CHARACTERISATION OF HERPES SIMPLEX VIRUS TYPE 1 TS MUTANTS WHICH HAVE STRUCTURAL DEFECTS

by

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SUMMARY

Four temperature-sensitive DNA$^+$ mutants, ts1201, ts1203, ts1204 and ts1208, each of which contain a lesion in HSV DNA fragment EcoRI f (mu 0.312-0.415), have been characterised in this study.

Ts1204 has a temperature-sensitive lesion located within a 400bp region between mu 0.322-0.324 on the HSV genome. This mutant adsorbed normally to the cell surface at the NPT, but failed to penetrate the cell membrane. Ts1204 appeared to bind to specific cellular receptors for HSV-1, since high multiplicities of infection of the mutant blocked subsequent superinfection of cells by HSV-1, but not by HSV-2. The penetration defect could be overcome either by brief incubation of ts1204-infected cells at the PT before temperature upshift to the NPT, or by treatment of the cells with polyethylene glycol, a compound which promotes fusion of membranes. Upon continued incubation of mutant virus-infected cells at the NPT, low numbers of capsids were assembled. Although these capsids contained some internal structure, they did not contain DNA.

Another mutant, ts1208, lies in the same complementation group as ts1204. This mutant penetrated cells normally at the NPT but, like ts1204, assembled low numbers of capsids which did not contain DNA. Marker rescue experiments mapped the ts1208 lesion to the left of the ts1204 lesion, within BamHI u.

The ability of high multiplicities of ts1204 to block superinfection of cells by HSV-1 but not by HSV-2 was utilised to determine the virus polypeptides involved in the recognition of specific cell surface receptors. A series of intertypic recombinant viruses, which induced both HSV-1- and HSV-2-specific envelope glycoproteins, were all found to be capable of penetrating cells previously infected with ts1204 at the NPT. This result suggests that the virus attachment complex may be composed of more than

one glycoprotein, and that a mixture of both HSV-1 and HSV-2 glycoproteins are able to recognise and bind to cellular receptors specific for HSV-2, since no HSV-2 sequence was common to all recombinants. The alternative explanation that the recombinants contained undetected crossovers cannot, however, be ruled out.

Ts1203 has a ts lesion which maps in a 450bp fragment located between mu 0.377-0.380 on the HSV-1 genome. This mutant assembled large numbers of capsids at the NPT, but failed to encapsidate DNA. In this respect ts1203 resembled ts1201, a mutant which lies in a different complementation group but also has a defect in packaging of virus DNA into capsids (Preston et al., 1983). At the PT, DNA encapsidation was less efficient in ts1203-infected cells than in wild-type virus-infected cells. In addition, the defect in ts1203 appeared to be irreversible upon temperature downshift in the absence of further protein synthesis. Greater than 99% of the DNA synthesized in both ts1203- and ts1201-infected cells at the NPT was endless, suggesting that the unpackaged mutant virus DNA remained in a concatemeric form.

Complementation experiments, using other ts mutants which had lesions in HSV DNA fragment EcoRI f, showed that ts1203 represents a novel complementation group. In preparation for DNA sequencing experiments to determine the base change responsible for the ts1203 phenotype, the DNA fragment containing the ts1203 lesion, and the corresponding fragment from ts1203 rev-1, a spontaneous revertant of ts1203, have been cloned into plasmid vector pUC9.

Preston et al. (1983) demonstrated that ts1201 failed to process the polypeptide p40 to its lower molecular weight forms at the NPT. This result, together with the earlier observations by Gibson and Roizman (1974), strongly suggested that p40 was involved in the virus DNA encapsidation process. In contrast to ts1201, however, ts1203 was found to process p40 correctly at the NPT. Immune electron microscopic

experiments showed that p40 was associated with both ts1203 and ts1201 empty capsids at the NPT.

Experiments were performed to investigate the reason for the failure of p40 to be processed correctly in ts1201-infected cells at the NPT. It was found that the high MW forms of p40 were translocated normally from the cytoplasm to the nucleus in these cells. It was also shown that p40 synthesized in cells infected with HSV-1 strain 17 syn^+ was not phosphorylated to any detectable extent, in contrast to results obtained with other HSV-1 strains (Heilman, 1979; Braun et al., 1984). Thus, defects in either of these events are unlikely to be responsible for the failure of p40 to be processed correctly to its lower molecular weight forms in ts1201-infected cells at the NPT.

ABBREVIATIONS

ACG	acycloguanosine
APS	ammonium persulphate
BHK	baby hamster kidney cells
bp	base pairs
BSA	bovine serum albumin
BUdR	5-bromo-2'-deoxyuridine
BVdU	bromovinyl deoxyuridine
Ci	Curies
CI	complementation index
cm	centimetre
CMV	cytomegalovirus
CPE	cytopathic effect
cpm	counts per minute
dATP	deoxyadenosine triphosphate
dCTP	deoxycytidine triphosphate
dGTP	deoxyguanosine triphosphate
DMSO	dimethyl sulphoxide
DNA	deoxyribonucleic acid
DNase	deoxyribonuclease
ds	double-stranded
dTTP	deoxythymidine triphosphate
EBV	Epstein-Barr virus
E. coli	Escherichia coli
EDTA	sodium ethylene diamine tetra-acetic acid
EHV	equine herpesvirus
eop	efficiency of plating
g	grams
h	hour
HA	hydroxylamine

HCl	hydrochloric acid
HFL	human foetal lung
HSV	herpes simplex virus
HVS	herpesvirus saimiri
ICP	infected cell polypeptide
IE	immediate early
IgG	immunoglobulin G
K	kilo
kd	kilodalton
kb	kilobase
l	litre
M	molar
MI	mock-infected
min	minute
ml	millilitre
mM	millimolar
mm	millimetre
moi	multiplicity of infection
mRNA	messenger ribonucleic acid
mu	map units
MW	molecular weight
NA	nitrous acid
ng	nanograms
NP40	nonidet p40
NPC	nucleoprotein complex
NPT	non-permissive temperature
OD	optical density
ori	origin of viral DNA replication
PAA	phosphonoacetic acid
PAGE	polyacrylamide gel electrophoresis

PBS	phosphate buffered saline
PEG	polyethylene glycol
pfu	plaque forming units
pi	post infection
PRV	pseudorabies virus
PT	permissive temperature
RNA	ribonucleic acid
RNase	ribonuclease
rpm	revolutions per minute
RT	room temperature
SDS	sodium dodecyl sulphate
sec	seconds
syn	syncytial
syn^+	non-syncytial
TCA	trichloracetic acid
TEMED	N,N,N'-N'-tetramethylethylenediamine
TK^-	thymidine kinase-minus
TK^+	thymidine kinase-positive
ts	temperature-sensitive
ts^+	wild-type for temperature-sensitivity
UV	ultra-violet
V	volts
v/v	volume per volume
Vmw	molecular weight of virus-induced polypeptide
VP	virion protein
VZV	varicella zoster virus
w/v	weight per volume
wt	wild-type
uCi	microcuries
ug	microgram
ul	microlitre
%	percentage

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CHAPTER 1

The subject of this thesis is the characterisation of herpes simplex virus type 1 ts mutants with potential structural defects, in order to elucidate the functions of herpes simplex virus virion polypeptides. The aim of the Introduction is to provide background information on the general biology of herpes simplex virus. Particular reference will be made to the advances in understanding of the virus replication cycle gained from the analysis of ts mutants. A brief review of current herpes simplex virus genetics will also be provided.

INTRODUCTION

1.1 CLASSIFICATION OF HERPESVIRUSES

Members of the family Herpesviridae have been isolated from more than eighty organisms, ranging from many vertebrates to molluscs and even fungi (Nahmias, 1972). Herpesvirus virions are enveloped, and have a diameter of approximately 150-200nm. The genome is a linear ds DNA molecule which is enclosed within an icosahedral-shaped capsid. In addition, viruses in this family replicate in the cell nucleus, and acquire their envelope by budding through the nuclear membrane.

Three sub-families have been established on the basis of the biological properties of their members (Roizman et al., 1978; Matthews, 1982; Roizman, 1982).

Alphaherpesviruses usually cause acute, self-limiting diseases in their natural hosts. The primary infection is often followed by establishment of latent infection, frequently but not exclusively, in the ganglia. Latent virus can be reactivated by a variety of stimuli, giving rise to recurrent disease. In vitro, the virus replication cycle is relatively short (<24h), and results in rapid destruction of susceptible cells. These

viruses have a genome of MW 85-110x10^6.

