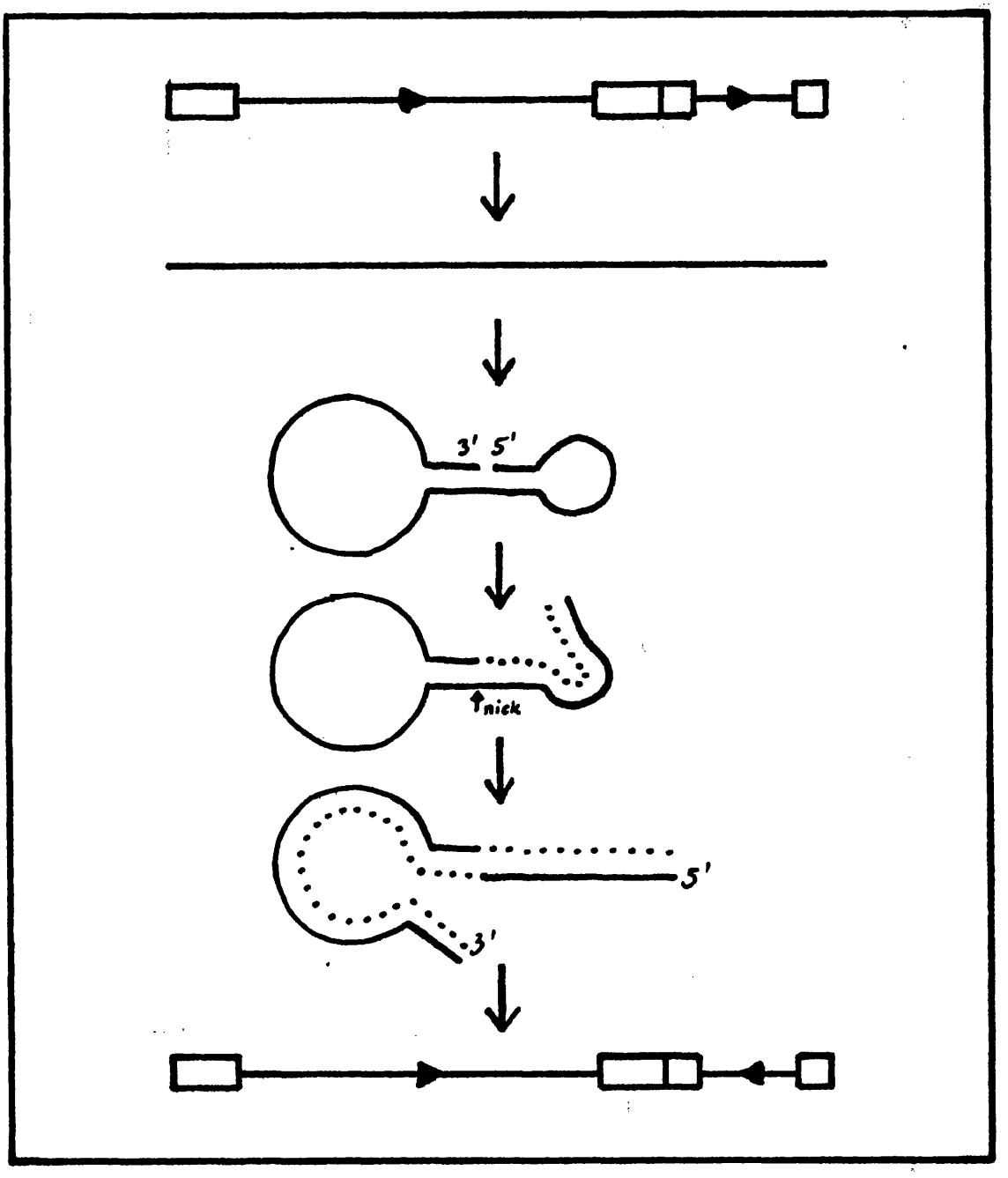
1



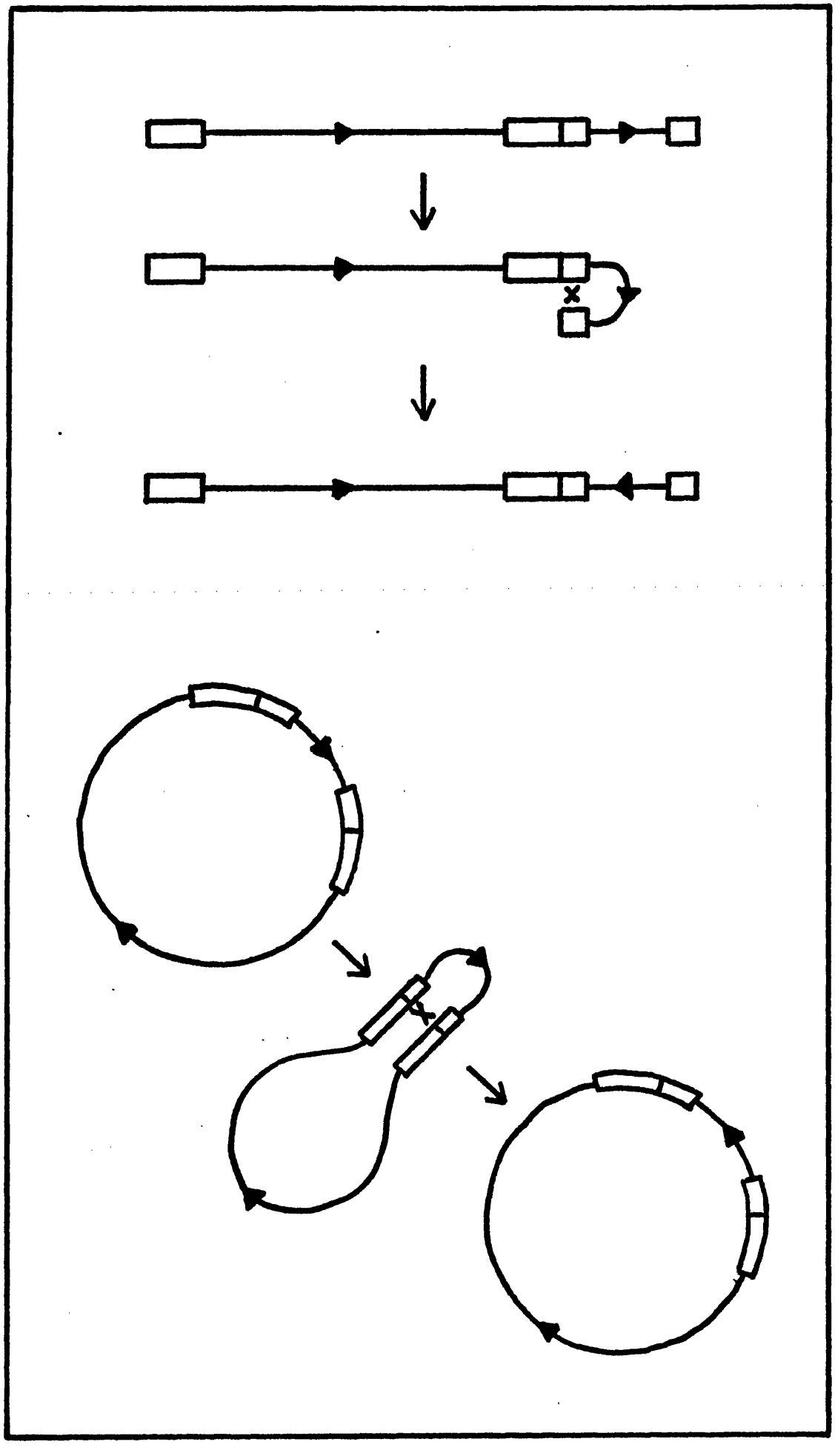
2



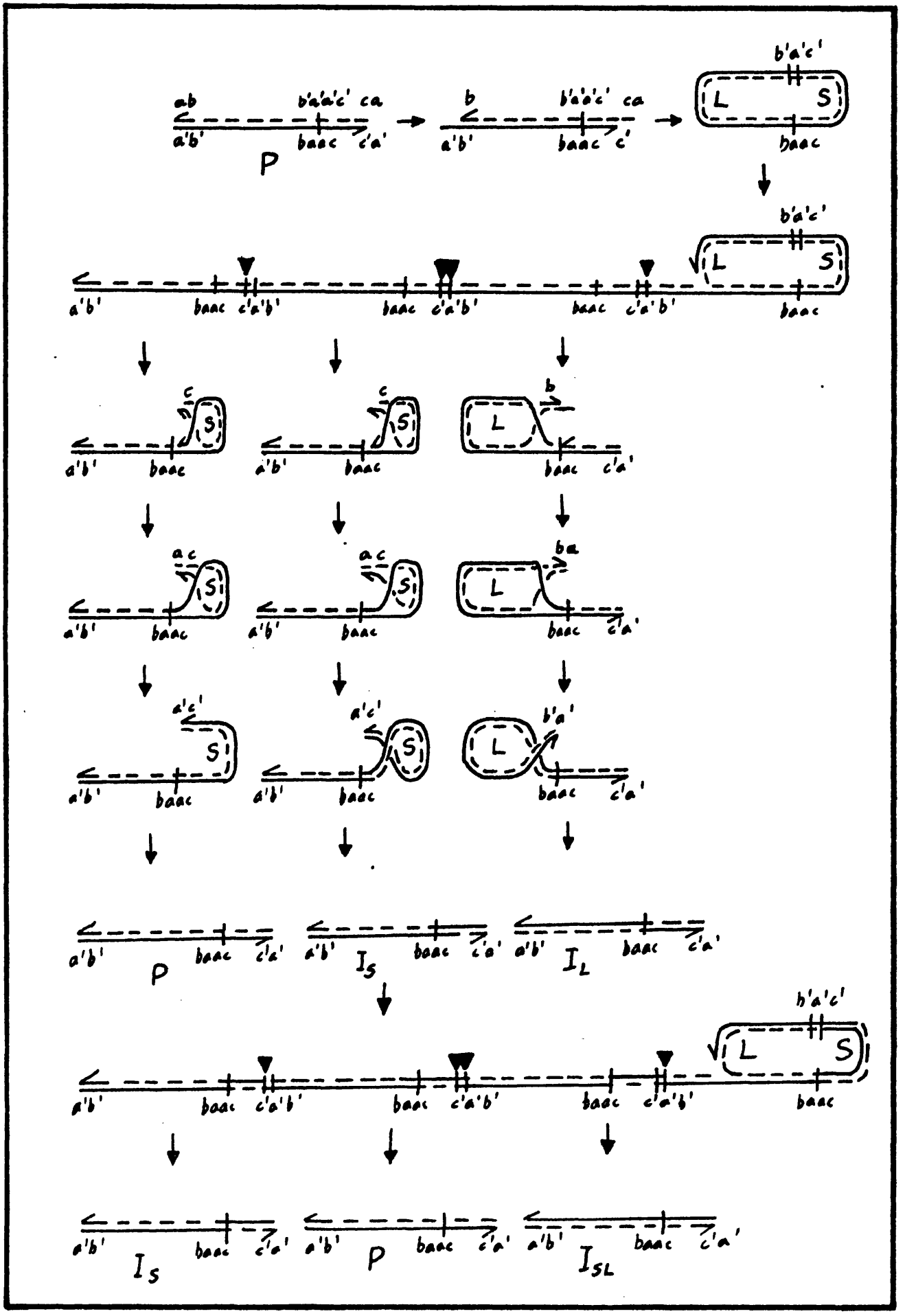
3A



3B



3C

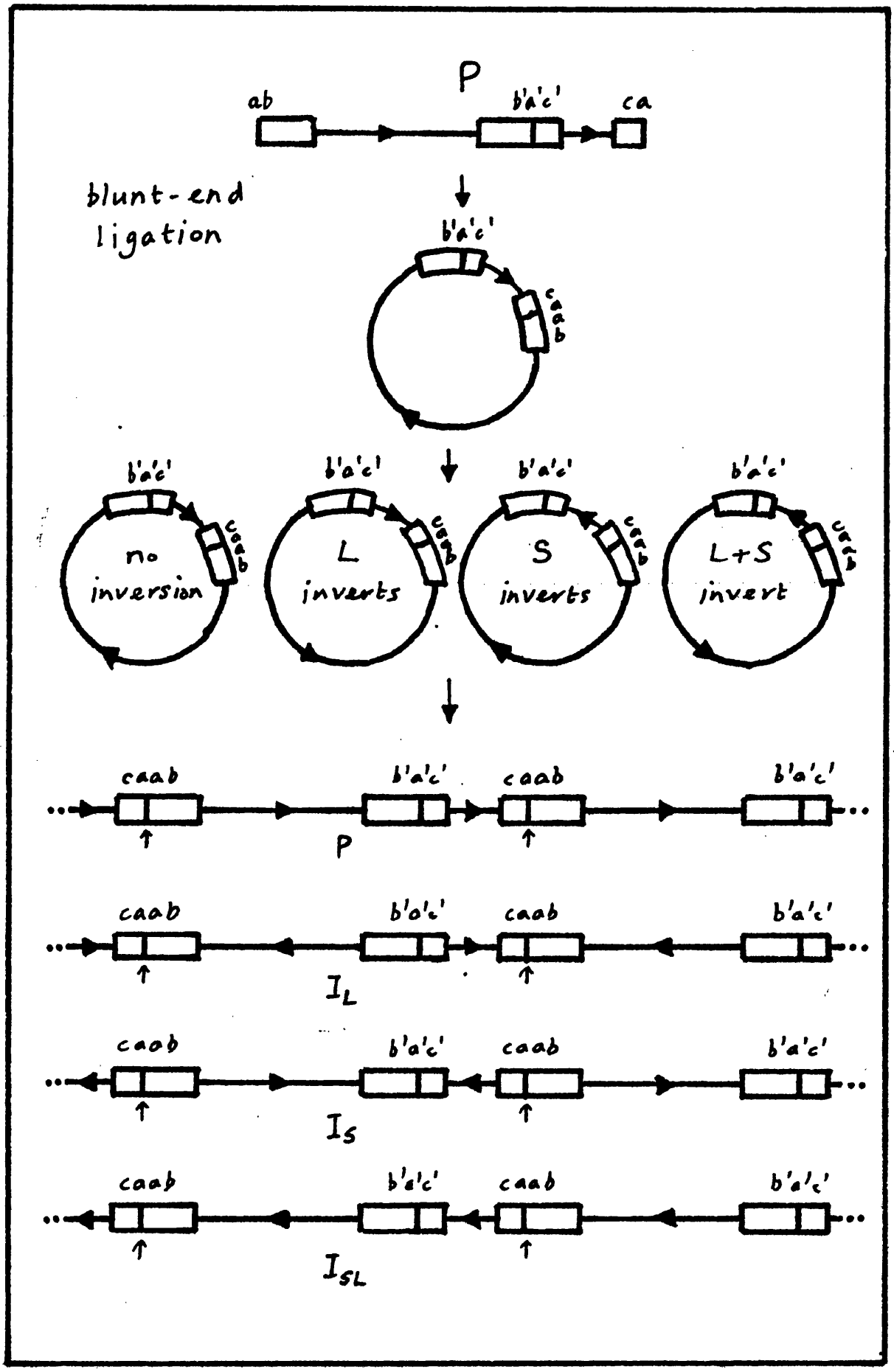

ab
b'a'a'c' ca
a'b'
baac
c'a'
P
b
b'a'a'c' ca
a'b'
baac
c'
b'a'c'
L
S
baac
a'b'
baac
c'a'b'
baac
c'a'b'
baac
c'a'b'
baac
c
S
b
L
ac
ba
a'c'
b'a'
c'a'
P
I_S
I_L
I_S
P
I_{SL}

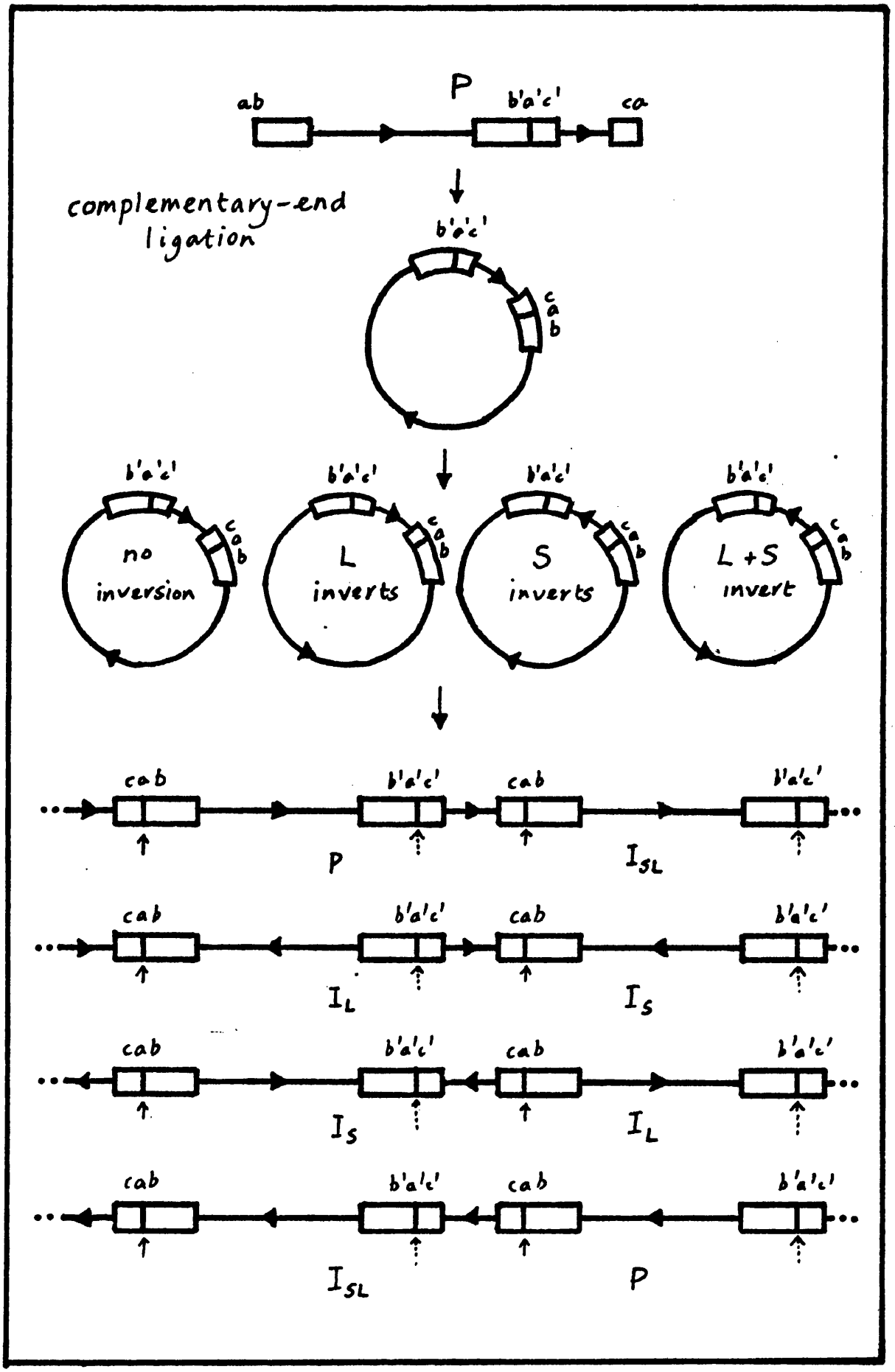
Comparison of the effects upon maturation of unit-length genomes of two mechanisms for generation of circular HSV DNA.