The prototype within this group is herpes simplex virus type 1 (HSV-1), which is primarily responsible for vesicular lesions of the mouth and lips in man, but also causes ocular keratitis, and occasionally encephalitis. Herpes simplex virus type 2 (HSV-2), which is normally isolated from genital lesions (Dowdle et al., 1967), is closely related to HSV-1. They share extensive homology in about 50% of their DNA sequences (Kieff et al., 1972), and their genomes are largely colinear (Wilkie et al., 1979; Davison and Wilkie, 1983). The disease associations for these two viruses, however, are not absolute. For example, HSV-1 can cause genital lesions and HSV-2 can be isolated from lip lesions. Other members of this sub-family include varicella zoster virus (VZV) which gives rise to chicken-pox in man, pseudorabies virus (PRV) (Aujeszky's disease) which infects pigs, and equine herpesviruses (EHV) types -1, -2 and -3.

Betaherpesviruses exhibit a narrow in vivo host range. These viruses normally cause asymptomatic infections in immunocompetent adults, however, severe generalised disease may occur in immunosuppressed patients and neonates. Latent virus has been demonstrated in secretory glands, lymphoreticular cells, kidneys and other tissues. The in vitro host range for viruses in this sub-family is narrow, and the replication cycle is relatively slow (>24h). A large genome (MW 130-150x10^6) is a unifying feature of this group, which includes cytomegaloviruses (CMV) isolated from man, non-human primates, pigs and mice.

Gammaherpesviruses form the lymphoproliferative virus group. These viruses have a narrow in vivo host range, and replication can be demonstrated in B or T lymphocytes, depending on the virus. Latency can be established in lymphoid tissue. In vitro, all members of this sub-family will replicate in lymphocytes. The genome size is similar to that of the Alphaherpesviruses. Epstein-Barr virus (EBV) is the prototype within this

group, and is the causative agent of infectious mononucleosis in man. This virus is also strongly associated with Burkitt's lymphoma and nasopharyngeal carcinoma. Other members of the sub-family include herpesvirus saimiri (HVS) which infects the squirrel monkey, and Marek's disease virus which causes an economically important disease of chickens.

1.2 STRUCTURAL FEATURES OF THE VIRION

1.2.1 Morphology

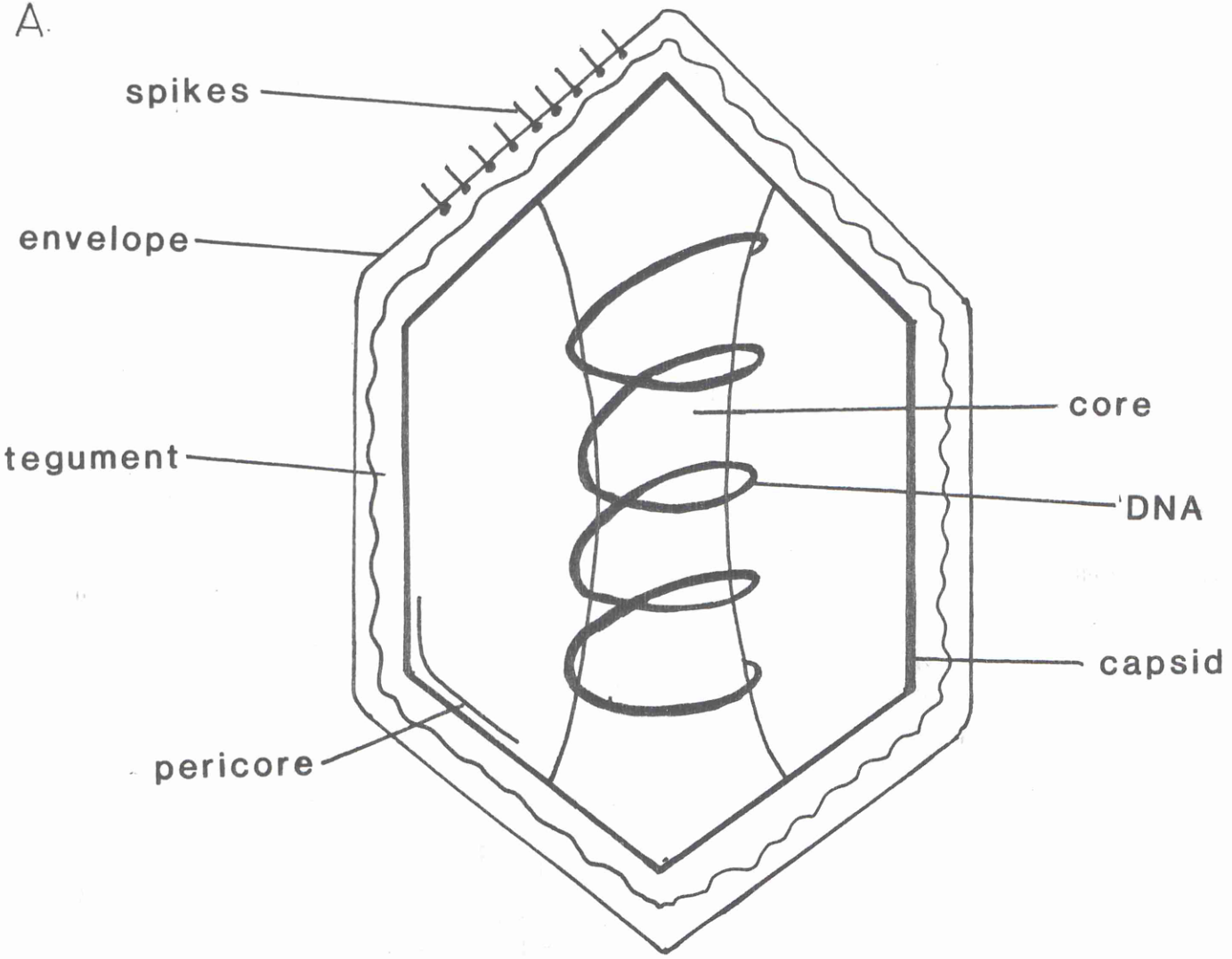
The herpesvirion is a large, complex, virus particle consisting of five structural components referred to as the core, pericore, capsid, tegument and envelope (Fig. 1).

(i) The virus envelope: The particle has an external lipid envelope (Wildy et al., 1960) which is normally acquired by budding of intranuclear particles through the inner nuclear membrane (Darlington and Moss, 1968). The envelope contains numerous spikes calculated to be approximately 8nm long (Wildy et al., 1960).

(ii) The tegument: Between the capsid and envelope lies an amorphous layer known as the tegument (Roizman and Furlong, 1974), which appears to be attached to the capsid, probably at the vertices, and to the inner surface of the envelope (Vernon et al., 1982). The thickness of the tegument varies among different herpesviruses, and is genetically determined (McCombs et al., 1971).

(iii) The capsid: The capsid is icosohedral in shape and has been calculated to consist of 162 hollow capsomers, comprising 150 hexamers with six-fold symmetry, and 12 pentamers (Wildy et al., 1960; Furlong, 1978). The overall diameter of the particle is

A.



B.

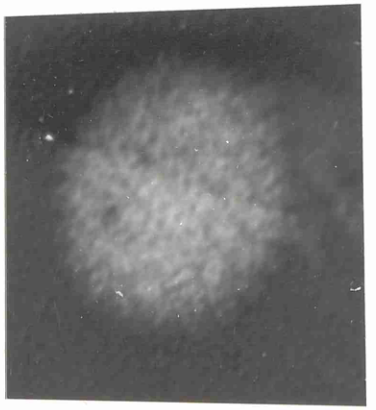


FIGURE 1

(a) Diagrammatic representation of a section through a herpesvirus particle (adapted from Roizman et al., 1975 and Perdue et al., 1976).

(b) HSV-1 strain 17 intranuclear capsid particle isolated by detergent treatment of BHK cells (see Section 2.2.6).

100nm (Wildy et al., 1960; Abodeely et al., 1970). Electron microscopy has revealed sub-structural components of the capsid such as intercapsomeric fibrils, 2nm in width, which appear to connect adjacent capsomeres (Vernon et al., 1974; Palmer et al., 1975). It is possible that these fibrils contribute to the remarkable stability of the capsid, which is resistant to disruption methods such as freezing and thawing, pH changes, trypsin treatment and detergents.