Blunt-end ligation produces a circle with dissimilar joints, whereas complementary-end ligation produces a circle with identical joints. Initial progeny molecules may invert neither, either or both segments to produce the four circular arrangements. In this diagram model 3B for site-specific intramolecular recombination has been invoked (Figure C4.27). Four arrangements of concatemers are then generated by rolling circle DNA synthesis.

Cleavage may occur at any joint of concatemers resulting from complementary-end ligation, so that each concatemer arrangement produces two linear genome arrangements. Cleavage could result from a single strand nick at the ends of the a sequence, followed by repair of the single stranded a sequence at the termini of unit-length molecules. It is necessary for only one segment to invert to produce the four genome arrangements, and isomerisation is a function of both inversion and cleavage.

Conversely, cleavage may occur only at alternate joints of concatemers resulting from blunt-end ligation, and each concatemer produces only one linear genome arrangement. Cleavage in this case could result from a double stranded cut between two a sequences (i.e. at the joint which is not cleaved in model 3C of Figure C4.27). It is necessary for both segments to invert to generate the four arramgements. Isomerisation is a function only of inversion. Alternatively, the cleavage mechanism may recognise both types of joint, and in this case the two models for circularisation have identical effects upon maturation (i.e. that of complementary-end ligation


P
ab
b'a'c'
ca
blunt-end ligation
b'a'c'
caab
b'a'c'
caab
no inversion
b'a'c'
caab
L inverts
b'a'c'
caab
S inverts
b'a'c'
caab
L+S invert
caab
b'a'c'
caab
b'a'c'
P
caab
b'a'c'
caab
b'a'c'
I_L
caab
b'a'c'
caab
b'a'c'
I_S
caab
b'a'c'
caab
b'a'c'
I_{SL}


P
ab
b'a'c'
ca
complementary-end ligation
b'a'c'
c a b
b'a'c'
no inversion
b'a'c'
L inverts
b'a'c'
S inverts
b'a'c'
L+S invert
cab
b'a'c'
cab
b'a'c'
P
I_SL
cab
b'a'c'
cab
b'a'c'
I_L
I_S
cab
b'a'c'
cab
b'a'c'
I_S
I_L
cab
b'a'c'
cab
b'a'c'
I_SL
P

R E F E R E N C E S

ALWINE, J.C., STEINHART, W.L. and HILL, C.W. (1974). Transcription of herpes simplex virus type 1 DNA in nuclei isolated from infected HEp-2 and KB cells. Virology 60, 302-307.

ANDERSON, K.P., STRINGER, J.R., HOLLAND, L.E. and WAGNER, E.K. (1979). Isolation and localization of herpes simplex virus type 1 mRNA. J. Virol. 30, 805-820.

ANDERSON, K.P., COSTA, R.H., HOLLAND, L.E. and WAGNER, E.K. (1980a). Characterization of herpes simplex virus type 1 RNA present in the absence of de novo protein synthesis. J. Virol. 34, 9-27.

ANDERSON, K.P., HOLLAND, L.E., GAYLORD, B.H. and WAGNER, E.K. (1980b). Isolation and translation of mRNA encoded by a specific region of the herpes simplex type 1 genome. J. Virol. 33, 749-759.

ANDERSON, K.P., FRINK, R.J., DEVI, G.B., GAYLORD, B.H., COSTA, R.H. and WAGNER, E.K. (1981). Detailed characterization of the mRNA mapping in the Hind fragment K region of the herpes simplex virus type 1 genome. J. Virol. 37, 1011-1027.

ARON, G.M., SCHAFFER, P.A., COURTNEY, R.J., BENYESH-MELNICK, M. and KIT, S. (1973). Thymidine kinase activity of herpes simplex virus temperature-sensitive mutants. Intervirology 1, 96-109.

ARON, G.M., PURIFOY, D.J.M. and SCHAFFER, P.A. (1975). DNA synthesis and DNA polymerase activity of herpes simplex virus type 1 temperature-sensitive mutants. J. Virol. 16, 498-507.

ASHER, Y., HELLER, M. and BECKER, Y. (1969). Incorporation of lipids into herpes simplex virus particles. J. gen. Virol. 4, 65-76.

ATKINSON, M.A., BARR, S. and TIMBURY, M.C. (1978). The fine structure of cells infected with temperature-sensitive mutants of herpes simplex type 2. J. gen. Virol. 40, 103-119

BACHENHEIMER, S.L. and ROIZMAN, B. (1972). Ribonucleic acid synthesis in cells infected with herpes simplex virus. VI. Polyadenylic acid sequences in viral messenger ribonucleic acid. J. Virol. 10, 875-879.

BACHENHEIMER, S.L., KIEFF, E.D., LEE, L.F. and ROIZMAN, B. (1972). Comparative studies of DNAs of Marek's disease and herpes simplex virus. In "Oncogenesis and Herpesviruses", pp. 74-81. Edited by P.M. Biggs, G. de Thé and L.N. Payne. IARC Scientific Publications, no. 2, Lyon.

BARAHONA, H., MELÉNDEZ, L.V. and MELNICK, J.L. (1974). A compendium of herpesviruses isolated from non-human primates. Intervirology 3, 175-192.

BARINGER, J.R. and SWOVELAND, P. (1973). Recovery of herpes simplex virus from human trigeminal ganglions. New England Journal of Medicine 288, 648-650.

BARTKOSKI, M. and ROIZMAN, B. (1976). RNA synthesis in cells infected with herpes simplex virus. XIII. Differences in the methylation patterns of viral RNA during the replicative cycle. J. Virol. 20, 583-588.

BARTKOSKI, M.J. Jr. and ROIZMAN, B. (1978). Regulation of herpesvirus macromolecular synthesis. VII. Inhibition of internal methylation of mRNA late in infection. Virology 85, 146-156.

BAUCKE, R.B. and SPEAR, P.G. (1979). Membrane proteins specified by herpes simplex virus. V. Identification of an Fc-binding glycoprotein. J. Virol. 32, 779-789.

BAYLISS, G.J., MARSDEN, H.S. and HAY, J. (1975). Herpes simplex virus proteins: DNA-binding proteins in infected cells and in the virus structure. Virology 68, 124-134.

BECKER, Y., DYM, H. and SAROV, I. (1968). Herpes simplex virus DNA. Virology 36, 184-192.

BECKER, Y. and ASHER, Y. (1975). In vitro synthesis of DNA in nuclei isolated from herpes simplex virus-infected cells, untreated and treated with metabolic inhibitors. Virology 63, 209-220.

BECKER, Y., ASHER, Y., WEINBERG-ZAHLERING, E., RABKIN, S., FRIEDMANN, A. and KESSLER, E. (1978). Defective herpes simplex virus DNA: circular and circular-linear molecules resembling rolling circles. J. gen. Virol. 40, 319-335.

BECKER, Y., CHEJANOVSKY, N. and FRIDLENDER, B. (1980). DNA-binding proteins in the nuclei of herpes simplex virus-infected, arginine-deprived, BSC-1 cells. J. gen. Virol. 50, 259-278.

BELL, D. (1974). DNA synthesis in 'nuclear monolayers' from BSC-1 cells infected with herpes virus. Nature (London) 248, 505-508.

BENOIST, C., O'HARE, K., BREATHNACH, R. and CHAMBON, P. (1980). The ovalbumin gene - sequence of putative control regions. Nucleic Acids Research 8, 127-142.

BEN-PORAT, T. and KAPLAN, A.S. (1962). The chemical composition of herpes simplex and pseudorabies viruses. Virology 16, 261-266.

BEN-PORAT, T. and KAPLAN, A.S. (1963). The synthesis and fate of pseudorabies virus DNA in infected mammalian cells in the stationary phase of growth. Virology 20, 310-317.

BEN-PORAT, T., DEMARCHI, J.M. and KAPLAN, A.S. (1974). Characterization of defective interfering viral particles present in a population of pseudorabies virions. Virology 61, 29-37.

BEN-PORAT, T., STEHN, B. and KAPLAN, A.S. (1976a). Fate of parental herpesvirus DNA. Virology 71, 412-422.

BEN-PORAT, T., KAPLAN, A.S., STEHN, B. and RUBENSTEIN, A.S. (1976b). Concatemeric forms of intracellular herpesvirus DNA. Virology 69, 547-560.

BEN-PORAT, T. and TOKAZEWSKI, S.A. (1977). Replication of herpesvirus DNA. II. Sedimentation characteristics of newly synthesized DNA. Virology 79, 292-301.

BEN-PORAT, T., BLANKENSHIP, M.L., DEMARCHI, J.M. and KAPLAN, A.S. (1977). Replication of herpesvirus DNA. III. Rate of DNA elongation. J. Virol. 22, 734-741.

BEN-PORAT, T. and RIXON, F.J. (1979). Replication of herpesvirus DNA. IV. Analysis of concatemers. Virology 94, 61-70.

BEN-PORAT, T., RIXON, F.J. and BLANKENSHIP, M.L. (1979). Analysis of the structure of the genome of pseudorabies virus. Virology 95, 285-294.

BEN-PORAT, T. and VEACH, R.A. (1980). Origin of replication of the DNA of a herpesvirus (pseudorabies). Proc. Natl. Acad. Sci. USA 77, 172-175.

BEN-PORAT, T., VEACH, R.A. and LADIN, B.F. (1980). Replication of herpesvirus DNA. VI. Virions containing either isomer of pseudorabies virus DNA are infectious. Virology 102, 370-380.

BENYESH-MELNICK, M., SCHAFFER, P.A., COURTNEY, R.J., ESPARZA, J. and KIMURA, S. (1974). Viral gene functions expressed and detected by temperature-sensitive mutants of herpes simplex virus. Cold Spring Harbor Symp. Quant. Biol. 39, 731-746.

BEN-ZEEV, A. and BECKER, Y. (1975). Symmetrical transcription of herpes simplex virus DNA in infected BSC-1 cells. Nature (London) 254, 719-722.

BEN-ZEEV, A., ASHER, Y. and BECKER, Y. (1976). Synthesis of herpes simplex virus-specified RNA by an RNA polymerase II in isolated nuclei *in vitro*. Virology 71, 302-311.

BEN-ZEEV, A. and BECKER, Y. (1977). Requirement of host cell RNA polymerase II in the replication of herpes simplex virus in α-amanitin-sensitive and -resistant cell lines. Virology 76, 246-253.

BERK, A.J. and SHARP, P.A. (1977). Sizing and mapping of early adenovirus mRNAs by gel electrophoresis of S1 endonuclease-digested hybrids. Cell 12, 721-732.

BIEGELEISEN, K. and RUSH, M.G. (1976). Association of herpes simplex virus type 1 DNA with host chromosomal DNA during productive infection. Virology 69, 246-257.

BIEGELEISEN, K., YANAGI, K. and RUSH, M.G. (1977). Further studies on the association of herpes simplex virus type 1 DNA with host DNA during productive infection. Virology 83, 221-225.

BISWAL, N. and MURRAY, B.K. (1974). Synthesis of herpes simplex virus type 1 (HSV-1) DNA in isolated nuclei. I. Conditions for isolation of nuclei capable of viral DNA synthesis. Intervirology 4, 1-13.

BISWAL, N., MURRAY, B.K. and BENYESH-MELNICK, M. (1974). Ribonucleotides in newly synthesized DNA of herpes simplex virus. Virology 61, 87-99.

BLOMBERG, J. (1979). Studies on the attachment of herpes simplex virus. Effect of trypsin treatment. Arch. Virol. 61, 201-206.

BLUE, W.T. and STOBBS, D.G. (1981). Isolation of a protein kinase induced by herpes simplex virus type 1. J. Virol. 38, 383-388.

BOLDEN, A., AUCKER, J. and WEISSBACH, A. (1975). Synthesis of herpes simplex virus, vaccinia virus and adenovirus DNA in isolated HeLa cell nuclei. I. Effect of viral-specific antisera and phosphonoacetic acid. J. Virol. 16, 1584-1592.

BONE, D.R., BROWN, M., CROMBIE, I. and FRANCKE, B. (1978). Viral DNA synthesis in cells infected with temperature-sensitive mutants of herpes simplex type 1. J. Virol. 28, 14-19.

BOOKOUT, J., HIRSCH, I., PURIFOY, D.J.M. and BISWAL, N. (1979). Herpes simplex virus types 1 and 2: comparison of the defective genomes and virus specific polypeptides. Virology 93, 598-604.

BOOKOUT, J.B. and LEVY, C.C. (1980). Comparative examination of the polypeptides of herpes simplex virus: types 1 and 2. Virology 101, 198-216.

BORNKAMM, G.W., DELIUS, H., FLECKENSTEIN, B., WERNER, F.J. and MULDER, C. (1976). Structure of herpesvirus saimiri genomes: arrangement of heavy and light sequences in the M genome. J. Virol. 19, 154-161.

BORNKAMM, G.W., DELIUS, H., ZIMBER, U., HUDEWENTZ, J. and EPSTEIN, M.A. (1980). Comparison of Epstein-Barr virus strains of different origin by analysis of the viral DNAs. J. Virol. 35, 603-618.

BOYER, H.W. and ROULLAND-DUSSOIX, D. (1969). A complementation analysis of the restriction and modification of DNA in Escherichia coli. J. mol. Biol. 41, 459-472.

BRONSON, D.L., GRAHAM, B.J., LUDWIG, H., BENYESH-MELNICK, M. and BISWAL, N. (1972). Studies on the relatedness of herpes viruses through DNA-RNA hybridisation. Biochim. Biophys. Acta 259, 24-34.

BRONSON, D.L., DREESMAN, G.R., BISWAL, N. and BENYESH-MELNICK, M. (1973). Defective virions of herpes simplex virus. Intervirology 1, 141-153.

BROWN, S.M., RITCHIE, D.A. and SUBAK-SHARPE, J.H. (1973). Genetic studies with herpes simplex type 1. The isolation of temperature sensitive mutants, their ordering into complementation groups and recombinational analysis leading to a linkage map. J. gen. Virol. 18, 329-346.

BROWN, S.M. and RITCHIE, D.A. (1975). Genetic studies with herpes simplex virus type 1. Analysis of mixed plaque-forming virus and its bearing on genetic recombination. Virology 64, 32-42.

BROWN, S.M. and JAMIESON, A.T. (1977). Location of non temperature-sensitive genes on the genetic map of herpes simplex virus type 1. In "Oncogenesis and Herpesviruses III", part I, pp. 33-39. Edited by G. de Thé, W. Henle and F. Rapp. IARC Scientific Publications, no. 24, Lyon.

BUCHAN, A. and WATSON, D.H. (1969). The immunological specificity of thymidine kinases in cells infected by viruses of the herpes group. J. gen. Virol. 4, 461-463.

BUCHAN, A., WATSON, D.H., DUBBS, D.R. and KIT, S. (1970). Serological study of a mutant herpesvirus unable to stimulate thymidine kinase. J. Virol. 5, 817-818.

BUCHMAN, T.G. and ROIZMAN, B. (1978a). Anatomy of bovine mammilitis virus DNA. I. Restriction endonuclease maps of four populations of molecules that differ in the relative orientation of their long and short components. J. Virol. 25, 395-407.

BUCHMAN, T.G. and ROIZMAN, B. (1978b). Anatomy of bovine mammilitis virus DNA. II. Size and arrangements of the deoxynucleotide sequences. J. Virol. 27, 239-254.

BUCHMAN, T.G., ROIZMAN, B., ADAMS, G. and STOVER, H. (1978). Restriction endonuclease fingerprinting of herpes simplex DNA: a novel epidemiological tool applied to a nosocomial outbreak. J. infect. Dis. 138, 488-498.

BUCHMAN, T.G., SIMPSON, T., NOSAL, C., ROIZMAN, B. and NAHMIAS, A. (1980). The structure of herpes simplex virus DNA and its application to molecular epidemiology. Annals of New York Acad. Sci. 354, 279-290.

BUSBY, D.W.G., HOUSE, W. and MacDONALD, J.R. (1964). In "Virological Techniques", Churchill, London.

BZIK, D.J. and PERSON, S. (1981). Dependence of herpes simplex virus type 1-induced cell fusion on cell type. Virology 110, 35-42.