(iv) The core: A cylindrical core structure, around which the viral DNA is tightly spooled, is held within the capsid particle (Furlong et al., 1972; Nii and Yasuda, 1975). The core is probably attached to the inner surface of the capsid at its poles (Nazerian, 1974; Haguenau and Michelson-Fiske, 1975). The existence of a pericore or inner capsid, which surrounds the core, has been suggested on the basis of electron microscopic observations of negatively stained, disrupted herpesvirus capsids (Abodeely et al., 1970; Palmer et al., 1975; Vernon et al., 1976). Although several core morphologies have been visualised by electron microscopy (Perdue et al., 1976), it is likely that several of these are aberrant forms, or result from staining procedures, and it is difficult to define steps in core maturation from this evidence alone.

1.2.2 Structure of the viral genome

The HSV-1 genome is a linear ds DNA molecule of MW 95-100x10^6 or approximately 150-155kbp in length (Becker et al., 1968; Frenkel and Roizman, 1971; Kieff et al., 1971; Grafstrom et al., 1974; Wagner et al., 1974; Clements et al., 1976; Davison, 1981). The DNA molecule consists of two unique sequence components, U_L (long) and US (short), each

of which is bounded by inverted repeat sequences TR_L, IR_L, IR_S and TR_S (Fig. 2) (Sheldrick and Berthelot, 1974). A 250-550bp sequence, designated the a sequence, is present as a direct repeat at both termini, and in an inverted orientation at the L-S junction (Sheldrick and Berthelot, 1974; Wadsworth et al., 1975; Skare and Summers, 1977; Wilkie, 1976; Davison and Wilkie, 1981). Sequence analysis of the L-S junction and adjacent regions has revealed the existence of tandemly reiterated DNA sequences within the a sequence itself (Fig. 3) (Davison and Wilkie, 1981; Mocarski and Roizman, 1981). Single a sequences are flanked by 20bp direct repeats (DR1), whilst multiple a sequences, which are present at the L-S junction and L-terminus, but not at the S-terminus, share a single DR1 sequence between them (Wagner and Summers, 1978; Locker and Frenkel, 1979). The a sequence is involved in DNA encapsidation and inversion of the L and S segments (see Sections 1.5.2 and 1.7.2). As a consequence of inversion, four isomeric forms of the genome are generated which differ in the relative orientation of U_S and U_L. These arrangements are designated P (prototype), I_S (inversion of the S component), I_L (inversion of the L component), and I_{SL} (inversion of both S and L components (Fig. 2) (Delius and Clements, 1976; Hayward et al., 1975; Morse et al., 1977). Although the overall base composition of the HSV-1 genome is 67% G+C moles (Kieff et al., 1971), this value varies for specific regions of the DNA molecule. For example, the short repeat sequence has a G+C content of approximately 78% (Davison and Wilkie, 1981; Murchie and McGeoch, 1982). These values differ widely from that of BHK C13 DNA which has a G+C content of 42% (Subak-Sharpe et al., 1966).

DNA sequence analysis of the short region of the HSV-1 genome has revealed several interesting features about gene arrangement (McGeoch et al., 1985). This region has quite a compact gene layout, with approximately 79% of the DNA coding for proteins. Overlapping genes have

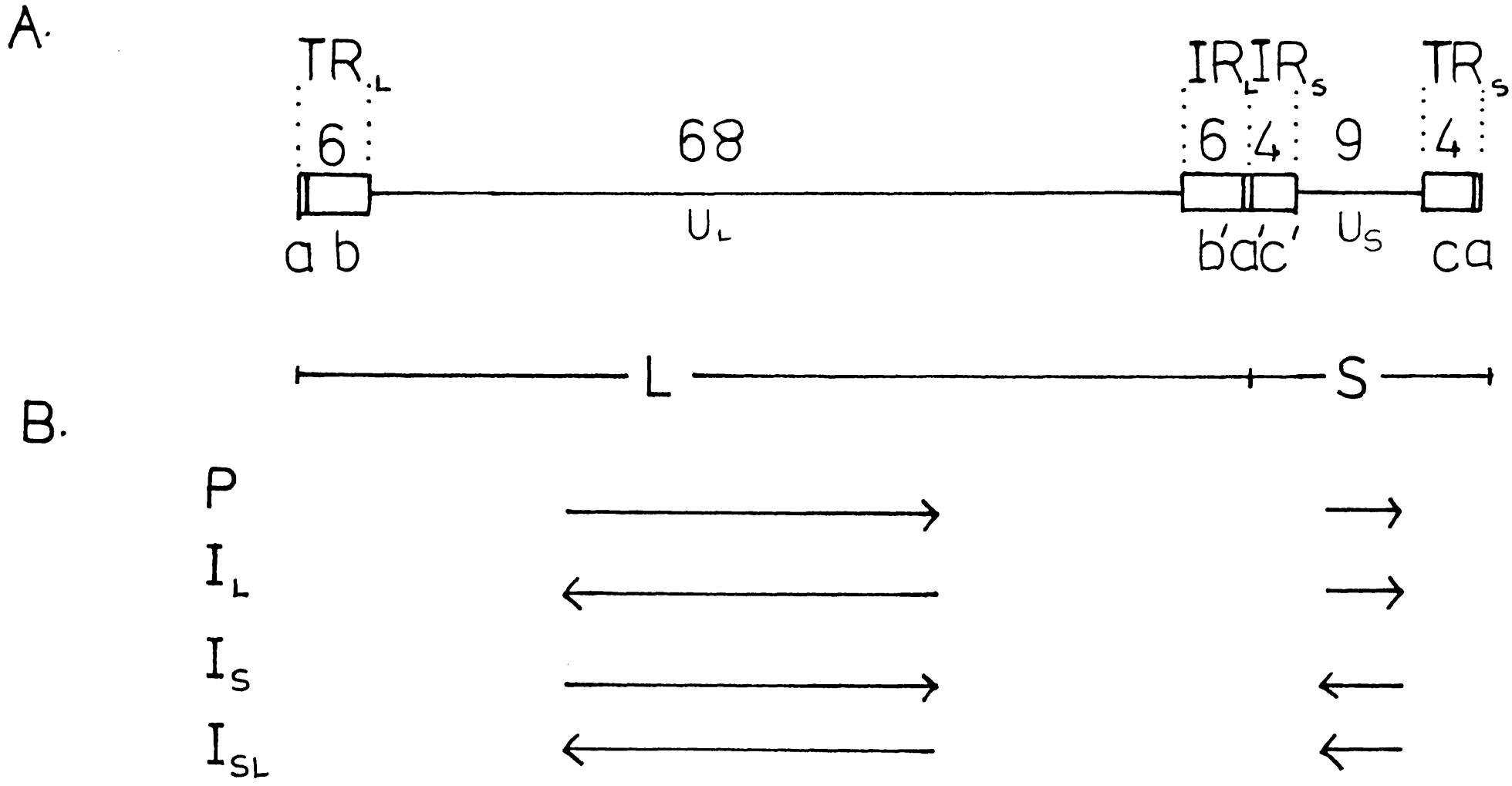

A.
TR L
IR L IR S
TR S
6
68
6
4
9
4
U L
U S
a b
b′ a′ c′
c a
L
S
B.
P
I L
I S
I SL

FIGURE 2

(a) The genome arrangement of HSV-1. The long region (L) is composed of a unique region (U_L) bounded by a terminal sequence (TR_L) which is repeated internally in an inverted orientation (IR_L). Similarly the short region (S) is composed of a unique region (U_S) which is bounded by a terminal sequence (TR_S) which is repeated internally in an inverted orientation (IR_S). Terminally redundant sequences are designated a (a'). The remaining sequences within TR_L/IR_L and TR_S/IR_S are designated b/b' and c/c' respectively.

(b) The four genome isomers:

P = prototype orientation

I_L = inversion of long region (L)

I_S = inversion of short region (S)

I_{SL} = inversion of short and long regions

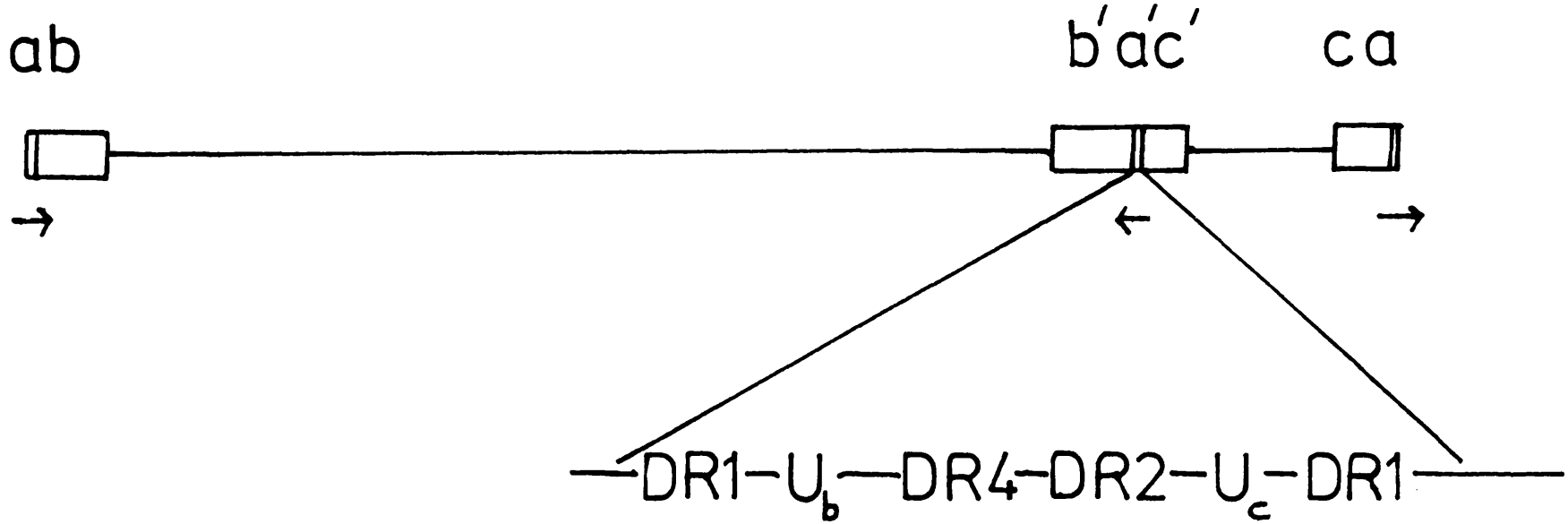

ab
b′a′c′
ca
DR1–Ub–DR4–DR2–Uc–DR1

FIGURE 3

Structure of the a sequence of HSV-1 strain F (taken from Chou and Roizman, 1985). The components of the a sequence are described in the text. Arrows indicate orientation of the a sequence.

been identified with 3'-cotermini and unique 5'-termini (Rixon and McGeoch, 1984; McGeoch et al., 1985) and, in addition, genes on opposite DNA strands have been found to overlap. Tandemly reiterated sequences both in non-coding (Murchie and McGeoch, 1982; Davison and Wilkie, 1981) and coding regions (Rixon and McGeoch, 1984) have also been found.

1.2.3 Structural polypeptides

Although more than thirty HSV-induced polypeptides have been designated as structural components of the virion (Spear and Roizman, 1972; Heine et al., 1974; Marsden et al., 1976), it is difficult to distinguish whether some minor polypeptides are essential for virion structural integrity or are passively acquired during capsid maturation.

Capsid polypeptides

Polypeptides present in herpesvirus capsids have been determined by the purification of intranuclear particles. Under the electron microscope, various types of capsids can be detected in the nuclei of virus-infected cells, in thin section. These capsids can be broadly classified into three types: empty capsids which lack any core structure, intermediate capsids which lack DNA but contain some internal structure and heavy capsids which contain a complete DNA core (Fig. 4). In EHV, all three forms have been purified by velocity gradient centrifugation techniques (Perdue et al., 1975) whereas in HSV, only two capsid populations have been resolved (Gibson and Roizman, 1972; Heilman et al., 1979; Cohen et al., 1980). Table 1 summarises the approximate MW of the HSV-1 capsid polypeptides, and compares the polypeptide nomenclatures used by each group. It is generally accepted that HSV-1 capsids contain seven polypeptides ranging in MW from 155,000 to 12,000. Capsids which lack DNA contain five virus-specific polypeptides, whereas capsids which contain

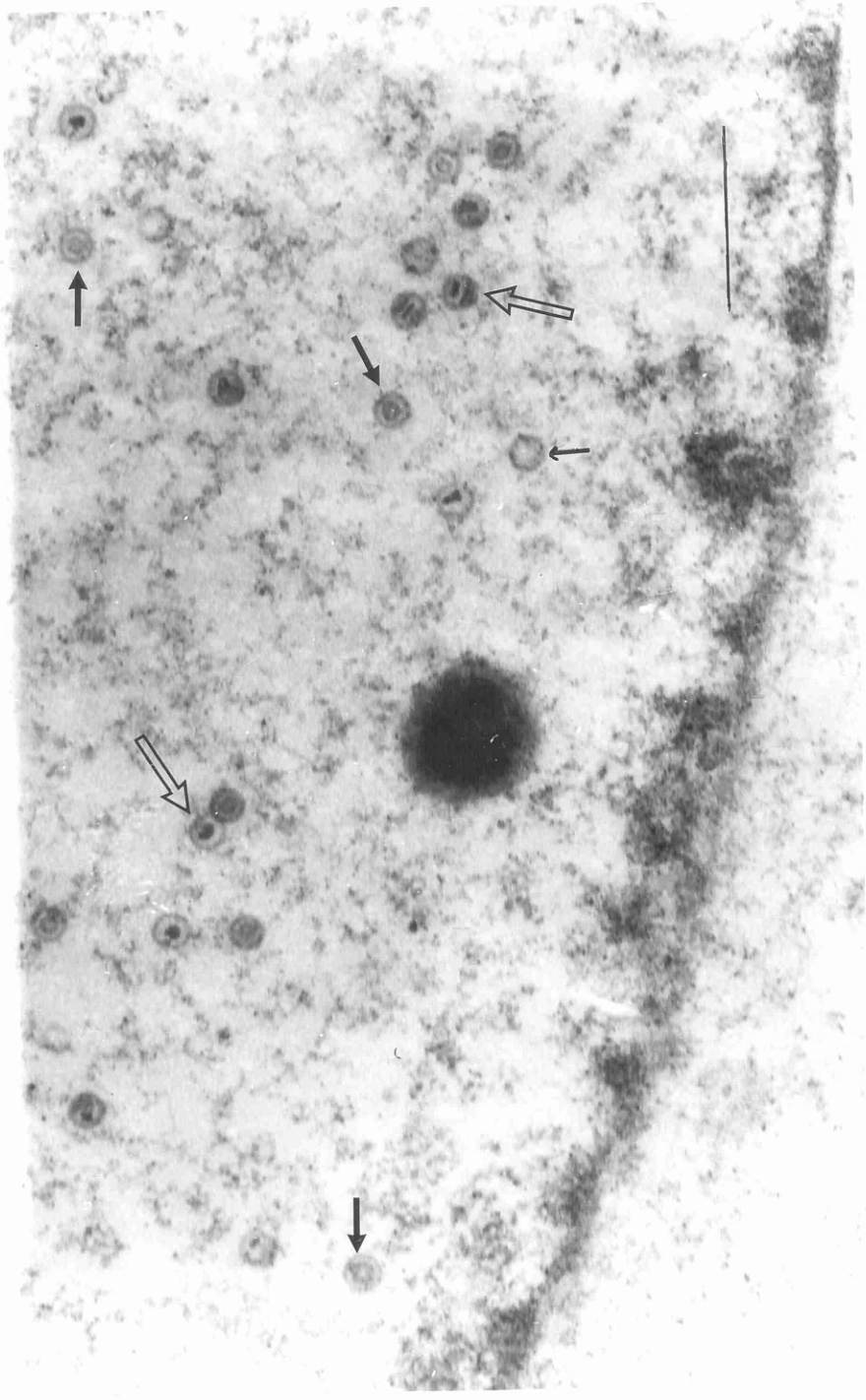


FIGURE 4

Types of intranuclear capsid particles detected in thin-section preparations of wt HSV-1-infected HFL cells. HFL cells were infected at 38.5° with 5 pfu per cell of wt HSV-1 strain 17, harvested at 9h pi and prepared for electron microscopy.

Narrow arrows represent capsids which lack any internal structure.

Broad arrows represent capsids which lack DNA but contain some internal structure.

Open arrows represent capsids which contain an electron-dense DNA core.

The bar represents 0.5uM.

TABLE 1

Apparent molecular weight ($\times 10^3$)	Nomenclatures		
	1	2	3
155	VP5	p155	NC-1
50	VP19C	p50	NC-2
40	VP21/22/22a	p40	NC-3/4
33-32	VP23	p32	NC-5
26-25	VP24	p25	NC-6
12	ND	p12	NC-7

TABLE 1

Approximate MWs of HSV-1 capsid polypeptides and comparison of capsid polypeptide nomenclatures. Apparent MWs are an average from all these groups listed.