CABRAL, G.A. and SCHAFFER, P.A. (1976). Electron microscope studies of temperature-sensitive mutants of herpes simplex virus type 2. J. Virol. 18, 727-737.

CABRAL, G.A., COURTNEY, R.J., SCHAFFER, P.A. and MARCIANO-CABRAL, F. (1980). Ultrastructural characterization of an early, non-structural polypeptide of herpes simplex virus type 1. J. Virol. 33, 1192-1198.

CAMACHO, A. and SPEAR, P.G. (1978). Transformation of hamster embryo fibroblasts by a specific fragment of the herpes simplex virus genome. Cell 15, 993-1002.

CATCHESIDE, D.G. and CORCORAN, D. (1973). Control of non-allelic recombination in Neurospora crassa. Australian J. biol. Sci. 26, 1337-1353.

CHAI, L. (1971). Architecture of the DNA molecule within Epstein-Barr virus (EBV). Proc. Amer. Assoc. for Cancer Res. 12, abstract no. 71.

CHAN, T-S. (1977). Induction of deoxycytidine deaminase activity in mammalian cell lines by infection with herpes simplex virus type 1. Proc. Natl. Acad. Sci. USA 74, 1734-1738.

CHARTRAND, P., STOW, N.D., TIMBURY, M.C. and WILKIE, N.M. (1979). Physical mapping of paa^r mutations of herpes simplex virus type 1 and 2 by intertypic marker rescue. J. Virol. 31, 265-276.

CHARTRAND, P., CRUMPACKER, C.S., SCHAFFER, P.A. and WILKIE, N.M. (1980). Physical and genetic analysis of the herpes simplex virus DNA polymerase locus. Virology 103, 311-326.

CHARTRAND, P., WILKIE, N.M. and TIMBURY, M.C. (1981). Physical mapping of temperature-sensitive mutations of herpes simplex virus type 2 by marker rescue. J. gen. Virol. 52, 121-133.

CHOUSTERMAN, S., LACASA, M. and SHELDRICK, P. (1979). Physical map of the channel catfish virus genome: location of sites for restriction endonucleases EcoRI, HindIII, HpaI and XbaI. J. Virol. 31, 73-85.

CHU, C-T., PARRIS, D.S., DIXON, R.A.F., FARBER, F.E. and SCHAFFER, P.A. (1979). Hydroxylamine mutagenesis of HSV DNA and DNA fragments: introduction of mutations into selected regions of the viral genome. Virology 98, 168-181.

CHURCHILL, A.E. and BIGGS, P.M. (1967). Agent of Marek's disease in tissue culture. Nature (London) 215, 528-530.

CLEMENTS, J.B., CORTINI, R. and WILKIE, N.M. (1976). Analysis of herpesvirus DNA substructure by means of restriction endonucleases. J. gen. Virol. 30, 243-256.

CLEMENTS, J.B., WATSON, R.J. and WILKIE, N.M. (1977). Temporal regulation of herpes simplex virus type 1 transcription: location of transcripts on the viral genome. Cell 12, 275-285.

CLEMENTS, J.B., McLAUCHLAN, J. and McGEOCH, D.J. (1979). Orientation of herpes simplex virus type 1 immediate early mRNAs. Nucleic Acids Research 7, 77-91.

CLEWELL, D.B. and HELINSKI, D.R. (1970). Properties of a supercoiled deoxyribonucleic acid-protein relaxation complex and strand specificity of the relaxation event. Biochemistry 9, 4428-4440.

COHEN, G.H., VAUGHAN, R.K. and LAWRENCE, W.C. (1971). Deoxyribonucleic acid synthesis in synchronized mammalian KB cells infected with herpes simplex virus. J. Virol. 7, 783-791.

COHEN, G.H., KATZE, M., HYDREAN-STERN, C. and EISENBERG, R.J. (1978). Type-common CP-1 antigen of herpes simplex virus is associated with a 59,000-molecular weight envelope glycoprotein. J. Virol. 27, 172-181.

COHEN, G.H., LONG, D. and EISENBERG, R.J. (1980a). Synthesis and processing of glycoproteins gD and gC of herpes simplex virus type 1. J. Virol. 36, 429-439.

COHEN, G.H., PONCE DE LEON, M., DIGGELMANN, H., LAWRENCE, W.C., VERNON, S.K. and EISENBERG, R.J. (1980b). Structural analysis of the capsid polypeptides of herpes simplex virus types 1 and 2. J. Virol. 34, 521-531

COHEN, S.N., CHANG, A.C.Y. and HSU, L. (1972). Nonchromosomal antibiotic resistance in bacteria: genetic transformation of Escherichia coli by R-factor DNA. Proc. Natl. Acad. Sci. USA 69, 2110-2114.

COLBÈRE-GARAPIN, F., CHOUSTERMAN, S., HORODNICEANU, F., KOURILSKY, P. and GARAPIN, A-C. (1979). Cloning of the active thymidine kinase gene of herpes simplex virus type 1 in Escherichia coli K-12. Proc. Natl. Acad. Sci. USA 76, 3755-3759.

CONLEY, A.J., KNIPE, D.M., JONES, P. and ROIZMAN, B. (1981). Molecular genetics of herpes simplex virus. VII. Characterization of a temperature-sensitive mutant produced by in vitro mutagenesis and defective in DNA synthesis and accumulation of γ polypeptides. J. Virol. 37, 191-206.

COOPER, G.M. (1973). Phosphorylation of 5-bromodeoxycytidine in cells infected with herpes simplex virus. Proc. Natl. Acad. Sci. USA 70, 3788-3792.

CORTINI, R. and WILKIE, N.M. (1978). Physical maps for HSV type 2 DNA with five restriction endonucleases. J. gen. Virol. 39, 259-280.

COSTA, R.H., DEVI, B.G., ANDERSON, K.P., GAYLORD, B.H. and WAGNER, E.K. (1981). Characterization of a major late herpes simplex virus type 1 mRNA. J. Virol. 38, 483-496.

COSTANZO, F., CAMPADELLI-FIUME, G., FOA-TOMASI, L. and CASSAI, E. (1977). Evidence that herpes simplex virus DNA is transcribed by cellular RNA polymerase B. J. Virol. 21, 996-1001.

COURTNEY, R.J. and POWELL, K.L. (1975). Immunological and biochemical characterization of polypeptides induced by herpes simplex virus type 1 and 2. In "Oncogenesis and Herpesviruses II", part I, pp. 63-73. Edited by G. de Thé, M.A. Epstein and H. zur Hausen. IARC Scientific Publications, no. 11, Lyon.

COURTNEY, R.J., SCHAFFER, P.A. and POWELL, K.L. (1976). Synthesis of virus specific polypeptides by temperature-sensitive mutants of herpes simplex virus type 1. Virology 75, 306-318.

CREMER, K.J., SUMMERS, W.C. and GESTELAND, R.F. (1977). Cell-free synthesis of herpes simplex virus proteins. J. Virol. 22, 750-757.

CREMER, K., BODEMER, M. and SUMMERS, W.C. (1978). Characterization of the mRNA for herpes simplex virus thymine kinase by cell-free synthesis of active enzyme. Nucleic Acids Research 5, 2333-2344.

CROMBIE, I.K. (1975). Genetic and biochemical studies with herpes simplex virus type 1. PhD thesis, University of Glasgow.

CRUMPACKER, C.S., CHARTRAND, P., SUBAK-SHARPE, J.H. and WILKIE, N.M. (1980). Resistance of herpes simplex virus to acycloguanosine - genetic and physical analysis. Virology 105, 171-184.

DALES, S. and SILVERBERG, H. (1969). Viropexis of herpes simplex virus by HeLa cells. Virology 37, 475-480.

DARAI, G. and MUNK, K. (1973). Human embryonic lung cells abortively infected with herpesvirus hominis type 2 show some properties of cell transformation. Nature New Biol. 241, 268-269.

DARBY, G., LARDER, B.A., BASTOW, K.F. and FIELD, H.J. (1980). Sensitivity of viruses to phosphorylated 9-(2-hydroxy-ethoxymethyl) guanine revealed in TK-transformed cells. J. gen. Virol. 48, 451-454.

DARBY, G., FIELD, H.J. and SALISBURY, S.A. (1981). Altered substrate specificity of herpes simplex virus thymidine kinase confers acyclovir-resistance. Nature (London) 289, 81-83.

DARLINGTON, R.W. and MOSS, L.H. III (1968). Herpesvirus envelopment. J. Virol. 2, 48-55.

DELIUS, H. and CLEMENTS, J.B. (1976). A partial denaturation map of herpes simplex virus type 1 DNA: evidence for inversions of the unique DNA regions. J. gen. Virol. 33, 125-133.

DEMARCHI, J., BLANKENSHIP, M., BROWN, G. and KAPLAN, A.S. (1978). Size and complexity of human cytomegalovirus DNA. Virology 89, 643-646.

DENHARDT, D.T. (1966). A membrane filter technique for the detection of complementary DNA. Biochem. biophys. Res. Commun. 23, 641-646.

DHAR, R., SUBRAMANIAN, K.N., PAN, J. and WEISSMAN, S.M. (1977). Structure of a large segment of the genome of simian virus 40 that does not encode known proteins. Proc. Natl. Acad. Sci. USA 74, 827-831.

DIXON, R.A.F. and FARBER, F.E. (1980). Channel catfish virus: physicochemical properties of the viral genome and identification of viral polypeptides. Virology 103, 267-278.

DIXON, R.A.F. and SCHAFFER, P.A. (1980). Fine-structure mapping and functional analysis of temperature-sensitive mutants in the gene encoding the herpes simplex virus type 1 immediate-early protein VP175. J. Virol. 36, 189-203.

DOLYNIUK, M., PRITCHETT, R. and KIEFF, E. (1976). Proteins of Epstein-Barr virus. I. Analysis of the polypeptides of purified enveloped Epstein-Barr virus. J. Virol. 17, 935-949.

DUFF, R. and RAPP, F. (1971). Properties of hamster embryo fibroblasts transformed in vitro after exposure to ultraviolet-irradiated herpes simplex virus type 2. J. Virol. 8, 469-477.

DUMAS, A.M., GEELEN, J.L.M.C., MARIS, W. and VAN DER NOORDAA, J. (1980). Infectivity and molecular weight of varicella-zoster virus DNA. J. gen. Virol. 47, 233-235.

EASTON, A.J. and CLEMENTS, J.B. (1980). Temporal regulation of herpes simplex virus type 2 transcription and characterization of virus immediate-early mRNAs. Nucleic Acids Research 8, 2627-2645.

EBERLE, R. and COURTNEY, R.J. (1980a). Preparation and characterization of specific antisera to individual glycoprotein antigens comprising the major glycoprotein region of herpes simplex virus type 1. J. Virol. 35, 902-917.

EBERLE, R. and COURTNEY, R.J. (1980b). gA and gB glycoproteins of herpes simplex virus type 1: two forms of a single polypeptide. J. Virol. 36, 665-675.

ECKER, J.R. and HYMAN, R.W. (1981). Analysis of interruptions in the phosphodiester backbone of herpes simplex virus DNA. Virology 110, 213-216.

EGLIN, R.P., SHARP, F., MacLEAN, A.B., MacNAB, J.C.M., CLEMENTS, J.B. and WILKIE, N.M. (1981). Detection of RNA complementary to herpes simplex virus DNA in human cervical squamous cell neoplasms. Cancer Research, in press (September 1981).

EISENBERG, R.J., HYDREAN-STERN, C. and COHEN, G.H. (1979). Structural analysis of precursor and product forms of type-common envelope glycoprotein D (CP-1 antigen) of herpes simplex virus type 1. J. Virol. 31, 608-620.

EISENBERG, R.J., PONCE DE LEON, M. and COHEN, G.H. (1980). Comparative structural analysis of glycoprotein gD of herpes simplex virus types 1 and 2. J. Virol. 35, 428-435.

ELION, G.B., FURMAN, P.A., FYFE, J.A., DE MIRANDA, P., BEAUCHAMP, L. and SCHAEFFER, H.J. (1977). Selectivity of action of an antiherpetic agent, 9-(2-hydroxy-ethoxymethyl) guanine. Proc. Natl. Acad. Sci. USA 74, 5716-5720.

ENQUIST, L.W., VANDE WOUDE, G.F., WAGNER, M., SMILEY, J.R. and SUMMERS, W.C. (1979). Construction and characterization of a recombinant plasmid encoding the gene for the thymidine kinase of herpes simplex type 1 virus. Gene 7, 335-342.

EPSTEIN, M.A. (1962a). Observations on the fine structure of mature herpes simplex virus and on the composition of its nucleoid. J. exp. Med. 115, 1-9.

EPSTEIN, M.A. (1962b). Observations on the mode of release of herpes virus from infected HeLa cells. J. cell. Biol. 12, 589-597.

EPSTEIN, M.A. and HOLT, S.J. (1963). Electron microscopic observations on the surface adenosine triphosphatase-like enzymes of HeLa cells infected with herpes virus. J. cell. Biol. 19, 337-347.

ERICKSON, J.S. and KAPLAN, A.S. (1973). Synthesis of proteins in cells infected with herpesvirus. IX. Sulfated proteins. Virology 55, 94-102.

ESPARZA, J., PURIFOY, D.J.M., SCHAFFER, P.A. and BENYESH-MELNICK, M. (1974). Isolation, complementation and preliminary phenotypic characterization of temperature-sensitive mutants of herpes simplex virus type 2. Virology 57, 554-565.

ESPARZA, J., BENYESH-MELNICK, M. and SCHAFFER, P.A. (1976). Intertypic complementation and recombination between temperature-sensitive mutants of herpes simplex virus types 1 and 2. Virology 70, 372-384.

FALK, L., DEINHARDT, F., NONOYAMA, M., WOLFE, L.G., BERGHOLTZ, C., LAPIN, B., YAKOVLEVA, L., AGRBA, V., HENLE, G. and HENLE, W. (1976). Properties of a baboon lymphotropic herpesvirus related to Epstein-Barr virus. Int. J. Cancer 18, 798-807.

FALKE, D., SIEGERT, R. and VOGEL, W. (1959). Elektronenmikroskopische Befunde zur Frage der Doppelmembranbildung des Herpes-simplex-virus. Arch. ges. Virusforsch. 9, 484-496.

FALKE, D., NEHRBASS, W., BRAUER, D. and MULLER, W.E.G. (1981). Adenylic acid:deoxythymidine 5'-phosphotransferase : evidence for the existence of a novel herpes simplex virus-induced enzyme. J. gen. Virol. 53, 247-255.

FARBER, F.E. (1976). Comparison of DNA facilitators in the uptake and intracellular fate of infectious herpes simplex virus type 2 DNA. Biochim. biophys. Acta 454, 410-41

FARNHAM, A.E. and NEWTON, A.A. (1959). The effect of some environmental factors on herpes virus grown in HeLa cells. Virology 7, 449-461.

FAWCETT, D.W. (1956). Electron microscope observations of intracellular virus-like particles associated with the cells of the Lucké renal adenocarcinoma. J. biophys. biochem. Cytol. 2, 725-742.

FENWICK, M.L. and WALKER, M.J. (1978). Suppression of the synthesis of cellular macromolecules by herpes simplex virus. J. gen. Virol. 41, 37-51.

FENWICK, M.L., WALKER, M.J. and PETKEVICH, J.M. (1978). On the association of virus proteins with the nuclei of cells infected with herpes simplex virus. J. gen. Virol. 39, 519-529.

FENWICK, M.L. and WALKER, M.J. (1979). Phosphorylation of a ribosomal protein and of virus-specific proteins in cells infected with herpes simplex virus. J. gen. Virol. 45, 397-405.

FENWICK, M., MORSE, L.S. and ROIZMAN, B. (1979). Anatomy of herpes simplex virus DNA. XI. Apparent clustering of functions effecting rapid inhibition of host DNA and protein synthesis. J. Virol. 29, 825-827.

FENWICK, M., WALKER, M. and MARSHALL, L. (1980). Some characteristics of an early protein (ICP22) synthesized in cells infected with herpes simplex virus. J. gen. Virol. 47, 333-342.

FIELD, H.J. and DARBY, G. (1978). The pathogenicity of thymidine kinase-deficient mutant of herpes simplex virus in mice. J. Hygiene (Cambridge) 81, 267-277.

FIELD, H.J. and DARBY, G. (1980). The pathogenicity for mice of strains of herpes simplex virus which are resistant to acyclovir in vitro and in vivo. Antimicrobial Agents and Chemotherapy 17, 209-216.

FINNIE, E.P., LITTLEJOHNS, I.R. and ACLAND, H.M. (1976). Mortalities in Parma wallabies (Macropus parma) associated with probable herpesvirus. Australian Veterinary J. 52, 294.

FLANAGAN, J.F. (1967). Virus-specific ribonucleic acid synthesis in KB cells infected with herpes simples virus. J. Virol. 1, 583-590.

FLAVELL, R.A., BIRFELDER, E.J., SANDERS, J.P.M. and BORST, P. (1974a). DNA-DNA hybridization on nitrocellulose filters. I. General considerations and non-ideal kinetics. Eur. J. Biochem. 47, 535-543.

FLAVELL, R.A., BORST, P. and BIRFELDER, E.J. (1974b). DNA-DNA hybridization on nitrocellulose filters. 2. Concatenation effects. Eur. J. Biochem. 47, 545-548.

FLECKENSTEIN, B. and WOLF, H. (1974). Purification and properties of herpesvirus saimiri DNA. Virology 58, 55-64.

FLECKENSTEIN, B., BORNKAMM, G.W. and LUDWIG, H. (1975). Repetitive sequences in complete and defective genomes of herpesvirus saimiri. J. Virol. 15, 398-406.

FLECKENSTEIN, B., BORNKAMM, G.W., MULDER, C., WERNER, F-J., DANIEL, M.D., FALK, L.A. and DELIUS, H. (1978). Herpesvirus ateles DNA and its homology with herpesvirus saimiri nucleic acid. J. Virol. 25, 361-373.