Nomenclature 1 is taken from Gibson and Roizman (1972). These workers designated capsid polypeptides according to the virion protein (VP) nomenclature of Spear and Roizman (1972). ND = not detected.

Nomenclature 2 is taken from Heilman et al. (1979) and is a general nomenclature used for both HSV-1 and HSV-2 capsid polypeptides by these workers.

Nomenclature 3 is taken from Cohen et al. (1980).

DNA contain two additional polypeptides, VP21 and VP22a, which are probably related (Gibson and Roizman, 1974; Cohen et al., 1980).

Localisation of the structural polypeptides within the capsid was attempted by Vernon et al. (1981) using immune electron microscopy. The major capsid protein, Vmw155, was distributed over the entire capsid surface, and is probably the major constituent of the hexamers. Vmw50 was located on the capsid surface at the vertices in this study. Braun et al. (1984a), however, reported that this polypeptide was an internal component of the capsid and, in addition, was capable of binding DNA. These results suggest that Vmw50 may be a constituent of the core, involved in DNA packaging and/or anchorage. Immune electron microscopy also showed that Vmw40 coated the entire surface of capsids. The other nucleocapsid polypeptides could not be precisely localised by immune electron microscopy, and may occupy internal locations.

Nucleocapsid polypeptide p40 has been subjected to detailed structural and functional analysis since its presence in capsids containing DNA and absence from empty capsids suggested a role in DNA encapsidation for this polypeptide (Gibson and Roizman, 1972, 1974). The genetically and immunologically related family of polypeptides designated p40 (Heilman et al., 1979; Zweig et al., 1980) is recognised under alternative polypeptide nomenclatures as VP22a (Gibson and Roizman, 1972) and ICP35 (Braun et al., 1983, 1984b). Generally, between four and six related polypeptides, ranging in molecular weight from 37,000 to 50,000 can be resolved by one-dimensional SDS-PAGE (Zweig et al., 1980; Braun et al., 1984b). On the basis of virus infected-cell fractionation experiments, Braun et al. (1984b) reported that the slowest migrating forms of p40 (ICP35 a to d) were the cytoplasmic precursors to faster migrating nuclear products (ICP35 e and f). Two-dimensional PAGE revealed the presence of approximately twenty related polypeptides within the family,

although the nature of the structural changes responsible for alterations in isoelectric properties and electrophoretic mobilities are poorly understood (Braun et al., 1984b). The fastest migrating polypeptides in the family are the predominant forms of p40 found in full nucleocapsids (Heilman, 1982; Braun et al., 1984b). Surface iodination of capsids showed that these polypeptides coat the entire capsid surface (Braun et al., 1984b). Since a ts mutant of HSV-1, ts1201, which fails to encapsidate virus DNA at the NPT, also fails to process p40 to its lower MW forms, it seems likely that this processing step is essential for encapsidation of DNA.

Analogous polypeptides have been identified in PRV (Ladin et al., 1982), CMV strains Colburn (Gibson, 1981) and AD169 (Irmiere and Gibson, 1985), EHV-1 (Perdue et al., 1975) and possibly HVS (Randall et al., 1983).

Tegument polypeptides

Assignment of structural polypeptides to the tegument is based on their presence in capsid-tegument structures (de-enveloped virions), and their absence from capsids extracted from nuclei in the presence of detergents (Gibson and Roizman, 1972; Lemaster and Roizman, 1980). Several of these may, however, be constituents of the underside of the envelope. Of note is the large 273,000 MW polypeptide (Heine et al., 1974), the function of which is unclear, and the 65,000 MW "virion component" which is responsible for switch-on of IE transcription (see Section 1.4.2; Roizman and Furlong, 1974). The presence of a virion-associated protein kinase activity in the tegument has been described (Lemaster and Roizman, 1980). Stevely et al. (1985), however, reported similarities between the virion kinase and host caesin kinase II, suggesting that this enzyme may not be virus-coded.

Envelope polypeptides

Although the HSV virion envelope is normally acquired by budding through the host cell nuclear membrane, it is enriched for virus-specified polypeptides (Spear and Roizman, 1972; Heine et al., 1972; Honess and Roizman, 1975). Four major glycoproteins have been identified in HSV-1- and HSV-2-infected cells and in virion envelopes: gB, gC, gD and gE (Spear, 1976; Bauke and Spear, 1979; Para et al., 1980; Eberle and Courtney, 1980; Para et al., 1982; Zezulak and Spear, 1983; Cohen et al., 1984). The map positions of these glycoproteins are shown in figure 5. Minor glycoproteins gH (Buckmaster et al., 1984), and gY (Palfreyman et al., 1983), have been detected in HSV-1-infected cells, and g92K (Marsden et al., 1984) and gG (Roizman et al., 1984) have been detected in HSV-2-infected cells. The latter two glycoproteins are, in fact, identical (Oloffson et al., submitted for publication), and the HSV-1 counterpart for this HSV-2 glycoprotein has recently been identified (Frame et al., 1985b). As yet, these glycoproteins have not been assigned to the virion envelope. Several putative glycoprotein genes have been identified in the short unique (U_S) region of the HSV-1 genome, and it is possible that these may encode integral polypeptides of the virion envelope (McGeoch et al., 1985).

1.3 THE LYTIC CYCLE OF HSV

1.3.1 Adsorption and penetration

Infection of cells with HSV is initiated by adsorption of virus particles onto the cell surface, followed by penetration of virus through the cell membrane into the cytoplasm.

Adsorption is a rapid, relatively temperature-independent process, which occurs maximally within the first 30min after addition of virus to cells (Hochberg and Becker, 1968). The initial virus-cell interaction is probably

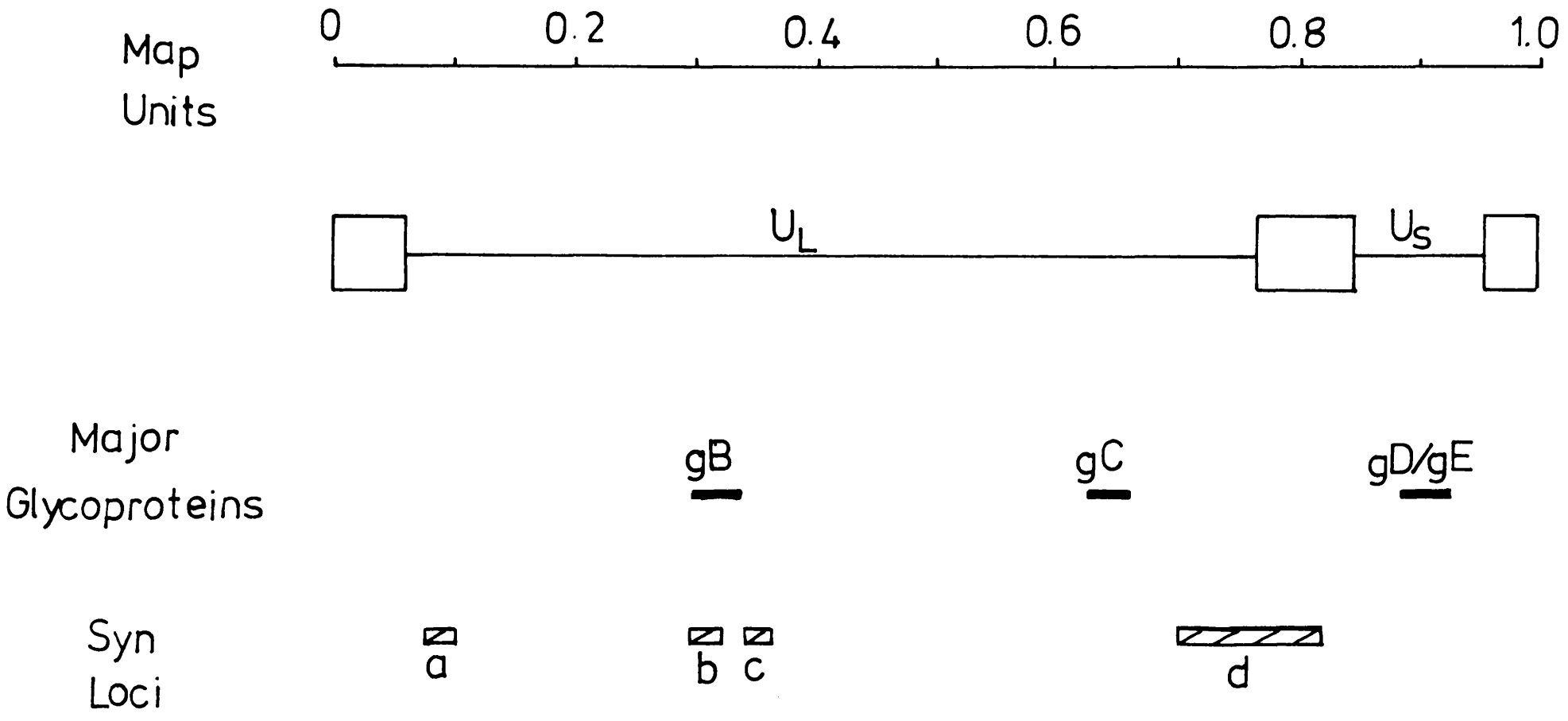

Map Units
0
0.2
0.4
0.6
0.8
1.0
U_L
U_S
Major Glycoproteins
gB
gC
gD/gE
Syn Loci
a
b
c
d

FIGURE 5

Map locations of the major HSV-1 glycoproteins and syn loci on the HSV-1 genome.

Citations for map positions are given in the text.

weak and electrostatic in nature, since adsorbed virions can be removed from the cell surface by heparin, a negatively charged polysaccharide (Hochberg and Becker, 1968). The subsequent irreversible multivalent virus-cell attachment probably involves many virus glycoprotein-cell surface receptor interactions (Kohn, 1979). Biochemical evidence for this multivalent attachment was obtained by Rosenthal et al. (1984) who demonstrated that binding of HSV to cell surfaces caused a reduction in the mobility of cell surface proteins, similar to that caused by multivalent attachment of ligands such as lectins or anti-immunoglobulins (Gall and Edelman, 1981). This effect, which occurs prior to virus penetration, is thought to be caused by the cross-linking of cellular receptor proteins to cytoskeletal elements. Interestingly, type-specific cell surface receptors for HSV-1 and HSV-2 have been described by Vahlne et al. (1979) on the basis of competition assays with heterotypic and UV-irradiated virus.

The virion polypeptide(s) which interact with cellular receptors have not yet been identified, however, such attachment polypeptides are most likely to be one or more of the virus envelope glycoproteins. The ability of polyclonal and monoclonal antibodies, directed against HSV-1 glycoproteins gC and gD, to inhibit HSV-1 adsorption to cells, suggests that these glycoproteins are involved in virion attachment (Fuller and Spear, 1985). Since glycoprotein gE binds the Fc portion of IgG (Baucke and Spear, 1979), the observation that high concentrations of the Fc portion of IgG partially inhibit attachment of virus to cells indicates that gE may also play a role in virus adsorption (Para et al., 1982; Fuller and Spear, 1985). Other work has implicated gB in this process. Artificial liposomes or 'virosomes', enriched for HSV glycoproteins, bound to cells more efficiently when gB was present than when it was absent (Johnson et al., 1984).

In contrast to adsorption, penetration of virions through the cell

membrane is a temperature-dependent event (Farnam and Newton, 1959; Duyckinck-Smith and de Harven, 1974). Electron microscopic studies of virus-infected cells suggested that internalisation of virions occurs predominantly by fusion of the viral envelope with the cell membrane (Morgan et al., 1968; Abodeely et al., 1970), rather than by phagocytosis of the virus particle, as proposed by Dales and Silverberg (1969) and Hummeler et al. (1969). Biochemical evidence for the fusion model of virus penetration was obtained by Para et al. (1980), who showed that glycoprotein gE was transferred from virion envelopes to the cell membrane, when cells were infected with virus in the presence of a protein synthesis inhibitor.

Glycoprotein gB has been implicated as one likely candidate for a virus polypeptide involved in the membrane fusion event. Characterisation of ts mutants, tsB5 and tsJ12, revealed that at the NPT these mutants assemble virions containing gB-deficient envelopes. These progeny virions adsorb to cells normally, but fail to penetrate the cell membrane (Sarmiento et al., 1979; Little et al., 1980). The finding that polyethylene glycol (PEG), a membrane fusion promoter, significantly enhanced the infectivity of the adsorbed virions, gives support to the fusion model of virus entry. Genetic analysis of the structural gene for gB identified a locus affecting rate of virus entry at 31^{o}, and a syn locus responsible for fusion of neighbouring HSV-infected cells (DeLuca et al., 1981, 1982; Bond et al., 1982; Bzik et al., 1984). These data suggest that gB has an important function in virus penetration.

Although glycoprotein gC is not essential for virus infectivity (Holland et al., 1984), this polypeptide may be involved in the negative regulation of gB-promoted membrane fusion. Recombinants which lack gC in their envelopes entered cells at a faster rate than viruses which contain gC (DeLuca et al., 1982). In addition, HSV-1 strains, which synthesize low

levels of gC relative to gB in a permissive cell line, were capable of penetrating the non-permissive rat cell line XC, whereas viruses which induce normal amounts of gC remained on the cell surface (Epstein et al., 1984).

Thus, although the precise mechanisms remain unclear, it seems reasonable to hypothesize that the initial virus-cell interaction is weak, but is followed by multivalent attachments between virion envelope and the correct cellular receptor, such that fusion between the closely aligned membranes can occur. The virus envelope glycoproteins play important roles in both these processes.

1.3.2 Uncoating of the viral genome

The term uncoating refers to the release of the viral genome from the capsid prior to DNA replication. Uncoating of the HSV-1 genome is a poorly understood process. Capsids can be detected in the perinuclear region of the cell within 15-30min of virus penetration (Hummeler et al., 1969; Miyamoto and Morgan, 1971). Early reports that the capsids undergo sequential degradation during transport to the nucleus (Morgan et al., 1968) have not been substantiated, and it is likely that release of the genome from the capsid occurs only at the nuclear membrane. A study in which metabolic inhibitors were added to cells from the time of virus infection, indicated that de novo RNA and protein synthesis are not required for virus uncoating. This finding suggested that either a structural component of the capsid and/or cellular enzymes are responsible for the process (Hochberg and Becker, 1969). The observation that the pentamers were frequently missing from preparations of flattened capsid sheets led to the proposal by Vernon et al. (1976) that removal of these structures allows exit of the viral DNA from the capsid. Nuclease-sensitive input virus DNA can be detected in the nucleus between 15-60min

pi (Hochberg and Becker, 1969; Hummeler et al., 1969), although the mechanism of translocation is not clear. Analysis of ts mutant tsB7, which fails to uncoat viral DNA at the NPT, has provided further clues as to the mechanism whereby input virus DNA reaches the nucleus. In tsB7-infected cells at the NPT mutant capsids containing DNA, accumulated at the nuclear pores and viral DNA was only released upon temperature downshift to the PT. Under these conditions, intermediate uncoating forms with partially extruding DNA cores were seen (Knipe et al., 1981; Batterson et al., 1983), indicating that nucleocapsids themselves did not penetrate the nucleus before release of the viral genome. Juxtaposition of tsB7 capsids at the nuclear pores suggests that viral DNA enters the nucleus using an active cellular transport system.

1.3.3 Suppression of cellular macromolecular synthesis

Infection of cells with HSV results in a decrease in host cell DNA, RNA and protein synthesis. Recent work, however, has centred on the inhibition of host protein synthesis. An immediate consequence of infection is the disaggregation of polyribosomes, an event which is mediated by a component of the infecting virion. This process coincides with the rapid inhibition of host protein synthesis (Roizman et al., 1965; Sydiskis and Roizman, 1966, 1967; Fenwick and Walker, 1978; Nishioka and Silverstein, 1978; Silverstein and Engelhardt, 1979). A virion component is also responsible for the inactivation of host mRNAs, a process which is generally more rapid in HSV-2-infected cells than in HSV-1-infected cells (Fenwick and Clarke, 1982; Fenwick and McMenamin, 1984; Schek and Bachenheimer, 1985). These events are referred to as 'early' shut-off of host protein synthesis, but it is not known whether a single virion structural component is involved in all of these processes. 'Delayed' inhibition of host polypeptide synthesis, on the other hand, is

dependent on virus gene expression, and results in the degradation of host transcripts (Nishioka and Silverstein, 1978; Hill et al., 1983).

The virion-associated shut-off of host polypeptide synthesis is not essential for virus replication, since viable mutants, which are defective in this function, have been isolated (Read and Frenkel, 1983). The virion function has been mapped to a region between 0.52 and 0.59mu on the HSV genome (Fenwick et al., 1979). The mechanism of action of the virion component is unclear, but it is thought that the polypeptide either acts directly as a virion-associated nuclease, or alternatively, acts indirectly by stimulating a cellular RNase activity.