FRANCKE, B., MOSS, H., TIMBURY, M.C. and HAY, J. (1978). Alkaline DNase activity in cells infected with a temperature-sensitive mutant of herpes simplex virus type 2. J. Virol. 26, 209-213.

FRANCKE, B. and MARGOLIN, J. (1981). Effect of novobiocin and other DNA gyrase inhibitors on virus replication and DNA synthesis in herpes simplex type 1-infected BHK cells. J. gen. Virol. 52, 401-404.

FRENKEL, N. and ROIZMAN, B. (1972a). Separation of the herpesvirus deoxyribonucleic acid duplex into unique fragments and intact strands on sedimentation in alkaline gradients. J. Virol. 10, 565-572.

FRENKEL, N. and ROIZMAN, B. (1972b). Ribonucleic acid synthesis in cells infected with herpes simplex virus: controls of transcription and RNA abundance. Proc. Natl. Acad. Sci. USA 69, 2654-2658.

FRENKEL, N., ROIZMAN, B., CASSAI, E. and NAHMIAS, A. (1972). A herpes simplex 2 DNA fragment and its transcription in human cervical cancer tissue. Proc. Natl. Acad. Sci. USA 69, 3784-3789.

FRENKEL, N., SILVERSTEIN, S., CASSAI, E. and ROIZMAN, B. (1973). RNA synthesis in cells infected with herpes simplex virus. VII. Control of transcription and of transcript abundancies of unique and common sequences of herpes simplex virus 1 and 2. J. Virol. 11, 886-892.

FRENKEL, N., JACOB, R.J., HONESS, R.W., HAYWARD, G.S., LOCKER, H. and ROIZMAN, B. (1975). Anatomy of herpes simplex virus DNA. III. Characterization of defective DNA molecules and biological properties of virus populations containing them. J. Virol. 16, 153-167.

FRENKEL, N., LOCKER, H., BATTERSON, W., HAYWARD, G.S. and ROIZMAN, B. (1976). Anatomy of herpes simplex virus DNA. VI. Defective DNA originates from the S component. J. Virol. 20, 527-531.

FRENKEL, N., LOCKER, H. and VLAZNY, D.A. (1980). Studies of defective herpes simplex viruses. Annals of New York Acad. Sci. 354, 347-370.

FRIEDMANN, A., COWARD, J.E., ROSENKRANZ, H.S. and MORGAN, C. (1975). Electron microscopic studies on assembly of herpes simplex virus upon removal of hydroxyurea block. J. gen. Virol. 26, 171-181.

FRIEDMANN, A., SHLOMAI, J. and BECKER, Y. (1977). Electron microscopy of herpes simplex virus DNA molecules isolated from infected cells by centrifugation in CsCl density gradients. J. gen. Virol. 34, 507-522.

FURLONG, D., SWIFT, H. and ROIZMAN, B. (1972). Arrangement of herpesvirus deoxyribonucleic acid in the core. J. Virol. 10, 1071-1074.

FURMAN, P.A., ST. CLAIR, M.H., FYFE, J.A., RIDEOUT, J.L., KELLER, P.M. and ELION, G.B. (1979). Inhibition of herpes simplex virus-induced DNA polymerase activity and viral DNA replication by 9-(2-hydroxyethoxymethyl) guanine and its triphosphate. J. Virol. 32, 72-77.

GALLAHER, W.R., LEVITAN, D.B. and BLOUGH, H.A. (1973). Effect of 2-deoxy-D-glucose on cell fusion induced by Newcastle disease and herpes simplex viruses. Virology 55, 193-201.

GALLOWAY, D.A. and SWAIN, M. (1980). Cloning of herpes simplex virus type 2 DNA fragments in a plasmid vector. Gene 11, 253-257.

GALLOWAY, D.A. and McDOUGALL, J.K. (1981). Transformation of rodent cells by a cloned DNA fragment of herpes simplex virus type 2. J. Virol. 38, 749-760.

GANNON, F., O'HARE, K., PERRIN, F., LEPENNEC, H.P., BENOIST, C., COCHET, M., BREATHNACH, R., ROYAL, A., GARAPIN, A., CAMI, B. and CHAMBON, P. (1979). Organisation and sequences at the 5' end of a cloned complete ovalbumin gene. Nature (London) 278, 428-433.

GARFINKLE, B. and McAUSLAN, B.R. (1974). Regulation of herpes simplex virus-induced thymidine kinase. Biochem. biophys. Res. Commun. 58, 822-829.

GEELEN, J.L.M.C., WALIG, C., WERTHEIM, P. and VAN DER NOORDAA, J. (1978). Human cytomegalovirus DNA. I. Molecular weight and infectivity. J. Virol. 26, 813-816.

GELB, L.D., HUANG, J.J. and WELLINGHOFF, W.J. (1980). Varicella-zoster virus transformation of hamster embryo cells. J. gen. Virol. 51, 171-177.

GERBER, P., PRITCHETT, R.F. and KIEFF, E. (1976). Antigens and DNA of a chimpanzee agent related to Epstein-Barr virus. J. Virol. 19, 1090-1099.

GIBSON, W. and ROIZMAN, B. (1971). Compartmentalization of spermine and spermidine in the herpes simplex virion. Proc. Natl. Acad. Sci. USA 68, 2818-2821.

GIBSON, W. and ROIZMAN, B. (1972). Proteins specified by herpes simplex virus. VIII. Characterization and composition of multiple capsid forms of subtypes 1 and 2. J. Virol. 10, 1044-1052.

GIBSON, W. and ROIZMAN, B. (1974). Proteins specified by herpes simplex virus. X. Staining and radiolabelling properties of B capsid and virion proteins in polyacrylamide gels. J. Virol. 13, 155-165.

GIRALDO, G., BETH, E., KOURILSKY, G., HENLE, W., HENLE, G., MIKÉ, V., HURAUX, J.M., ANDERSEN, H.K., GHARBI, M.R., KYALWAZI, S.K. and PUISSANT, A. (1975). Antibody patterns to herpesviruses in Kaposi's sarcoma: serological association of European Kaposi's sarcoma with cytomegalovirus. Int. J. Cancer 15, 839-848.

GIVEN, D. and KIEFF, E. (1978). DNA of Epstein-Barr virus. IV. Linkage map of restriction endonuclease fragments of the B95-8 and W91 strains of Epstein-Barr virus. J. Virol. 28, 524-542.

GIVEN, D. and KIEFF, E. (1979). DNA of Epstein-Barr virus. VI. Mapping of the internal tandem reiteration. J. Virol. 31, 315-324.

GIVEN, D., YEE, D., GRILM, K. and KIEFF, E. (1979). DNA of Epstein-Barr virus. V. Direct repeats at the ends of Epstein-Barr virus DNA. J. Virol. 30, 852-862.

GLUZMAN, Y., SAMBROOK, J.F. and FRISQUE, R.J. (1980). Expression of early genes of origin-defective mutants of simian virus 40. Proc. Natl. Acad. Sci. USA 77, 3898-3902.

GOEDDEL, D.V., LEVING, D.W., DULL, T.J., GROSS, M., LAWN, R.M., McCANDLISS, R., SEEBURG, P.H., ULLRICH, A., YELVERTON, E. and GRAY, P.J. (1981). The structure of eight distinct cloned human leukocyte interferon cDNAs. Nature (London) 290, 20-26.

GOLDIN, A.L., SANDRI-GOLDIN, R.M., LEVINE, M. and GLORIOSO, J.C. (1981). Cloning of herpes simplex virus type 1 sequences representing the whole genome. J. Virol. 38, 50-58

GOODHEART, C.R., PLUMMER, G. and WANER, J.L. (1968). Density differences of DNA of human herpes simplex viruses, types 1 and 2. Virology 35, 473-475.

GORDIN, H., OLSHEVSKY, U., ROSENKRANTZ, H.S. and BECKER, Y. (1973). Studies on herpes simplex virus DNA: denaturation properties. Virology 55, 280-284.

GRAFSTROM, R.H., ALWINE, J.C., STEINHART, W.L. and HILL, C.W. (1974). Terminal repetitions in herpes simplex virus type 1 DNA. Cold Spring Harbor Symp. Quant. Biol. 39, 679-68.

GRAFSTROM, R.H., ALWINE, J.C., STEINHART, W.L., HILL, C.W. and HYMAN, R.W. (1975). The terminal repetition of herpes simplex virus DNA. Virology 67, 144-157.

GRAHAM, B.J., BENGALI, Z. and VANDE WOUDE, G.F. (1978). Physical map of the origin of defective DNA in herpes simplex virus type 1 DNA. J. Virol. 25, 878-887.

GRAHAM, F.L., VELDHUISEN, G. and WILKIE, N.M. (1973). Infectious herpesvirus DNA. Nature New Biol. 245, 265-266.

GRAY, A., TOKUMARU, T. and SCOTT, T.F. (1958). Different cytopathogenic effects observed in HeLa cells infected with herpes simplex virus. Arch. ges. Virusforsch. 8, 60-76.

GRUNSTEIN, M. and HOGNESS, D.S. (1975). Colony hybridization: a method for the isolation of cloned DNAs that contain a specific gene. Proc. Natl. Acad. Sci. USA 72, 3961-3965.

HAARR, L. and MARSDEN, H.S. (1981). Two-dimensional gel analysis of HSV type 1-induced polypeptides and glycoprotein processing. J. gen. Virol. 52, 77-92.

HALLIBURTON, I.W. (1972). Biological comparisons of type 1 and type 2 herpes simplex viruses. In "Oncogenesis and Herpesviruses", pp. 432-438. Edited by P.M. Biggs, G. de Thé and L.N. Payne. IARC Scientific Publications, no. 2, Lyon.

HALLIBURTON, I.W. and TIMBURY, M.C. (1973). Characterization of temperature-sensitive mutants of herpes simplex virus type 2. Growth and DNA synthesis. Virology 54, 60-68.

HALLIBURTON, I.W. and ANDREW, J.C. (1976). DNA polymerase in pseudorabies virus infected cells. J. gen. Virol. 30, 145-148.

HALLIBURTON, I.W. and TIMBURY, M.C. (1976). Temperature-sensitive mutants of herpes simplex virus type 2: description of three new complementation groups and studies on the inhibition of host cell DNA synthesis. J. gen. Virol. 30, 207-221.

HALLIBURTON, I.W. (1980). Intertypic recombinants of herpes simplex viruses. J. gen. Virol. 48, 1-23.

HALLIBURTON, I.W., MORSE, L.S., ROIZMAN, B. and QUINN, K.E. (1980). Mapping of the thymidine kinase genes of type 1 and type 2 herpes simplex viruses using intertypic recombinants. J. gen. Virol. 49, 235-254.

HAY, J., KOTELES, G.J., KEIR, H.M. and SUBAK-SHARPE, H. (1966). Herpes virus specific ribonucleic acids. Nature (London) 210, 387-390.

HAY, J. and SUBAK-SHARPE, J.H. (1976). Mutants of herpes simplex virus types 1 and 2 that are resistant to phosphonoacetic acid induce altered DNA polymerase activities in infected cells. J. gen. Virol. 31, 145-148.

HAY, R.T. and HAY, J. (1980). Properties of herpesvirus-induced "immediate early" polypeptides. Virology 104, 230-234.

HAYWARD, G.S., FRENKEL, N. and ROIZMAN, B. (1975a). The anatomy of herpes simplex virus DNA: strain difference and heterogeneity in the location of restriction endonuclease sites. Proc. Natl. Acad. Sci. USA 72, 1768-1772

HAYWARD, G.S., JACOB, R.J., WADSWORTH, S.C. and ROIZMAN, B. (1975b). Anatomy of herpes simplex virus DNA: evidence for four populations that differ in the relative orientations of their long and short components. Proc. Natl. Acad. Sci. USA 72, 4243-4247.

HAYWARD, S.D., NOGEE, L. and HAYWARD, G.S. (1980). Organization of repeated regions within the Epstein-Barr virus DNA molecule. J. Virol. 33, 507-521.

HEINE, J.W., HONESS, R.W., CASSAI, E. and ROIZMAN, B. (1974). Proteins specified by herpes simplex virus. XII. The virion polypeptides of type 1 strains. J. Virol. 14, 640-651.

HEINE, V. and COTTLER-FOX, M. (1975). Electron microscopic observations on the composition of herpes-type viruses. In "Oncogenesis and Herpesviruses II", part I, pp. 103-110. Edited by G. de Thé, M.A. Epstein and H. zur Hausen. IARC Scientific Publications, no. 11, Lyon.

HELLER, M. and KIEFF, E. (1981). Colinearity between the DNAs of Epstein-Barr virus and herpesvirus papio. J. Virol. 37, 821-826.

HELLER, M., DAMBAUGH, T. and KIEFF, E. (1981a). Epstein-Barr virus DNA. IX. Variation among viral DNAs from producer and nonproducer infected cells. J. Virol. 38, 632-648.

HELLER, M., GERBER, P. and KIEFF, E. (1981b). Herpesvirus papio DNA is similar in organization to Epstein-Barr virus DNA. J. Virol. 37, 698-709.

HENLE, G., HENLE, W. and DIEHL, V. (1968). Relation of Burkitt's tumor-associated herpes-type virus to infectious mononucleosis. Proc. Natl. Acad. Sci. USA 53, 94-101.

HENLE, G., HENLE, W., CLIFFORD, P., DIEHL, V., KAFUKO, G.W., KIRYA, B.G., KLEIN, G., MORROW, R.H., MUNUBE, G.M.R., PIKE, M.C., TUKEI, P.M. and ZIEGLER, J.L. (1969). Antibodies to EB virus in Burkitt's lymphoma and control groups. J. Nat. Cancer Inst. 43, 1147-1157.

HILL, B.T. and WHATLEY, S. (1975). A simple, rapid microassay for DNA. FEBS Letters 56, 20-23.

HINZE, H.C. (1971). Induction of a lymphoid hyperplasia and lymphoma-like disease in rabbits by herpesvirus sylvilagus. Int. J. Cancer 8, 514-522.

HIRAI, K., IKUTA, K. and KATO, S. (1979). Comparative studies on Marek's disease virus and herpesvirus of turkey DNAs. J. gen. Virol. 45, 119-131.

HIRSCH, I. and VONKA, V. (1974). Ribonucleotides linked to DNA of herpes simplex virus type 1. J. Virol. 13, 1162-1168.

HIRSCH, I., REISCHIG, J., ROUBAL, J. and VONKA, V. (1975). Structure of herpes simplex virus DNA: topography of the molecule. I. Absence of circularly permuted sequences. Virology 65, 496-505.

HIRSCH, I., ROUBAL, J. and VONKA, V. (1976). Replicating DNA of herpes simplex virus type 1. Intervirology 7, 155-175.

HIRSCH, I., CABRAL, G., PATTERSON, M. and BISWAL, N. (1977). Studies on the intracellular replicating DNA of herpes simplex virus type 1. Virology 81, 48-61.

HOBART, P., CRAWFORD, R., SHEN, L., PICTET, R. and RUTTER, W.J. (1980). Cloning and sequence analysis of cDNAs encoding two distinct somatostatin precursors found in the endocrine pancreas of anglerfish. Nature (London) 288, 137-140.

HOCHBERG, E. and BECKER, Y. (1968). Adsorption, penetration and uncoating of herpes simplex virus. J. gen. Virol. 2, 231-241.

HOGGAN, M.D. and ROIZMAN, B. (1959). The isolation and properties of a variant of herpes simplex producing multinucleated giant cells in monolayer cultures in the presence of antibody. American J. Hygiene 70, 208-219.

HOLLAND, L.E., ANDERSON, K.P., STRINGER, J.R. and WAGNER, E.K. (1979). Isolation and localization of herpes simplex virus type 1 mRNA abundant before viral DNA synthesis. J. Virol. 31, 447-462.

HOLLAND, L.E., ANDERSON, K.P., SHIPMAN, C. Jr. and WAGNER, E.K. (1980). Viral DNA synthesis is required for the efficient expression of specific herpes simplex virus type 1 mRNA species. Virology 101, 10-24.

HOLMES, I.H. and WATSON, D.H. (1963). An electron microscope study of the attachment and penetration of herpes virus in BHK 21 cells. Virology 21, 112-123.

HONESS, R.W. and ROIZMAN, B. (1973). Proteins specified by herpes simplex virus. XI. Identification and relative molar rates of synthesis of structural and non-structural herpes virus polypeptides in the infected cell. J. Virol. 12, 1347-1365.

HONESS, R.W. and ROIZMAN, B. (1974). Regulation of herpesvirus macromolecular synthesis. I. Cascade regulation of the synthesis of three groups of viral proteins. J. Virol. 14, 8-19.

HONESS, R.W. and WATSON, D.H. (1974). Herpes simplex virus-specific polypeptides studied by polyacrylamide gel electrophoresis of immune precipitates. J. gen. Virol. 22, 171-185.

HONESS, R.W., POWELL, K.L., ROBINSON, D.J., SIM, C. and WATSON, D.H. (1974). Type specific and type common antigens in cells infected with herpes simplex virus type 1 and on the surfaces of naked and enveloped particles of the virus. J. gen. Virol. 22, 159-169.

HONESS, R.W. and ROIZMAN, B. (1975a). Regulation of herpesvirus macromolecular synthesis: sequential transition of polypeptide synthesis requires functional polypeptides. Proc. Natl. Acad. Sci. USA 72, 1276-1280.

HONESS, R.W. and ROIZMAN, B. (1975b). Proteins specified by herpes simplex virus. XIII. The glycosylation of viral polypeptides. J. Virol. 16, 1308-1326.

HONESS, R.W. and WATSON, D.H. (1977a). Herpes simplex virus resistance and sensitivity to phosphonoacetic acid. J. Virol. 21, 584-600.