HSV-induced inhibition of host DNA and RNA synthesis have been less extensively studied. Stenberg and Pizer (1982) observed that an IE gene function is responsible for the decrease in cellular RNA synthesis which accompanies HSV infection. Obviously, a decrease in the availability of cellular mRNA is a contributory factor in the inhibition of host protein synthesis. HSV infection also decreases the rate of host cell DNA synthesis (Roizman and Roane, 1964), however, the precise mechanisms underlying this inhibition remain unclear.

1.4 HSV mRNA SYNTHESIS

Transcription of HSV DNA takes place in the host cell nucleus (Wagner and Roizman, 1969). The observation that HSV transcription is indistinguishable from host cell mRNA synthesis in its sensitivity to -amanitin inhibition (Alwine et al., 1974; Ben-Zeev et al., 1976; Costanzo et al., 1977), suggests that HSV DNA is transcribed by a host cell DNA-dependent RNA polymerase II. This conclusion is supported by the finding that naked HSV DNA is infectious (Graham et al., 1973). In common with most eukaryotic mRNAs, HSV transcripts are capped at their 5'-termini and methylated at internal nucleosides (Moss et al., 1977). The DNA specifying

the 3'-termini of these transcripts contains the polyadenylation signal AATAAA, which is essential for correct processing of mRNAs (McKnight, 1980); Cole and Santangelo, 1983). A G+T-rich element, located approximately 30bp downstream from the polyadenylation signal of several HSV genes so far analysed, is also required for efficient formation of the mRNA 3'-terminus (McLauchlan et al., 19883; Whitton et al., 1983; McLauchlan et al., 1985; Cole and Stacy, 1985). This sequence is conserved in many mammalian genes (Taya et al., 1982; McLauchlan et al., 1985).

To date only four spliced HSV mRNAs have been identified, a finding at odds with the general view of eukaryotic mRNA processing, where splicing is commonplace. IE mRNAs -4 (Vmw68) and -5 (Vmw12) share a common 5' leader sequence in the short repeat segments of the virus genome, which is spliced to polypeptide coding sequences in U_S (Watson et al., 1981; Rixon and Clements, 1982; Watson and Vande-Woude, 1982). IE mRNA -1 (Vmw110) is also spliced (Perry et al., 1985). The gene encoding glycoprotein gC also gives rise to a family of spliced late mRNAs which share common 5'- and 3'- termini (Frink et al., 1981; Frink et al., 1983).

HSV mRNA is transported to the cytoplasm where it becomes associated with polyribosomes, and is translated (Wagner and Roizman, 1969).

1.4.1 Regulation of HSV gene expression

Virus-specific mRNAs and polypeptides have been classified into three broad groups, immediate-early (IE or α), early (β) and late (γ), whose synthesis is temporally regulated (Honess and Roizman, 1973, 1974, 1975; Swanstrom and Wagner, 1974; Clements et al., 1977; Jones and Roizman, 1979). Recent work on the control of HSV gene expression has

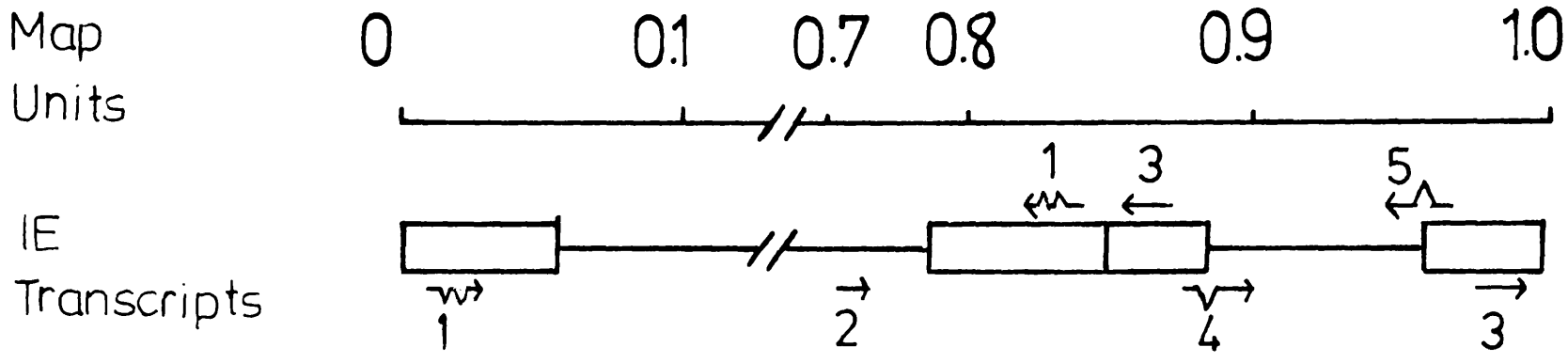
centred on the identification of cis- and trans- acting factors required for the cascade regulation of mRNA and polypeptide synthesis.

1.4.2 IE gene expression

IE transcripts are the first class of virus-specific mRNA detectable in the virus growth cycle, and do not require de novo virus protein synthesis for their production. Normally, in a lytic infection, IE mRNAs are present in small amounts, however, the five major IE transcripts can be made in large amounts in the presence of protein synthesis inhibitors such as cycloheximide or anisomycin, added from the time of virus infection (Clements et al., 1977; Jones et al., 1977; Clements et al., 1979; Watson et al., 1979; Anderson et al., 1980; Harris-Hamilton and Bachenheimer, 1985). Figure 6 shows the physical map location of genes encoding these transcripts.

In the course of a normal virus infection at 37^{o}, levels of IE transcripts reach a peak within the first 6h of infection, after which time they decline, but still remain detectable at late times after infection (Harris-Hamilton and Bachenheimer, 1985). Upon removal of the cycloheximide block, five major IE polypeptides are synthesized (Clements et al., 1977; Preston, 1979a; Watson et al., 1979; Anderson et al., 1980; Watson et al., 1981), which have apparent MWs of 175,000, 110,000, 68,000, 63,000 and 12,000 (Preston, 1979b; Watson et al., 1979; Preston et al., 1981). All the IE polypeptides, except Vmw12, are phosphorylated (Pereira et al., 1977; Marsden et al., 1978; Marsden et al., 1980) and, with the exception of Vmw12, all bind to DNA in vitro (Hay and Hay, 1980).

Stimulation of IE transcription is mediated by a component of the infecting virion. Post et al. (1981) successfully transformed cells from a TK^{-} phenotype to a TK^{+} phenotype using plasmids in which the HSV-TK gene structural sequences were fused to the 5'- non-coding regions of IE-3



IE mRNA-	Vmw	Splice
1	110	+
2	63	-
3	175	-
4	68	+
5	12	+

FIGURE 6

Genome map locations and orientations of the IE mRNAs (numbered 1 to 5) of HSV-1. The table indicates the presence or absence of splicing of each IE transcript, and the MW of the polypeptide produced from each mRNA species.

gene (Vmw175). Transcription from the IE-TK gene constructs was stimulated by infection with TK^- virus in the presence of cycloheximide. Further studies by Batterson and Roizman (1983) showed that this stimulation of IE transcription was mediated by one or more structural polypeptides located on the outside of the HSV capsid. Identification of the virion component was obtained by Campbell et al. (1984), using a short term expression assay in which cells were cotransfected with cloned HSV DNA fragments and IE-TK gene constructs. Stimulation of the synthesis was elicited by a subclone of HSV-1 DNA fragment BamHI f (mu 0.64-0.69, pMC1), which contains a single intact gene encoding the tegument protein, Vmw65. This transcriptional stimulation by Vmw65 was only observed for genes containing IE promoters.

Detailed analyses of IE-3 gene upstream promoter sequences have identified regulatory and promoter regions (Mackem and Roizman, 1980, 1982a, 1982b, 1982c). Important transcriptional elements include a TATA homology, located between positions -16 and -37 in the promoter, which is required for accurate initiation of transcription. In addition, proximal promoter sequences from position -37 to -108, which are required for transcriptional initiation in the absence of further upstream sequences, and a far-upstream region (-174 to -331), containing positive regulatory signals resembling enhancer sequences, have been identified (Cordingley et al., 1983; Preston and Tannahill, 1984; Lang et al., 1984). A consensus sequence, TAATGARAT, located within the far-upstream regions of all the IE gene promoters (Mackem and Roizman, 1982; Whitton and Clements, 1984), forms part of the target sequence for virion component stimulation (Preston et al., 1984). GC-rich regions flanking the TAATGARAT sequence appear to be important in modulating transcriptional activity, and may be involved in interactions with the cellular transcription factor Sp1 (Jones and Tjian, 1985; see Section 1.4.3).