HONESS, R.W. and WATSON, D.H. (1977b). Unity and diversity in the herpesviruses. J. gen. Virol. 37, 15-37.

HONESS, R.W., BUCHAN, A., HALLIBURTON, I.W. and WATSON, D.H. (1980). Recombination and linkage between structural and regulatory genes of herpes simplex virus type 1. J. Virol. 34, 716-742.

HUANG, A.S. and WAGNER, R.R. (1964). Penetration of herpes simplex virus into human epidermoid cells. Proc. Soc. exp. Biol. Med. 116, 863-869.

HUANG, E-S., CHEN, S.T. and PAGANO, J. (1973). Human cytomegalovirus. I. Purification and characterization of viral DNA. J. Virol. 12, 1473-1481.

HUANG, E-S. and PAGANO, J. (1974). Human cytomegalovirus. II. Lack of relatedness to DNA of herpes simplex I and II, Epstein-Barr virus and non-human strains of cytomegalovirus. J. Virol. 13, 642-645.

HUANG, E-S., KILPATRICK, B., LAKEMAN, F. and ALFORD, C.A. (1978). Genetic analysis of a cytomegalovirus-like agent isolated from human brain. J. Virol. 26, 718-723.

HUMMELER, K., TOMASSINI, N. and ZAJAC, B. (1969). Early events in herpes simplex virus infection: a radioautographic study. J. Virol. 4, 67-74.

HUSZAR, D. and BACCHETTI, S. (1981). Partial purification and characterization of the ribonucleotide reductase induced by herpes simplex virus infection of mammalian cells. J. Virol. 37, 580-588.

HUTTON, J.R. and WETMUR, J.G. (1973). Effect of chemical modification on the rate of renaturation of deoxyribonucleic acid. Deaminated and glyoxalated deoxyribonucleic acid. Biochemistry 12, 558-563.

HUTTON, J.R. (1977). Renaturation kinetics and thermal stability of DNA in aqueous solutions of formamide and urea. Nucleic Acids Research 4, 3537-3555.

HYMAN, R.W., BURKE, S. and KUDLER, L. (1976). A nearby inverted repeat of the terminal sequence of herpes simplex virus DNA. Biochem. biophys. Res. Commun. 68, 609-61

HYMAN, R.W., OAKES, J.E. and KUDLER, L. (1977). In vitro repair of the pre-existing nicks and gaps in herpes simplex virus DNA. Virology 76, 286-294.

HYMAN, R.W. (1980). Identification of proteins tightly bound to herpes simplex virus DNA. Virology 105, 254-255.

ILTIS, J.P., OAKES, J.E., HYMAN, R.W. and RAPP, F. (1977). Comparison of the DNAs of varicella zoster viruses isolated from clinical cases of varicella and herpes zoster. Virology 82, 345-352.

INGLIS, M.M. and NEWTON, A.A. (1981). Comparison of the activities of HSV-1 and cellular mRNAs as templates for in vitro translation. Virology 110, 1-15.

IWASAKA, T., ODA, H., MORI, R., MIYAZOZO, J. and NAGAFUCHI, S. (1979). Production of tubular structures in Vero cells infected with herpes simplex virus type 2: effects of ultraviolet light irradiation and antimetabolites. J. gen. Virol. 44, 57-67.

JACOB, R.J. and ROIZMAN, B. (1977). Anatomy of herpes simplex virus DNA. VIII. Properties of the replicating DNA. J. Virol. 23, 394-411.

JACOB, R.J., MORSE, L.S. and ROIZMAN, B. (1979). Anatomy of herpes simplex virus DNA. XII. Accumulation of head-to-tail concatemers in nuclei of infected cells and their role in the generation of the four isomeric arrangements of viral DNA. J. Virol. 29, 448-457.

JACQUEMONT, B. and ROIZMAN, B. (1975a). RNA synthesis in cells infected with herpes simplex virus. X. Properties of viral symmetric transcripts and of double-stranded RNA prepared from them. J. Virol. 15, 707-713.

JACQUEMONT, B. and ROIZMAN, B. (1975b). Ribonucleic acid synthesis in cells infected with herpes simplex virus: characterisation of viral high molecular weight nuclear RNA. J. gen. Virol. 29, 155-165.

JACQUEMONT, B. and HUPPERT, J. (1977). Inhibition of viral RNA methylation in herpes simplex virus type 1-infected cells by 5'-S-isobutyl-adenosine. J. Virol. 22, 160-167.

JACQUEMONT, B., GARCIA, A. and HUPPERT, J. (1980). Nuclear processing of viral high-molecular-weight RNA in cells infected with herpes simplex virus type 1. J. Virol. 35, 382-389.

JAMIESON, A.T. and SUBAK-SHARPE, J.H. (1974). Biochemical studies on herpes simplex virus-specified deoxypyrimidine kinase activity. J. gen. Virol. 24, 481-492.

JAMIESON, A.T., GENTRY, G.A. and SUBAK-SHARPE, J.H. (1974). Induction of both thymidine and deoxycytidine kinase activity by herpes viruses. J. gen. Virol. 24, 465-480.

JAMIESON, A.T., HAY, J. and SUBAK-SHARPE, J.H. (1976a). Herpesvirus proteins: induction of nucleoside phosphotransferase activity after herpes simplex virus infection. J. Virol. 17, 1056-1059.

JAMIESON, A.T., MacNAB, J.C.M., PERBAL, B. and CLEMENTS, J.B. (1976b). Virus specified enzyme activity and RNA species in herpes simplex virus type 1 transformed mouse cells. J. gen. Virol. 32, 493-508.

JAMIESON, A.T. and SUBAK-SHARPE, J.H. (1978). Interallelic complementation of mutants of herpes simplex virus deficient in deoxypyrimidine kinase activity. Virology 85, 109-117.

JARIWALLA, R.J., AURELIAN, L. and TS'O, P.O. (1980). Tumorigenic transformation induced by a specific fragment of DNA from herpes simplex type 2. Proc. Natl. Acad. Sci. USA 77, 2279-2283.

JEAN, J-H. and BEN-PORAT, T. (1976). Appearance *in vivo* of single-stranded complementary ends on herpesvirus DNA. Proc. Natl. Acad. Sci. USA 73, 2674-2678.

JEAN, J-H., BLANKENSHIP, M.L. and BEN-PORAT, T. (1977). Replication of herpesvirus DNA. I. Electron microscopic analysis of replicative structures. Virology 79, 281-291.

JEAN, J-H., YOSHIMURA, N., FARUKAWA, T. and POLTKIN, S.A. (1978). Intracellular forms of parental HCMV genome at early stages of the infectious process. Virology 86, 281-286.

JERKOFSKY, M.A., DOBERSEN, M.J. and GREER, S. (1980). Inhibition of the replication of human cytomegalovirus by bromodeoxycytidine in the absence of detectable increases in bromodeoxycytidine kinase activity. Intervirology 14, 233-238.

JOFRE, J.T., SCHAFFER, P.A. and PARRIS, D.S. (1977). Genetics of resistance to phosphonoacetic acid in strain KOS of herpes simplex virus type 1. J. Virol. 23, 833-836.

JOFRE, J.T., COURTNEY, R.J. and SCHAFFER, P.A. (1981). A dominant lethal temperature-sensitive mutant of herpes simplex virus type 1. Virology 111, 173-190.

JONES, P.C., HAYWARD, G.S. and ROIZMAN, B. (1977). Anatomy of herpes simplex virus DNA. VII. α RNA is homologous to noncontiguous sites in both the L and S components of viral DNA. J. Virol. 21, 268-276.

JONES, P.C. and ROIZMAN, B. (1979). Regulation of herpesvirus macromolecular synthesis. VIII. The transcription program consists of three phases during which both extent of transcription and accumulation of RNA in the cytoplasm are regulated. J. Virol. 31, 299-314.

JONGENEEL, C.V. and BACHENHEIMER, S.L. (1980). Replication of herpes simplex virus type 1 DNA in permeabilized infected cells. Nucleic Acids Research 8, 1661-1673.

KAERNER, H.C., MAICHLE, I.B., OTT, A. and SCHRODER, C.H. (1979). Origin of two different classes of defective HSV-1 Angelotti DNA. Nucleic Acids Research 6, 1467-1478.

KAPLAN, A.S. and VATTER, A.E. (1959). A comparison of herpes simplex and pseudorabies viruses. Virology 7, 394-407.

KAPLAN, A.S. and BEN-PORAT, T. (1964). Mode of replication of pseudorabies virus DNA. Virology 23, 90-95.

KAPLAN, A.S. and BEN-PORAT, T. (1976). Synthesis of proteins in cells infected with herpesvirus. XI. Sulfated, structural glycoproteins. Virology 70, 561-563.

KAZAMA, F.Y. and SCHORNSTEIN, K.L. (1972). Herpes-type virus particles associated with a fungus. Science 177, 696-697.

KEIR, H.M. and GOLD, E. (1963). DNA nucleotidyl transferase and deoxyribonuclease from cultured cells infected with herpes simplex virus. Biochim. biophys. Acta 72, 263-276.

KEIR, H.M., SUBAK-SHARPE, H., SHEDDEN, W.I.H., WATSON, D.H. and WILDY, P. (1966). Immunological evidence for a specific DNA polymerase produced after infection with herpes simplex virus. Virology 30, 154-157.

KELLER, J.M., SPEAR, P.G. and ROIZMAN, B. (1970). Proteins specified by herpes simplex virus. III. Viruses differing in their effect on the social behaviour of infected cells specify different membrane glycoproteins. Proc. Natl. Acad. Sci. USA 65, 865-871.

KELLER, J.M. (1976). The expression of the syn gene of herpes simplex virus type 1. II. Requirements of macromolecular synthesis. Virology 72, 402-409.

KHAN, N.C., MELNICK, J.L. and BISWAL, N. (1978). The fate of parental herpesvirus DNA after infection with normal and photoinactivated virions. J. gen. Virol. 39, 195-200.

KIEFF, E.D., BACHENHEIMER, S.L. and ROIZMAN, B. (1971). Size, composition and structure of the deoxyribonucleic acid of herpes simplex virus subtypes 1 and 2. J. Virol. 8, 125-132.

KIEFF, E.D., HOYER, B., BACHENHEIMER, S.L. and ROIZMAN, B. (1972). Genetic relatedness of type 1 and type 2 herpes simplex viruses. J. Virol. 9, 738-745.

KILLINGTON, R.A., YEO, J., HONESS, R.W., WATSON, D.H., DUNCAN, B.E., HALLIBURTON, I.W. and MUMFORD, J. (1977). Comparative analysis of the proteins and antigens of five herpesviruses. J. gen. Virol. 37, 297-310.

KILLINGTON, R.A., RANDALL, R.E., YEO, J., HONESS, R.W., HALLIBURTON, I.W. and WATSON, D.H. (1978). Observations on antigenic relatedness between viruses of the herpes simplex "neutroseron". In "Oncogenesis and Herpesviruses III" part I, pp. 185-194. Edited by G. de Thé, W. Henle and F. Rapp. IARC Scientific Publications, no. 24, Lyon.

KILPATRICK, B.A., HUANG, E-S. and PAGANO, J.S. (1976). Analysis of cytomegalovirus genomes with restriction endonucleases HindIII and EcoRI. J. Virol. 18, 1095-1105.

KILPATRICK, B.A. and HUANG, E-S. (1977). Human cytomegalovirus genome: partial denaturation map and organization of genome sequences. J. Virol. 24, 261-276.

KIM, K.S., SAPIENZA, V.J., CARP, R.I. and MOON, H.M. (1976a). Analysis of structural proteins of purified murine cytomegalovirus. J. Virol. 17, 906-915.

KIM, K.S., SAPIENZA, V.J., CARP, R.I. and MOON, H.M. (1976b). Analysis of structural proteins of purified human cytomegalovirus. J. Virol. 20, 604-611.

KINTNER, C.R. and SUGDEN, B. (1979). The structure of the termini of the DNA of Epstein-Barr virus. Cell 17, 661-671.

KIRKWOOD, J., GEERING, G. and OLD, L.J. (1972). Demonstration of group and type-specific antigens of herpes viruses. In "Oncogenesis and Herpesviruses", pp. 479. Edited by P.M. Biggs, G. de The and L.N. Payne. IARC Scientific Publications, no. 2, Lyon.

KIT, S. and DUBBS, D.R. (1963a). Acquisition of thymidine kinase activity by herpes simplex infected mouse fibroblast cells. Biochem. biophys. Res. Commun. 11, 55-59.

KIT, S. and DUBBS, D.R. (1963b). Non-functional thymidine kinase cistron in bromodeoxyuridine resistant strains of herpes simplex virus. Biochem. biophys. Res. Commun. 13, 500-504.

KLEMPERER, H.G., HAYNES, G.R., SHEDDEN, W.I.H. and WATSON, D.H. (1967). A virus specific thymidine kinase in BHK 21 cells infected with herpes simplex virus. Virology 31, 120-128.

KNIPE, D.M., RUYECHAN, W.T., ROIZMAN, B. and HALLIBURTON, I.W. (1978). Molecular genetics of herpes simplex virus: demonstration of regions of obligatory and nonobligatory identity within diploid regions of the genome by sequence replacement and insertion. Proc. Natl. Acad. Sci. USA 75, 3896-3900.

KNIPE, D.M., RUYECHAN, W.T., HONESS, R.W. and ROIZMAN, B. (1979). Molecular genetics of herpes simplex virus: the terminal a sequences of the L and S components are obligatorily identical and constitute a part of a structural gene mapping predominantly in the S component. Proc. Natl. Acad. Sci. USA 76, 4534-4538.

KNIPE, D.M., BATTERSON, W., NOSAL, C., ROIZMAN, B. and BUCHAN, A. (1981). Molecular genetics of herpes simplex virus. VI. Characterization of a temperature-sensitive mutant defective in the expression of all early viral gene products. J. Virol. 38, 539-547.

KNOPF, K-W. (1979). Synthesis of herpes simplex virus DNA in isolated chromatin. Biochemistry 18, 1776-1780.

KNOWLES, R.W. and PERSON, S. (1976). Effect of 2-deoxyglucose, glucosamine and mannose on cell fusion and the glycoproteins of herpes simplex virus. J. Virol. 18, 644-651.

KOLBER, A.R. (1975). In vitro synthesis of DNA in nuclei isolated from human lung cells infected with herpes simplex type II virus. J. Virol. 15, 322-331.

KOMANO, T. and SINSHEIMER, R.L. (1969). Preparation and purification of ØX-RF component I. Biochim. biophys. Acta 155, 295-298.

KOUSOULAS, K.G., PERSON, S. and HOLLAND, T.C. (1978). Timing of some of the molecular events required for cell fusion induced by herpes simplex virus type 1. J. Virol. 27, 505-51

KOZAK, M. and ROIZMAN, B. (1974). Regulation of herpesvirus macromolecular synthesis: nuclear retention of non-translated viral RNA sequences. Proc. Natl. Acad. Sci. USA 71, 4322-4326.

KOZAK, M. and ROIZMAN, B. (1975). RNA synthesis in cells infected with herpes simplex virus. IX. Evidence for accumulation of abundant symmetric transcripts in nuclei. J. Virol. 15, 36-40.

KUDLER, L. and HYMAN, R.W. (1979). Exonuclease III digestion of herpes simplex virus DNA. Virology 92, 68-81.

LADIN, B.F., BLANKENSHIP, M.L. and BEN-PORAT, T. (1980). Replication of herpesvirus DNA. V. Maturation of concatemeric DNA of pseudorabies virus to genome length is related to capsid formation. J. Virol. 33, 1151-1164.

LAKEMAN, A.D. and OSBORN, J.E. (1979). Size of infectious DNA from human and murine cytomegalovirus. J. Virol. 30, 414-416.

LANDO, D.L. and RYHINER, M-L. (1969). Pourvoir infectieux du DNA d'herpesvirus hominis en culture cellulaire. Compte Rendu de l'Academie de Sciences de Paris 269, 527-530.

LANDY, A. and ROSS, W. (1977). Viral integration and excision: structure of the lambda att sites. Science 197, 1147-1160.

LANGELIER, Y., DECHAMPS, M. and BUTTIN, G. (1978). Analysis of dCMP deaminase and CDP reductase levels in hamster cells infected by herpes simplex virus. J. Virol. 26, 547-555.

LEE, G.T-Y. and SPEAR, P.G. (1980). Viral and cellular factors that influence cell fusion induced by herpes simplex virus. Virology 107, 402-414.

LEE, L.F., KIEFF, E.D., BACHENHEIMER, S.L., ROIZMAN, B., SPEAR, P.G., BURMESTER, B.R. and NAZERIAN, K. (1971). Size and composition of Marek's disease virus deoxyribonucleic acid. J. Virol. 7, 289-294.

LEE, Y.S., TANAKA, A., LAU, R.Y., NONOYAMA, M. and RABIN, H. (1980). Comparative studies of herpesvirus papio (baboon herpesvirus) DNA and Epstein-Barr virus DNA. J. gen. Virol. 51, 245-253.

LEE, Y.S., NONOYAMA, M. and RABIN, H. (1981). Colinear relationships of herpesvirus papio DNA to Epstein-Barr virus DNA. Virology 110, 248-252.

LEINBACH, S.S and SUMMERS, W.C. (1980). The structure of herpes simplex virus type 1 DNA as probed by micrococcal nuclease digestion. J. gen. Virol. 51, 45-60.

LEMASTER, S. and ROIZMAN, B. (1980). Herpes simplex virus phosphoproteins. II. Characterization of the virion protein kinase and of the polypeptides phosphorylated in the virion. J. Virol. 35, 798-811.

LEUNG, W-C., DIMOCK, K., SMILEY, J. and BACCHETTI, S. (1980). Herpes simplex virus thymidine kinase transcripts are absent from both nucleus and cytoplasm during infection in the presence of cycloheximide. J. Virol. 36, 361-365.

LINDAHL, T., ADAMS, A., BJURSELL, G., BORNKAMM, G.W., KASCHKA-DIERICH, C. and JEHN, V. (1976). Covalently closed circular duplex DNA of Epstein-Barr virus in a human lymphoid cell line. J. mol. Biol. 102, 511-530.

LINNEMANN, C.C.Jr., BUCHMAN, T.G., LIGHT, I.J., BALLARD, J.L. and ROIZMAN, B. (1978). Transmission of herpes simplex virus type 1 in a nursery for the newborn: identification of viral isolates by DNA fingerprinting. Lancet 1, 964-966.

LOCKER, H. and FRENKEL, N. (1978). The DNA of serially passaged herpes simplex virus: organization, origin, and homology to viral RNA. In "Oncogenesis and Herpesviruses III", part I, pp. 75-85. Edited by G. de Thé, W. Henle and F. Rapp. IARC Scientific Publications, no. 24, Lyon.

LOCKER, H. and FRENKEL, N. (1979a). Structure and origin of defective genomes contained in serially passaged herpes simplex virus type 1 (Justin). J. Virol. 29, 1065-1077.

MACKEM, S. and ROIZMAN, B. (1980). Regulation of herpesvirus macromolecular synthesis: transcription-initiation sites and domains of α genes. Proc. Natl. Acad. Sci. USA 77, 7122-7126.

MAITLAND, N.J. and McDOUGALL, J.K. (1977). Biochemical transformation of mouse cells by fragments of herpes simplex virus DNA. Cell 11, 233-241.

MANIATIS, T., JEFFREY, A. and VAN DE SANDE, H. (1975). Chain length determination of small double- and single-stranded DNA molecules by polyacrylamide gel electrophoresis. Biochemistry 14, 3787-3794.

MANSERVIGI, R., SPEAR, P.G. and BUCHAN, A. (1977). Cell fusion induced by herpes simplex virus is promoted and suppressed by different glycoproteins. Proc. Natl. Acad. Sci. USA 74, 3913-3917.

MANTEI, N., BOLL, W. and WEISSMAN, C. (1979). Rabbit β-globin mRNA production in mouse L cells transformed with cloned rabbit β-globin chromosomal DNA. Nature (London) 281, 40-46.

MAO, J.C.H. and ROBISHAW, E.E. (1975). Mode of inhibition of herpes simplex virus DNA polymerase by phosphonoacetate. Biochemistry 14, 5475-5479.

MARCIANO-CABRAL, R., DIX, R.D., CABRAL, G.A. and COURTNEY, R.J. (1977). Effects of cytochalasin B on the maturation of herpes simplex virus type 1: an ultrastructural investigation. Virology 76, 860-865.

MAREK, J. (1907). Multiple nervenentzundung (polyneuritis) bei huhnern. Deutsche Tierarztliche Wochenschrift 15, 417-421.

MARSDEN, H.S., CROMBIE, I.K. and SUBAK-SHARPE, J.H. (1976). Control of protein synthesis in herpesvirus-infected cells: analysis of the polypeptides induced by wild-type and sixteen temperature-sensitive mutants of HSV strain 17. J. gen. Virol. 31, 347-372.

MARSDEN, H.S., STOW, N.D., PRESTON, V.G., TIMBURY, M.C. and WILKIE, N.M. (1978). Physical mapping of herpes simplex virus-induced polypeptides. J. Virol. 28, 624-642.

MATHEWS, R.E.F. (1979). Third report of the international committee on taxonomy of viruses. Classification and nomenclature of viruses. Intervirology 12, 129-296.

MAXAM, A.M. and GILBERT, W. (1980). Sequencing end-labelled DNA with base-specific chemical cleavages. Methods in Enzymology 65, 499-560.

MELÉNDEZ, L.V., HUNT, R.D., DANIEL, M.D., GARCIA, F.G. and FRASER, C.E.O. (1969). Herpesvirus saimiri. II. Experimentally induced malignant lymphoma in primates. Laboratory Animal Care 19, 378-386.

MELÉNDEZ, L.V., HUNT, R.D., KING, N.W., BARAHONA, H.H., DANIEL, M.D., FRASER, C.E.O. and GARCIA, F.G. (1972). Herpesvirus ateles, a new lymphoma virus of monkeys. Nature New Biol. 235, 182-184.

MELNICK, J.L., RABIN, E.R. and JENSON, A.B. (1968). Intracellular herpesvirus aggregate in the form of a pentagonal dipyramidal crystal-like structure. J. Virol. 2, 78-80.

MILLER, R.H., KUEHER, L., PRYSTOWSKY, J. and HYMAN, R.J. (1980). Comparison of foldback sequences of herpes simplex types 1 and 2 DNA. J. gen. Virol. 46, 51-62.

MILLER, R.L., ILTIS, J.P. and RAPP, F. (1977). Differential effect of arabinosylfuranosyl-thymine on the replication of human herpes-viruses. J. Virol. 23, 679-684.

MILLETTE, R.L. and KLAIBER, R. (1980). Gene expression of herpes simplex virus. II. UV radiological analysis of viral transcription units. J. Virol. 34, 604-614.

MIOVIC, M.L. and PIZER, L.I. (1980). Characterization of RNA synthesized in isolated nuclei of herpes simplex virus type 1-infected KB cells. J. Virol. 33, 567-571.

MIYAMOTO, K. and MORGAN, C. (1971). Structure and development of viruses as observed in the electron microscope. IX. Entry and uncoating of herpes simplex virus. J. Virol. 8, 910-918.

MOCARSKI, E.S., POST, L.E. and ROIZMAN, B. (1980). Molecular engineering of the herpes simplex virus genome: insertion of a second L-S junction into the genome causes additional inversions. Cell 22, 243-255.

MORGAN, C., JONES, E.P., HOLDEN, M. and ROSE, H.M. (1958). Intranuclear crystals of herpes simplex virus observed with the electron microscope. Virology 5, 568-571.

MORGAN, C., ROSE, H.M., HOLDEN, M. and JONES, E.P. (1959). Electron microscopic observations on the development of herpes simplex virus. J. exp. Med. 110, 643-656.

MORGAN, C., ROSE, H.M. and MEDNIS, B. (1968). Electron microscopy of herpes simplex virus. I. Entry. J. Virol. 2, 507-516.

MORRISON, J.M. and KEIR, H.M. (1968). A new DNA-endonuclease in cells infected with herpes virus: partial purification and properties of the enzyme. J. gen. Virol. 3, 337-347.

MORSE, L.S., BUCHMAN, T.G., ROIZMAN, B. and SCHAFFER, P.A. (1977). Anatomy of herpes simplex virus DNA. IX. Apparent exclusion of some parental DNA arrangements in the generation of intertypic (HSV-1 x HSV-2) recombinants. J. Virol. 24, 231-248.

MORSE, L., PEREIRA, L., ROIZMAN, B. and SCHAFFER, P.A. (1978). Anatomy of herpes simplex virus (HSV) DNA. X. Mapping of viral genes by analysis of polypeptides and functions specified by HSV-1 x HSV-2 recombinants. J. Virol. 26, 389-410.

MOSMANN, T.R. and HUDSON, J.B. (1973). Some properties of the genome of murine cytomegalovirus (MCV). Virology 54, 135-149.

MOSS, B., GERSCHOWITZ, A., STRINGER, J.R., HOLLAND, L.E. and WAGNER, E.K. (1977). 5'-terminal and internal methylated nucleosides in herpes simplex virus type 1 mRNA. J. Virol. 23, 234-239.

MOSS, H., CHARTRAND, P., TIMBURY, M.C. and HAY, J. (1979). Mutant of herpes simplex type 2 with temperature-sensitive lesions affecting virion thermostability and DNase activity: identification of the lethal mutation and physical mapping of the nuc⁻ lesion. J. Virol. 32, 140-146.

MOUTTET, M-E., GUÉTARD, D. and BÉCHET, J.M. (1979). Random cleavage of intranuclear herpes simplex virus DNA by micrococcal nuclease. FEBS Letters 100, 107-109.

MULLER, W.E.G., ZAHN, R.K., ARENDES, J. and FALKE, D. (1979a). Oligoribonucleotide initiators for herpes simplex virus DNA synthesis *in vivo* and *in vitro*. Virology 98, 200-210.

MULLER, W.E.G., ZAHN, R.K., ARENDES, J. and FALKE, D. (1979b). Phosphorylation of arabinofuranosylthymine in non-infected and herpesvirus (TK^+ and TK^-)-infected cells. J. gen. Virol. 43, 261-271.

MUNYON, W., KRAISEDBURD, E., DAVIS, D. and MANN, J. (1971). Transfer of thymidine kinase to thymidine kinaseless L cells by infection with ultraviolet irradiated herpes simplex virus. J. Virol. 7, 813-820.

MURPHY, F.A., HARRISON, A.K. and WHITFIELD, S.G. (1967). Intranuclear formation of filaments in herpes virus hominis infection of mice. Arch. ges. Virusforsch. 21, 463-468.

MURRAY, B.K. and BISWAL, N. (1974). Synthesis of herpes simplex virus type 1 (HSV-1) DNA in isolated nuclei. II. Covalent linkage of RNA to nascent viral DNA. Intervirology 4, 14-22.

MURRAY, B.K., BENYESH-MELNICK, M. and BISWAL, N. (1974). Early and late viral-specific polyribosomal RNA in herpesvirus-1 and -2 infected rabbit kidney cells. Biochim. biophys. Acta 361, 209-220.

MURRAY, B.K., BISWAL, N., BOOKOUT, J.B., LANFORD, R.E., COURTNEY, R.J. and MELNICK, J.L. (1975). Cyclic appearance of defective interfering particles of herpes simplex virus and the concomitant accumulation of early polypeptide VP175. Intervirology 5, 173-184.

MURRAY, K. and MURRAY, N.E. (1975). Phage lambda receptor chromosomes for DNA fragments made with restriction endonuclease III of Haemophilus influenzae and restriction endonuclease I of Escherichia coli. J. mol. Biol. 98, 551-564.

NAIB, Z.M., NAHMIAS, A.J. and JOSEY, W.E. (1966). Cytology and histopathology of cervical herpes simplex infection. Cancer 19, 1026-1031.

NAZERIAN, K. (1974). DNA configuration in the core of Marek's disease virus. J. Virol. 13, 1148-1150.

NEUBAUER, R.H., RABIN, H., STRAND, B.C., NONOYAMA, M. and NELSON-REES, W.A. (1979). Establishment of a lymphoblastoid cell line and isolation of an Epstein-Barr (EBV) related virus of gorilla origin. J. Virol. 31, 845-848.

NII, S., MORGAN, C. and ROSE, H.M. (1968a). Electron microscopy of herpes simplex virus. II. Sequence of development. J. Virol. 2, 517-536.

NII, S., ROSENKRANZ, H.S., MORGAN, C. and ROSE, H.M. (1968b). Electron microscopy of herpes simplex virus. III. Effect of hydroxyurea. J. Virol. 2, 1163-1171.

NII, S. (1971). Electron microscopic observations on FL cells infected with herpes simplex virus. II. Envelopment. Biken J. 14, 325-348.

NII, S. and YASUDA, I. (1975). Detection of viral cores having toroid structures in eight herpesviruses. Biken J. 18, 41-46.

NISHIOKA, Y. and SILVERSTEIN, S. (1977). Degradation of cellular mRNA during infection by herpes simplex virus. Proc. Natl. Acad. Sci. USA 74, 2370-2374.

NISHIOKA, Y. and SILVERSTEIN, S. (1978). Requirement of protein synthesis for the degradation of host mRNA in Friend erythroleukemia cells infected with herpes simplex virus type 1. J. Virol. 27, 619-627.

NORRILD, B., LUDWIG, H. and ROTT, R. (1978). Identification of a common antigen of herpes simplex virus, bovine herpes mammilitis virus and B virus. J. Virol. 26, 712-717.

NORRILD, B. and VESTERGAARD, B.F. (1979). Immunoelectrophoretic identification and purification of herpes simplex virus antigens released from infected cells in tissue culture. Intervirology 11, 104-110.

OAKES, J.E., ILTIS, J.P., HYMAN, R.W. and RAPP, F. (1977). Analysis by restriction enzyme cleavage of human varicella-zoster virus DNAs. Virology 82, 353-361.

ODA, H., MORI, R., MIYAZONO, J. and IWASAKA, T. (1979). Effects of antimetabolites on the production of tubular structures in Vero cells infected with herpes simplex virus type 2. Arch. Virol. 62, 175-187.

O'DONOVAN, C. and ROIZMAN, B. (1961). Differentiation of the process of cell infection with free herpes simplex virus from the recruitment of cells into virus-induced syncytia in X-rayed cell cultures. Virology 15, 374-376.

OKADA, K., FUJIMOTO, Y., NAKANISHI, Y.H., ONUMA, M. and MIKAMI, T. (1979). Further study on the three-dimensional structure of the core of Marek's disease and herpesvirus of turkey. Arch. Virol. 59, 137-144.

OLD, L.J., BOYSE, E.A., OETTGEN, H.F., DE HARVEN, E., GEERING, G., WILLIAMSON, B. and CLIFFORD, P. (1966). Precipitating antibody in human serum to an antigen present in cultured Burkitt's lymphoma cells. Proc. Natl. Acad. Sci. USA 56, 1699-1704.

OLSHEVSKY, U. and BECKER, Y. (1972). Surface glycopeptides in the envelope of herpes simplex virions. Virology 50, 277-279.

PARA, M.F., BAUCKE, R.B. and SPEAR, P.G. (1980). Immunoglobulin G(Fc)-binding receptors on virions of herpes simplex type 1 and transfer of these receptors to the cell surface by infection. J. Virol 34, 512-520.

PARRIS, D.S., DIXON, R.A.F. and SCHAFFER, P.A. (1980). Physical mapping of herpes simplex virus type 1 ts mutants by marker rescue: correlation of the physical and genetic maps. Virology 100, 275-287.

PAULS, F.P. and DOWDLE, W.R. (1967). A serological study of herpesvirus hominis strains by microneutralisation tests. J. Immun. 98, 941-947.

PEDERSEN, M., TALLEY-BROWN, S. and MILLETTE, R.L. (1981). Gene expression of herpes simplex virus. III. Effect of arabinosyl-adenine on viral polypeptide synthesis. J. Virol. 38, 712-719.

PELLICER, A., WIGLER, M., AXEL, R. and SILVERSTEIN, S. (1978). The transfer and stable integration of the HSV thymidine kinase gene into mouse cells. Cell 14, 133-141.

PENNINGTON, T.H. and McCREA, M.A. (1977). Processing of a pseudorabies virus-induced protein which is glycosylated, sulphated and excreted. J. gen. Virol. 34, 155-165.

PERDUE, M.L., KEMP, M.C., RANDALL, C.C. and O'CALLAGHAN, D.J. (1974). Studies on the molecular anatomy of the L-M strain of equine herpes virus type 1. Proteins of the nucleocapsid and intact virion. Virology 59, 201-216.

PEREIRA, L., WOLFF, M.H., FENWICK, M. and ROIZMAN, B. (1977). Regulation of herpesvirus macromolecular synthesis. V. Properties of α polypeptides made in HSV-1 and HSV-2 infected cells. Virology 77, 733-749.

PIGNATTI, P.F., CASSAI, E. and BERTAZZONI, U. (1979). Herpes simplex virus DNA synthesis in a partially purified soluble extract from infected cells. J. Virol. 32, 1033-1036.

PIGNATTI, P.F. and CASSAI, E. (1980). Analysis of herpes simplex virus nucleoprotein complexes extracted from infected cells. J. Virol. 36, 816-828.

PIZER, L.I., COHEN, G.H. and EISENBERG, R.J. (1980). Effect of tunicamycin on herpes simplex virus glycoproteins and infectious virus production. J. Virol. 34, 142-153.

PLUMMER, G. (1964). Serological comparison of the herpes viruses. British J. exp. Pathol. 45, 135-141.

PONCE DE LEON, M., EISENBERG, R.J. and COHEN, G.H. (1977). Ribonucleotide reductase from herpes simples virus (types 1 and 2) infected and uninfected KB cells: properties of the partially purified enzymes. J. gen. Virol. 36, 163-173.

POST, L.E., CONLEY, A.J., MOCARSKI, E.S. and ROIZMAN, B. (1980). Cloning of reiterated and nonreiterated herpes simplex virus 1 sequences as BamHI fragments. Proc. Natl. Acad. Sci. USA 77, 4201-4205.

POST, L.E., MACKEM, S. and ROIZMAN, B. (1981). Regulation of α genes of herpes simplex virus: expression of chimeric genes produced by fusion of thymidine kinase with α gene promoters. Cell 24, 555-566.

POWELL, D.J. (1979). Structure and expression of the pseudorabies virus genome. PhD thesis, University of Glasgow.

POWELL, K.L., BUCHAN, A., SIM, C. and WATSON, D.H. (1974). Type-specific protein in herpes simplex virus envelope reacts with neutralising antibody. Nature (London) 249, 360-361.

POWELL, K.L. and WATSON, D.H. (1975). Some structural antigens of herpes simplex virus type 1. J. gen. Virol. 29, 167-178.

POWELL, K.L. and PURIFOY, D.J.M. (1976). DNA-binding proteins of cells infected by herpes simplex virus type 1 and type 2. Intervirology 7, 225-239.

POWELL, K.L. and PURIFOY, D.J.M. (1977). Nonstructural proteins of herpes simplex virus. I. Purification of the induced DNA polymerase. J. Virol. 24, 618-626.

PRESTON, C.M. and NEWTON, A.A. (1976). The effects of herpes simplex virus type 1 on cellular DNA dependent RNA polymerase activities. J. gen. Virol. 33, 471-482.

PRESTON, C.M. (1977). Cell-free synthesis of herpes simplex virus-coded pyrimidine deoxyribonucleoside kinase enzyme. J. Virol. 23, 455-460.

PRESTON, C.M. (1979). Control of herpes simplex virus type 1 mRNA synthesis in cells infected with wild-type virus or the temperature-sensitive mutant tsK. J. Virol. 29, 275-284.

PRESTON, C.M. and McGEOCH, D.J. (1981). Identification and mapping of the two polypeptides encoded within the herpes simplex virus type 1 thymidine kinase gene sequences. J. Virol. 38, 593-605.

PRESTON, V.G., DAVISON, A.J., MARSDEN, H.S., TIMBURY, M.C., SUBAK-SHARPE, J.H. and WILKIE, N.M. (1978). Recombinants between herpes simplex virus types 1 and 2: analyses of genome structures and expression of immediate-early polypeptides. J. Virol. 28, 499-517.

PRINGLE, C.R., HOWARD, D.K. and HAY, J. (1973). Temperature-sensitive mutants of pseudorabies virus with differential effects on viral and host DNA synthesis. Virology 55, 495-505.

PRITCHETT, R.F., HAYWARD, S.D. and KIEFF, E. (1975). DNA of Epstein-Barr virus. I. Comparative studies of the DNA of Epstein-Barr virus from HR-1 and B95-8 cells: size, structure and relatedness. J. Virol. 15, 556-569.

PRITCHETT, R.F. (1980). DNA nucleotide sequence heterogeneity between the Towne and AD169 strains of cytomegalovirus. J. Virol. 36, 152-161.

PURCHASE, H.G., OKAZAKI, W. and BURMESTER, B.R. (1971). Field trials with the herpes virus of turkeys (HVT), strain FC 126, as a vaccine against Marek's disease. Poultry Science 50, 775-783.

PURIFOY, D.J.M. and BENYESH-MELNICK, M. (1975). DNA polymerase induction by DNA-negative temperature-sensitive mutants of herpes simplex virus type 2. Virology 68, 374-386.

PURIFOY, D.J.M. and POWELL, K.L. (1977). Herpes simplex virus DNA polymerase as the site of phosphonoacetate sensitivity: temperature-sensitive mutants. J. Virol. 24, 470-477.

PURIFOY, D.J.M. and POWELL, K.L. (1981). Temperature-sensitive mutants in two distinct complementation groups of herpes simplex virus type 1 specify thermolabile DNA polymerase. J. gen. Virol. 54, 217-222.

RAAB-TRAUB, N., PRITCHETT, R. and KIEFF, E. (1978). DNA of Epstein-Barr virus. III. Identification of restriction enzyme fragments which contain DNA sequences which differ among strains of EBV. J. Virol. 27, 388-398.

RAAB-TRAUB, N., DAMBAUGH, T. and KIEFF, E. (1980). DNA of Epstein-Barr virus. VIII. Analysis and molecular epidemiology of the "additional" DNA of two Burkitt tumor isolates of EBV. Cell 22, 257-267.

RABIN, H., NEUBAUER, R.H., HOPKINS, R.F. III and NONOYAMA, M. (1978). Further characterization of a herpesvirus-positive orangutan cell line and comparative aspects of in vitro transformation with lymphotropic old world primate herpesviruses. Intl. J. Cancer. 21, 762-767.

RAND, T.H. and BEN-PORAT, T. (1980). Distribution of sequences homologous to the DNA of herpes simplex virus, types 1 and 2, in the genome of pseudorabies virus. Intervirology 13, 48-53.

RAPP, F., ILTIS, J.P., OAKES, J.E. and HYMAN, R.W. (1977). A novel approach to study the DNA of herpes zoster virus. Intervirology 8, 272-280.

RAWLS, W.E., LAUREL, D., MELNICK, J.L., GLICKSMAN, J.M. and KAUFMAN, R.H. (1968). A search for viruses in smegma, premalignant and early malignant cervical tissues. The isolation of herpesviruses with distinct antigenic properties. American J. Epidemiol. 87, 647-655.

REYES, G.R., LA FEMINA, R., HAYWARD, S.D. and HAYWARD, G.S. (1979). Morphological transformation by DNA fragments of human herpes viruses: evidence for two distinct transforming regions in HSV-1 and HSV-2 and lack of correlation with biochemical transfer of the thymidine kinase gene. Cold Spring Harbor Symp. Quant. Biol. 44, 629-641.

RICE, M., STRINGER, J., SWANSTROM, R. and WAGNER, E.K. (1976). The association of the infecting DNA of herpes simplex virus with cellular components. Virology 70, 185-189.

RICHARDS, J.C., HYMAN, R.W. and RAPP, F. (1979). Analysis of the DNAs from seven varicella-zoster virus isolates. J. Virol. 32, 812-821.

RIGBY, P.W.J., DIECKMANN, M., RHODES, C. and BERG, P. (1977). Labelling deoxyribonucleic acid to high specific activity in vitro by nick-translation with DNA polymerase I. J. mol. Biol. 113, 237-251.

RITCHIE, D.A., BROWN, S.M., SUBAK-SHARPE, J.H. and JAMIESON, A.T. (1977). Heterozygosis and genetic recombination in herpes simplex type 1 virus. Virology 82, 323-333.

RIXON, F.J. (1977). Studies on herpes simplex virus DNA synthesis. PhD thesis, University of Glasgow.

ROBERTS, R.J. (1981). Restriction and modification enzymes and their recognition sequences. Nucleic Acids Research 9, r75-r96.

ROIZMAN, B. and ROANE, P.R.Jr. (1964). The multiplication of herpes simplex virus. II. The relation between protein synthesis and the duplication of viral DNA in infected HEp-2 cells. Virology 22, 262-269.

ROIZMAN, B., BORMAN, G.S. and KAMALI-ROUSTA, M. (1965). Macromolecular synthesis in cells infected with herpes simplex virus. Nature 206, 1374-1375.

ROIZMAN, B. and FURLONG, D. (1974). The replication of herpesviruses. In "Comprehensive Virology", vol. 3, pp. 229-403. Edited by H. Fraenkel-Conrat and R.R. Wagner. Plenum Press, New York.

ROIZMAN, B., KOZAK, M., HONESS, R.W. and HAYWARD, G. (1974). Regulation of herpesvirus macromolecular synthesis: evidence for multilevel regulation of herpes simplex 1 RNA and protein synthesis. Cold Spring Harbor Symp. Quant. Biol. 39, 687-701.

ROIZMAN, B., CARMICHAEL, S., DE THÉ, G.B., MASIC, M., NAHMIAS, A., PLOWRIGHT, W., RAPP, F., SHELDRICK, P., TAKASHI, M., TERNI, M. and WOLFE, K. (1978). Provisional classification of herpesviruses. In "Oncogenesis and Herpesviruses III", vol. II, pp. 1079-1082. Edited by G. de Thé, F. Rapp and W. Henle. IARC Scientific Publications, no. 24, Lyon.

ROIZMAN, B. (1979). The structure and isomerization of herpes simplex virus genomes. Cell 16, 481-494.

ROIZMAN, B., JACOB, R.J., KNIPE, D.M., MORSE, L.S. and RUYECHAN, L.S. (1979). On the structure, functional equivalence, and replication of the four arrangements of herpes simplex virus DNA. Cold Spring Harbor Symp. Quant. Biol. 43, 809-826.

ROLTON, H.A. and KEIR, H.M. (1974). Deoxycytidylate deaminase. Evidence for a new enzyme in cells infected by the virus of herpes simplex. Biochem. J. 143, 403-409.

ROYSTON, I. and AURELIAN, L. (1970). Immunofluorescent detection of herpesvirus antigens in exfoliated cells from human cervical carcinoma. Proc. Natl. Acad. Sci. USA 67, 204-212.

RUBENSTEIN, A.S., GRAVELL, M. and DARLINGTON, R. (1972). Protein kinase in enveloped herpes simplex virions. Virology 50, 287-290.

RUBENSTEIN, A.S. and KAPLAN, A.S. (1975). Electron microscopic studies of the DNA of defective and standard pseudorabies virions. Virology 66, 385-392.

RUSSELL, W.C., GOLD, E., KEIR, H.M., OMURA, H., WATSON, D.H. and WILDY, P. (1964). The growth of herpes simplex virus and its nucleic acid. Virology 22, 103-110.

RUYECHAN, W.T., MORSE, L.S., KNIPE, D.M. and ROIZMAN, B. (1979). Molecular genetics of herpes simplex virus. II. Mapping of the major viral glycoproteins and of the genetic loci specifying the social behaviour of infected cells. J. Virol. 677-697.

RYMO, L. and FORSELUM, S. (1978). Cleavage of Epstein-Barr virus DNA by restriction endonucleases EcoRI, HindIII and BamHI. Nucleic Acids Research 5, 1387-1402.

SANDRI-GOLDIN, R.M., LEVINE, M. and GLORIOSO, J.C. (1981). Method for induction of mutations in physically defined regions of the herpes simplex virus genome. J. Virol. 38, 41-49.

SAROV, I. and FRIEDMANN, A. (1976). Electron microscopy of human cytomegalovirus DNA. Arch. Virol. 50, 343-347.

SARMIENTO, M., HAFFEY, M. and SPEAR, P.A. (1979). Membrane proteins specified by herpes simplex viruses. III. Role of glycoprotein VP7 (B2) in virion infectivity. J. Virol. 29, 1149-1158.

SASAKI, Y., SASAKI, R., COHEN, G.H. and PIZER, L.I. (1974). RNA polymerase activity and inhibition in herpesvirus-infected cells. Intervirology 3, 147-161.

SATO, S., HUTCHISON, C.A. III and HARRIS, J.I. (1977). A thermostable sequence-specific endonuclease from Thermus aquaticus. Proc. Natl. Acad. Sci. USA 74, 542-546.

SCHAFFER, P.A., ARON, G.M., BISWAL, N. and BENYESH-MELNICK, M. (1973). Temperature-sensitive mutants of herpes simplex virus type 1: isolation, complementation and partial characterization. Virology 52, 57-71.

SCHAFFER, P.A., TEVETHIA, M.J. and BENYESH-MELNICK, M. (1974). Recombination between temperature-sensitive mutants of herpes simplex virus type 1. Virology 58, 219-228.

SCHAFFER, P.A., CARTER, V.C. and TIMBURY, M.C. (1978). Collaborative complementation study of temperature-sensitive mutants of herpes simplex virus types 1 and 2. J. Virol. 27, 490-504.

SCHILDKRAUT, C. and LIFSON, S. (1965). Dependence of the melting temperature of DNA on salt concentration. Biopolymers 3, 195-208.

SCHILDKRAUT, I., COOPER, G.M. and GREER, S. (1975). Selective inhibition of the replication of herpes simplex virus by 5-halogenated analogues of deoxycytidine. Molecular Pharmacology 11, 153-158.

SCHLEHOFER, J.R., HAMPL, H. and HABERMEHL, K-O. (1979). Differences in the morphology of herpes simplex virus infected cells: I. Comparative scanning and transmission electron microscopic studies on HSV-1 infected HEp-2 and chick embryo fibroblast cells. J. gen. Virol. 44, 433-442.

SCHMECKPEPER, B.J. and SMITH, K.D. (1972). Use of formamide in nucleic acid reassociation. Biochemistry 11, 1319-1326.

SCHWARTZ, J. and ROIZMAN, B. (1969). Concerning the egress of herpes simplex virus from infected cells: electron and light microscopic observations. Virology 38, 42-49.

SEIF, I., KHOURY, G. and DHAR, R. (1979). BKV splice sequences based on analysis of preferred donor and acceptor sites. Nucleic Acids Research 6, 3387-3398.

SHELDRICK, P., LAITHIER, M., LANDO, D. and RYHINER, M.L. (1973). Infectious DNA from herpes simplex virus: infectivity of double and single-stranded molecules. Proc. Natl. Acad. Sci. USA 70, 3621-3625.

SHELDRICK, P. and BERTHELOT, N. (1974). Inverted repetitions in the chromosome of herpes simplex virus. Cold Spring Symp. Quant. Biol. 39, 667-678.

SHENK, T. (1973). Construction of a viable SV40 variant containing two functional origins of DNA replication. Cell 13, 791-798.

SHLOMAI, J. and BECKER, Y. (1975). Analysis of herpes simplex virus DNA synthesized in nuclei isolated from BSC-1 cells. Virology 68, 275-279.

SHLOMAI, J., FRIEDMANN, A. and BECKER, Y. (1976). Replicative intermediates of herpes simplex virus DNA. Virology 69, 647-659.

SHLOMAI, J., ASHER, Y. and BECKER, Y. (1977). In vitro synthesis of herpes simplex virus DNA in nuclei isolated from infected BSC-1 cells. J. gen. Virol. 34, 223-234.

SILVERSTEIN, S., BACHENHEIMER, S.L., FRENKEL, N. and ROIZMAN, B. (1973). Relationship between post-transcriptional adenylation of herpes simplex RNA and messenger RNA abundance. Proc. Natl. Acad. Sci. USA 70, 2101-2104.

SILVERSTEIN, S., MILLETTE, R., JONES, P. and ROIZMAN, B. (1976). RNA synthesis in cells infected with herpes simplex virus. XII. Sequence complexity and properties of RNA differing in extent of adenylation. J. Virol. 18, 977-991.

SILVERSTEIN, S. and ENGELHARDT, D.L. (1979). Alterations in the protein synthetic apparatus of cells infected with herpes simplex virus. Virology 95, 334-342.

SIM, C. and WATSON, D.H. (1973). The role of type specific and cross-reacting structural antigens in the neutralization of herpes simplex virus types 1 and 2. J. gen. Virol. 19, 217-233.

SKARE, J., SUMMERS, J.P. and SUMMERS, W.C. (1975). Structure and function of herpesvirus gen[illegible] I. C[illegible] of five HSV-1 and two HSV-2 strains by cleavage sites on herpes simplex virus type 1 DNA. J. Virol. 25, 726-732.

SKARE, J. and SUMMERS, W.C. (1977). Structure and function of herpesvirus genomes. II. EcoRI, XbaI, and HindIII endonuclease cleavage sites on herpes simplex virus type 1 DNA. Virology 76, 581-595.

SMILEY, J.R. (1980). Construction in vitro and rescue of thymidine kinase-deficient deletion mutation of herpes simplex virus. Nature (London) 285, 333-335.

SMILEY, J.R., WAGNER, M.J., SUMMERS, W.P. and SUMMERS, W.C. (1980). Genetic and physical evidence for the polarity of transcription of the thymidine kinase gene of herpes simplex virus. Virology 102, 83-93.

SMITH, G.R., SCHULTZ, D.W. and CRASEMAN, J.M. (1980). Generalized recombination: nucleotide sequence homology between Chi recombinational hotspots. Cell 19, 785-793.

SMITH, J.D. and DE HARVEN, E. (1973). Herpes simplex virus and cytomegalovirus replication in WI-38 cells. I. Sequence of virus replication. J. Virol. 12, 919-930.

SMITH, K.O. (1964). Relationship between the envelope and the infectivity of herpes simplex virus. Proc. Soc. exp. Biol. Med. 115, 814-816.

SOEHNER, R.L., GENTRY, G.A. and RANDALL, C.C. (1965). Some physicochemical characteristics of equine abortion virus nucleic acid. Virology 26, 394-405.

SOUTHERN, E.M. (1975). Detection of specific sequences among DNA fragments separated by gel electrophoresis. J. mol. Biol. 98, 503-517.

SPEAR, P.G. and ROIZMAN, B. (1968). The proteins specified by herpes simplex virus. I. Time of synthesis, transfer into nuclei, and properties of proteins made in productively infected cells. Virology 36, 545-555.

SPEAR, P.G. and ROIZMAN, B. (1970). The proteins specified by herpes simplex virus. IV. The site of glycosylation and accumulation of viral membrane proteins. Proc. Natl. Acad. Sci. USA 66, 730-737.

SPEAR, P.G., KELLER, J.M. and ROIZMAN, B. (1970). Proteins specified by herpes simplex virus. II. Viral glycoproteins associated with cellular membranes. J. Virol. 5, 123-131.

SPEAR, P.G. and ROIZMAN, B. (1972). Proteins specified by herpes simplex virus. V. Purification and structural proteins of the herpesvirion. J. Virol. 9, 143-159.

SPEAR, P.G. (1976). Membrane proteins specified by herpes simplex viruses. I. Identification of four glycoprotein precursors and their products in type 1 infected cells. J. Virol. 17, 991-1008.

SPEAR, P.G., SARMIENTO, M. and MANSERVIGI, R. (1978). The structural proteins and glycoproteins of herpesviruses: a review. In "Oncogenesis and Herpesviruses III", vol. I, pp. 157-167. Edited by G. de Thé, W. Henle and F. Rapp. IARC Scientific Publications, no. 24, Lyon.

SPRING, S.B. and ROIZMAN, B. (1968). Herpes simplex virus products in productive and abortive infection. III. Differentiation of infectious virus derived from nucleus and cytoplasm with respect to stability and size. J. Virol. 2, 979-985.

STACKPOLE, C.W. (1969). Herpes-type virus of the frog renal adenocarcinoma. I. Virus development in tumor transplants maintained at low temperatures. J. Virol. 4, 75-93.

STEGMAN, B., ZENTGRAF, H., OTT, A. and SCHRODER, C.H. (1978). Synthesis and packaging of herpes simplex virus DNA in the course of virus passages at high multiplicity. Intervirology 10, 228-240.

STEIN, S., TODD, P. and MAHONEY, J. (1970). Infectivity of herpes simplex virus with and without envelopes. Canadian J. Microbiol. 16, 953-957.

STERZ, H., LUDWIG, H. and ROTT, R. (1974). Immunologic and genetic relationship between herpes simplex virus and bovine mammilitis virus. Intervirology 2, 1-13.

STEVELY, W.S. (1975). Virus-induced proteins in pseudorabies-infected cells. II. Proteins of the virion and nucleocapsid. J. Virol. 16, 944-950.

STEVELY, W.S. (1977). Inverted repetition in the chromosome of pseudorabies virus. J. Virol. 22, 232-234.

STINSKI, M.F., MOCARSKI, E.S. and THOMSEN, D.R. (1979). DNA of human cytomegalovirus: size, heterogeneity and defectiveness resulting from serial undiluted passage. J. Virol. 31, 231-239.

STOKER, M.G.P. (1958). Mode of intercellular transfer of herpes virus. Nature (London) 182, 1525-1526.

STOKER, M.G.P. and NEWTON, A.A. (1959). The effect of herpes virus on HeLa cells dividing parasynchronously. Virology 7, 438-448.

STOW, N.D. and WILKIE, N.M. (1976). An improved technique for obtaining enhanced infectivity with herpes simplex virus type 1 DNA. J. gen. Virol. 33, 447-458.

STOW, N.D. and WILKIE, N.M. (1978). Physical mapping of temperature-sensitive mutations of herpes simplex virus type 1 by intertypic marker rescue. Virology 90, 1-11.

STOW, N.D., SUBAK-SHARPE, J.H. and WILKIE, N.M. (1978). Physical mapping of herpes simplex virus type 1 mutations by marker rescue. J. Virol. 28, 182-192.

STRINGER, J.R., HOLLAND, L.E., SWANSTROM, R.I., PIVO, K. and WAGNER, E.K. (1977). Quantitation of herpes simplex virus type 1 RNA in infected cells. J. Virol. 21, 889-901.

STRINGER, J.R., HOLLAND, L.E. and WAGNER, E.K. (1978). Mapping early transcripts of herpes simplex virus type 1 by electron microscopy. J. Virol. 27, 56-73.

STRNAD, B.C. and AURELIAN, L. (1976). Proteins of herpesvirus type 2. I. Virion, nonvirion and antigenic polypeptides in infected cells. Virology 69, 438-452.

STROBEL-FIDLER, M. and FRANCKE, B. (1980). Alkaline deoxyribonuclease induced by herpes simplex virus type 1: composition and properties of the purified enzyme. Virology 103, 493-501.

SUBAK-SHARPE, H., BÜRK, R.R, CRAWFORD, L.V., MORRISON, J.M., HAY, J. and KEIR, H.M. (1966). An approach to evolutionary relationships of mammalian DNA viruses through analysis of the pattern of nearest neighbour base sequences. Cold Spring Harbor Symp. Quant. Biol. 31, 737-748.

SUBAK-SHARPE, J.H. (1969). In "Proceedings of the First International Congress for Virology, Helsinki, 1968", p. 252. Edited by J.L. Melnick. Karger, Basel.

SUBAK-SHARPE, J.H., BROWN, S.M., RITCHIE, D.A., TIMBURY, M.C., MacNAB, J.C.M., MARSDEN, H.S. and HAY, J. (1974). Genetic and biochemical studies with herpesviruses. Cold Spring Harbor Symp. Quant. Biol. 39, 717-730.

SUBRAMANIAN, K.N. and SHENK, T. (1978). Definition of the boundaries of the origin of DNA replication in simian virus 40. Nucleic Acids Research 5, 3635-3642.

SUGDEN, B., SUMMERS, W.C. and KLEIN, G. (1976). Nucleic acid renaturation and restriction endonuclease cleavage analyses show that the DNAs of a transforming and a nontransforming strain of Epstein-Barr virus share approximately 90% of their nucleotide sequences. J. Virol. 18, 765-775.

SUGDEN, B. (1977). Comparison of Epstein-Barr viral DNAs in Burkitt lymphoma biopsy cells and in cells clonally transformed in vitro. Proc. Natl. Acad. Sci. USA 74, 4651-4655.

SUGINO, W.M. and KINGSBURY, D.T. (1976). DNA homologies between strains of herpes simplex virus. Virology 71, 605-608.

SUMMERS, W.P., WAGNER, M. and SUMMERS, W.C. (1975). Possible peptide chain termination mutants in thymidine kinase gene of a mammalian virus, herpes simplex virus. Proc. Natl. Acad. Sci. USA 72, 4081-4084.

SUTCLIFFE, J.G. (1978). pBR322 restriction maps derived from the DNA sequence: accurate DNA size markers up to 4361 nucleotide pairs long. Nucleic Acids Research 5, 2721-2728.

SWANSTROM, R.I. and WAGNER, E.K. (1974). Regulation of synthesis of herpes simplex type 1 virus mRNA during productive infection. Virology 60, 522-533.

SWANSTROM, R.I., PIVO, K. and WAGNER, E.K. (1975). Restricted transcription of herpes simplex virus genome occurring early after infection and in the presence of metabolic inhibitors. Virology 66, 140-150.

SYDISKIS, R.J. and ROIZMAN, B. (1966). Polysomes and protein synthesis in cells infected with a DNA virus. Science 153, 76-78.

SYDISKIS, R.J. and ROIZMAN, B. (1968). The sedimentation profiles of cytoplasmic polyribosomes in mammalian cells productively and abortively infected with herpes simplex virus. Virology 34, 562-565.

TALLEY-BROWN, S. and MILLETTE, R.L. (1979). Gene expression of herpes simplex virus. I. Analysis of cytoplasmic RNAs in infected Xeroderma Pigmentosum cells. J. Virol. 31, 733-740.

TANAKA, T. and WEISBLUM, B. (1975). Construction of a colicin El-R factor composite plasmid in vitro: means for amplification of deoxyribonucleic acid. J. Bact. 121, 354-362.

THOMAS, M. and DAVIS, R.W. (1975). Studies on the cleavage of bacteriophage lambda DNA with EcoRI restriction endonuclease. J. mol. Biol. 91, 315-328.

THOULESS, M.E. (1972). Serological properties of thymidine kinase produced in cells infected with type 1 or type 2 herpes virus. J. gen. Virol. 17, 307-315.

THOULESS, M.E. and WILDY, P. (1975). Deoxypyrimidine kinases of herpes simplex viruses types 1 and 2: comparison of serological and structural properties. J. gen. Virol. 26, 159-170.

TIMBURY, M.C. (1971). Temperature sensitive mutants of herpes simplex virus type 2. J. gen. Virol. 13, 373-376.

TIMBURY, M.C. and SUBAK-SHARPE, J.H. (1973). Genetic interactions between temperature-sensitive mutants of types 1 and 2 herpes simplex virus. J. gen. Virol. 18, 347-357.

TIMBURY, M.C. and CALDER, L. (1976). Temperature-sensitive mutants of herpes simplex virus type 2: a provisional linkage map based on recombination analysis. J. gen. Virol. 30, 179-186.

TJIAN, R. (1978). Protein-DNA interactions at the origin of simian virus 40 DNA replication. Cold Spring Harbor Symp. Quant. Biol. 43, 655-662.

TWIGG, A.J. and SHERRATT, D.J. (1980). Trans-complementable copy-number mutants of plasmid Col El. Nature (London) 283, 216-218.

VAHLNE, A., SVENNERHOLM, B. and LYCKE, E. (1979). Evidence for herpes simplex type-selective receptors on cellular plasma membranes. J. gen. Virol. 44, 217-225.

VERNON, S.K., LAWRENCE, W.C. and COHEN, G.H. (1974). Morphological components of herpesvirus. I. Intercapsomeric fibrils and the geometry of the capsid. Intervirology 4, 237-248.

VLAZNY, D.A. and FRENKEL, N. (1981). Replication of herpes simplex virus DNA: localization of replicative recognition signals within defective virus genomes. Proc. Natl. Acad. Sci. USA 78, 742-746.

WADSWORTH, S., JACOB, R.J. and ROIZMAN, B. (1975). Anatomy of herpes simplex virus DNA. II. Size, composition and arrangement of inverted terminal repetitions. J. Virol. 15, 1487-1497.

WADSWORTH, S., HAYWARD, G.S. and ROIZMAN, B. (1976). Anatomy of herpes simplex virus DNA. V. Terminally repetitive sequences. J. Virol. 17, 503-512.

WAGNER, E.K. and ROIZMAN, B. (1969a). RNA synthesis in cells infected with herpes simplex virus. II. Evidence that a class of viral mRNA is derived from a high molecular weight precursor synthesized in the nucleus. Proc. Natl. Acad. Sci. USA 64, 626-633.

WAGNER, E.K. and ROIZMAN, B. (1969b). Ribonucleic acid synthesis in cells infected with herpes simplex virus. I. Patterns of ribonucleic acid synthesis in productively infected cells. J. Virol. 4, 36-46.

WAGNER, E.K. (1972). Evidence for transcriptional control of the herpes simplex virus genome in infected human cells. Virology 47, 502-506.

WAGNER, E.K., SWANSTROM, R.I. and STAFFORD, M.G. (1972). Transcription of herpes simplex virus genome in human cells. J. Virol. 10, 675-682.

WAGNER, M., SKARE, J. and SUMMERS, W.C. (1974). Analysis of DNA of defective herpes simplex virus type 1 by restriction endonuclease cleavage and nucleic acid hybridization. Cold Spring Harbor Symp. Quant. Biol. 39, 683-686.

WAGNER, M.J. and SUMMERS, W.C. (1978). Structure of the joint region and the termini of the DNA of herpes simplex virus type 1. J. Virol. 27, 374-387.

WAGNER, M.J., SHARP, J.A. and SUMMERS, W.C. (1981). Nucleotide sequence of the thymidine kinase gene of herpes simplex virus 1. Proc. Natl. Acad. Sci. USA 78, 1441-1445.

WARD, R.L. and STEVENS, J.G. (1975). Lifetimes of mRNA molecules directing the synthesis of viral proteins in herpes simplex virus-infected cell. J. Virol. 15, 81-89.

WATHEN, M.W., THOMSEN, D.R. and STINSKI, M. (1981). Temporal regulation of human cytomegalovirus transcription at immediate-early and early times after infection. J. Virol. 38, 446-459.

WATSON, R.J. and CLEMENTS, J.B. (1977). Virus transcript mapping studies in cells infected with temperature-sensitive mutants of herpes simplex virus type 1. In "Oncogenesis and Herpesviruses III", vol. I, pp. 313-326. Edited by G. de Thé, W. Henle and F. Rapp. IARC Scientific Publications, no. 24, Lyon.

WATSON, R.J. and CLEMENTS, J.B. (1978). Characterization of transcription-deficient temperature-sensitive mutants of herpes simplex virus type 1. Virology 91, 364-379.

WATSON, R.J., PRESTON, C.M. and CLEMENTS, J.B. (1979). Separation and characterization of herpes simplex virus type 1 immediate-early mRNAs. J. Virol. 31, 42-52.

WATSON, R.J. and CLEMENTS, J.B. (1980). A herpes simplex virus type 1 function continuously required for early and late virus RNA synthesis. Nature (London) 285, 329-331.

WATSON, R.J., SULLIVAN, M. and VANDE WOUDE, G.F. (1981). Structures of two spliced herpes simplex virus type 1 immediate-early mRNAs which map at the junctions of the unique and reiterated regions of the virus DNA S component. J. Virol. 37, 431-444.

WAUBKE, R., ZUR HAUSEN, H. and HENLE, W. (1968). Chromosomal and autoradiographic studies of cells infected with herpes simplex virus. J. Virol. 2, 1047-1054.

WERNER, F-J., BORNKAMM, G.W. and FLECKENSTEIN, B. (1977). Episomal viral DNA in a herpesvirus saimiri-transformed lymphoid cell line. J. Virol. 22, 794-803.

WESTSTRATE, M.W., GEELEN, J.L.M.C. and VAN DER NOORDAA, J. (1980). Human cytomegalovirus DNA: physical maps for the restriction endonucleases BglII, HindIII and XbaI. J. gen. Virol. 49, 1-22.

WHARTON, J.H., HENRY, B.E. and O'CALLAGHAN, D.J. (1981). Equine cytomegalovirus: cultural characteristics and properties of viral DNA. Virology 109, 106-119.

WIGLER, M., SILVERSTEIN, S., LEE, L.S., PELLICER, A., CHENG, Y.C. and AXEL, R. (1977). Transfer of purified herpes virus thymidine kinase gene to cultured mouse cells. Cell 11, 223-232.

WIGLER, M., SWEET, R., SIM, G.K., WOLD, B., PELLICER, A., LACY, E., MANIATIS, T., SILVERSTEIN, S. and AXEL, R. (1979). Transformation of mammalian cells with genes from procaryotes and eucaryotes. Cell 16, 777-785.

WILCOX, K.W., KOHN, A., SKLYANSKAYA, E. and ROIZMAN, B. (1980). Herpes simplex virus phosphoproteins. I. Phosphate cycles on and off some viral polypeptides and can alter their affinity for DNA. J. Virol. 33, 167-182.

WILDY, P. (1955). Recombination with herpes simplex virus. J. gen. Microbiol. 13, 346-360.

WILDY, P., RUSSELL, W.C. and HORNE, R.W. (1960). The morphology of herpes virus. Virology 12, 204-222.

WILDY, P., SMITH, C., NEWTON, A.A. and DENDY, P. (1961). Quantitative cytological studies on HeLa cells infected with herpes virus. Virology 15, 486-500.

WILKIE, N.M. (1973). The synthesis and substructure of herpesvirus DNA: the distribution of alkali-labile single strand interruptions in HSV-1 DNA. J. gen. Virol. 21, 453-467.

WILKIE, N.M., CLEMENTS, J.B., MacNAB, J.C.M. and SUBAK-SHARPE, J.H. (1974). The structure and biological properties of herpes simplex virus DNA. Cold Spring Harbor Symp. Quant. Biol. 39, 657-666.

WILKIE, N.M. (1976). Physical maps for herpes simplex virus type 1 DNA for restriction endonucleases Hind III, Hpa-1 and X.bad. J. Virol. 20, 222-233.

WILKIE, N.M. and CORTINI, R. (1976). Sequence arrangement in herpes simplex virus type 1 DNA: identification of terminal fragments in restriction endonuclease digests and evidence for inversion in redundant and unique sequences. J. Virol. 20, 211-221.

WILKIE, N.M., CORTINI, R. and CLEMENTS, J.B. (1977a). Structural studies and physical maps for the herpes simplex virus genome. J. antimicrobial Chemotherapy 3 (Suppl. A), 47-62.

WILKIE, N.M., STOW, N.D., MARSDEN, H.S., PRESTON, V., CORTINI, R., TIMBURY, M.C. and SUBAK-SHARPE, J.H. (1977b). Physical mapping of herpes simplex virus coded functions and polypeptides by marker rescue and analysis of HSV-1/HSV-2 intertypic recombinants. In "Oncogenesis and Herpesviruses III", vol. I, pp. 11-31. Edited by G. de Thé, W. Henle and F. Rapp. IARC Scientific Publications, no. 24, Lyon.

WILKIE, N.M., CLEMENTS, J.B., BOLL, W., MANTEI, N., LONSDALE, D. and WEISSMANN, C. (1979a). Hybrid plasmids containing an active thymidine kinase gene of herpes simplex virus 1. Nucleic Acids Research 7, 859-877.

WILKIE, N.M., DAVISON, A., CHARTRAND, P., STOW, N.D., PRESTON, V.G. and TIMBURY, M.C. (1979b). Recombination in herpes simplex virus: mapping of mutations and analysis of intertypic recombinants. Cold Spring Harbor Symp. Quant. Biol. 43, 827-840.

WILKIE, N.M., EGLIN, R.P., SANDERS, P.G. and CLEMENTS, J.B. (1980). The association of herpes simplex virus with squamous carcinoma of the cervix, and studies of the virus thymidine kinase gene. Proc. Royal Soc. 210, 411-421.

WOHLRABB, F. and FRANCKE, B. (1980). Deoxyribopyrimidine triphosphatase activity specific for cells infected with herpes simplex virus type 1. Proc. Natl. Acad. Sci. USA 77, 1872-1876.

WOLF, H. and ROIZMAN, B. (1978). The regulation of γ (structural) polypeptide synthesis in herpes simplex virus types 1 and 2 infected cells. In "Oncogenesis and Herpesviruses III", vol. I, pp. 327-336. Edited by G. de Thé, W. Henle and F. Rapp. IARC Scientific Publications, no. 24, Lyon.

WOLF, K. and DARLINGTON, R.W. (1971). Channel catfish virus: a new herpesvirus of ictalurid fish. J. Virol. 8, 525-533.

WU, M., HYMAN, R.W. and DAVIDSON, N. (1979). Electron microscopic mapping of proteins bound to herpes simplex virus DNA. Nucleic Acids Research 6, 3427-3441.

YAMAMOTO, S., KABUTA, J., IMAMOTO, M. and MATSUMOTO, H. (1975). Genetic analysis of polykaryocytosis by herpes simplex virus. I. Experiments using recombinants from intertypic cross. Kurume Med. J. 22, 71-78.

YAMAMOTO, S. and KABUTA, H. (1977). Genetic analysis of polykarycytosis by herpes simplex virus. III. Complementation and recombination between non-fusing mutants and construction of a linkage map with regard to the fusion function. Kurume Med. J. 24, 163-172.

YAMANISHI, K., OGINO, T. and TAKAHASHI, M. (1975). Induction of cellular DNA synthesis by a temperature-sensitive mutant of herpes simplex virus type 2. Virology 67, 450-462.

YANAGI, K., RUSH, M.G. and BIEGELEISEN, K. (1979). Integration of herpes simplex virus type 1 DNA into the DNA of growth-arrested BHK-21 cells. J. gen. Virol. 44, 657-667.

YEO, J., KILLINGTON, R.A., WATSON, D.H. and POWELL, K.L. (1981). Studies on cross-reactive antigens in the herpesviruses. Virology 108, 256-266.

ZWEIG, M., HEILMAN, C.J.Jr. and HAMPAR, B. (1979). Identification of disulphide-linked protein complexes in the nucleocapsids of herpes simplex virus type 2. Virology 94, 442-450.