1.4.3 Early gene expression

One or more functional IE polypeptides are required for the switch from IE to early gene expression, and for the continuous synthesis of early transcripts which reaches a maximum before the onset of virus DNA replication (Honess and Roizman, 1974, 1975). Vmw175 was first implicated in the switch-on of early gene transcription by the characterisation of tsK (Preston, 1979b), a mutant with a ts lesion in the gene encoding Vmw175 (Stow et al., 1978; Davison et al., 1984). Infection of cells with this mutant at the NPT led to overproduction of IE polypeptides. In addition, Vmw175 was not processed correctly to its higher MW forms in these cells, and the abnormal polypeptide was not completely translocated from cytoplasm to nucleus (Preston, 1979b). The normal progression to early gene expression occurred upon temperature downshift of tsK-infected cells to the PT, in the presence of cycloheximide to prevent further protein synthesis (Watson and Clements, 1978; Preston, 1979b). However, the mutant phenotype could be reintroduced upon subsequent temperature upshift. These results suggest that functional Vmw175 is required for a normal transition from IE to early polypeptide synthesis, and that this activity is required throughout the virus growth cycle. Similar findings have been obtained for other mutants with ts defects in Vmw175 (Parris et al., 1978; Dixon and Schaffer, 1980).

Alternative experimental approaches have clearly implicated IE polypeptides in the transactivation of early genes. Expression of early genes, stably integrated into mammalian cell genomes, is induced by infection with Vmw175 mutants of HSV-1 at the PT but not at the NPT. This result confirms that these early genes are dependent on the presence of functional Vmw175 for their efficient expression (Kit et al., 1978; Sandri-Goldin et al., 1983). Conversely, cells which constitutively expressed Vmw175 allowed the transactivation of several early genes

introduced by virus infection in the absence of viral protein synthesis (Person et al., 1985). The fact that one early gene was not expressed under these conditions indicates that additional factors, for example, other IE polypeptides, are required for the efficient transactivation of a subset of early genes. Cotransfection of cells with plasmids encoding early genes and immediate-early genes, singly and in various combinations, has provided additional evidence that both Vmw175 and Vmw110 play essential roles in the transactivation of early genes. Everett (1984) reported that Vmw110, together with Vmw175, could stimulate early gene expression to a greater level than was achieved by the action of Vmw175 alone. However, Vmw110 alone was unable to activate early gene transcription. In contrast, both O'Hare and Hayward (1985) and Quinlan and Knipe (1985) showed evidence that these two IE polypeptides could act separately to induce early gene expression, although their activity was apparently synergistic. Tentative evidence also exists that Vmw12 can augment early gene activation by Vmw175 and Vmw110 (O'Hare and Hayward, 1984).

Recent characterisation of ts mutants with lesions in the gene encoding Vmw63 (ICP27), has indicated that this polypeptide has a regulatory function. The polypeptide is required after the onset of early gene expression (Sacks et al., 1985), since drastically reduced levels of late virus polypeptides are synthesized in cells infected at the NPT with these mutants. Together with the fact that some ts mutants with lesions in Vmw175 are permissive for early gene expression (DeLuca et al., 1984), these results support the idea that several IE polypeptides have regulatory functions which are required throughout the virus replication cycle.

Several elegant analyses of early gene promoter regions have been undertaken with the aim of identifying cis- and trans- acting transcriptional control signals. Investigation of the TK promoter region by both deletion analysis and insertion of clustered sets of point mutations at random

locations, enabled three distinct cis-acting control signals to be identified within the 105bp upstream sequence required for efficient transcription of the TK gene (McKnight et al., 1981; McKnight and Kingsbury, 1982; McKnight et al., 1984). These included a proximal TATA homology, and two upstream distal signals which exhibited sequence homology and were required for quantitative transcriptional control. Experiments designed to determine early gene promoter sequences which respond to transactivation by virus IE polypeptides yielded conflicting results. Zipser et al. (1981) and Elkareh et al. (1985) reported that functional domains which had differential roles in constitutive and virus-induced expression of the gene, could be defined within the TK promoter region. Everett (1984) and McKnight et al. (1985), however, using genes containing the gD and TK promoters respectively in trans-induction assays, could not detect any specific sequences required for transactivation by IE polypeptides.

Recent experiments revealed that the same early gene upstream sequences are required for efficient expression of early genes in constitutive (frog oocyte) and regulated (HSV-infected fibroblast cell) environments (Eisenberg et al., 1985). Taken together with evidence that the mammalian transcription factor Sp1 binds strongly to IE and early gene promoters (Jones and Tjian, 1985), these findings indicate that IE polypeptides transactivate early genes in conjunction with, or by modification of, cellular transcription factors.

Two classes of early polypeptides, $\beta 1$ and $\beta 2$, have been distinguished on the basis of their synthesis in the presence of different amino acid analogues (Pereira et al., 1977) and the analysis of polypeptides induced by tsK at the NPT (Preston, 1979b). The early class of polypeptides includes many enzymes involved in virus nucleic acid metabolism and several structural polypeptides.

1.4.4 Autoregulation of IE gene expression

An added complexity to the pattern of mRNA and polypeptide synthesis in HSV-infected cells, is the apparent negative regulation of IE gene expression by the IE gene products themselves, i.e. autoregulation. In the course of a normal infection, IE polypeptide synthesis declines once early polypeptide synthesis is underway. On the basis of temperature-shift experiments with tsK and other ts mutants with lesions in Vmw175 (Preston, 1979b; Dixon and Schaffer, 1980), it appeared that Vmw175 was responsible for this switch-off of IE gene transcription. However, Vmw175 alone may not be sufficient to maintain the negative regulation of IE gene expression for the following resions: (i) ts mutants have been isolated which synthesize functional Vmw175, yet still overproduce IE polypeptides at the NPT (Sacks et al., 1985), and (ii) both a structural component of the infecting virion (Read and Frenkel, 1983), and a late gene function (DeLuca et al., 1984), have been implicated in the switch-off of IE gene expression.

1.4.5 Late transcription

Late or γ mRNAs have been subdivided into two classes: γ_1, ($\beta\gamma$ or early-late) transcripts, which are easily detectable in the absence of virus DNA replication but which reach maximum abundance once DNA synthesis has begun (Clements et al., 1977), and β_2 (true-late) transcripts, whose synthesis is strongly dependent on DNA replication (Powell et al., 1975; Holland et al., 1980). Functional IE and early polypeptides are required for expression of late genes (Honess and Roizman, 1975; Watson and Clements, 1980). Transactivation of the late promoter for Vmw155 (VP5) has been described (Dennis and Smiley, 1984), but as yet the overall mechanism responsible for late gene expression is poorly understood. Early and late genes can be distinguished in in vitro transcription systems

prepared from uninfected cells (Frink et al., 1981). In the absence of transactivation, early genes are expressed whereas late genes remain transcriptionally silent. One possible explanation is that late promoters are intrinsically weak, requiring transactivation for efficient expression. Late genes may also be subject to negative control by the major DNA binding protein Vmw136 (ICP8), since ts mutants with lesions in this gene exhibit increased levels of late gene expression at the NPT (Godowski and Knipe, 1983, 1985). An increase in gene dosage may also play a significant role in late gene expression, as a result of template amplification when the virus genome is replicated.

1.5 REPLICATION OF VIRAL DNA

Since much information concerning the replication of herpesvirus DNA has been gained from studies on PRV, as well as HSV, the following section encompasses aspects of both PRV and HSV DNA replication.

Jacob and Roizman (1977) reported that less than 5% of input HSV DNA entered the replication cycle, whereas greater than 80% of parental PRV genomes undergo replication (Ben-Porat et al., 1976). Over 90% of mature herpesvirus DNA contains single-stranded nicks or gaps, many of which are repaired during replication (Frenkel and Roizman, 1972; Wilkie, 1973; Wadsworth et al., 1976; Hyman et al., 1977; Ben-Porat and Rixon, 1979). Replacement of short stretches of DNA with ribonucleotides has also been reported (Biswal et al., 1974).

Semi-conservative HSV DNA replication at 37^{o} can be detected in the BHK cell nucleus as early as 3h pi, reaches a maximum between 9-11h pi, and is virtually completed by 16h pi (Rixon, 1977). Electron microscopic analysis of parental DNA molecules, before the onset of, and during the first round of DNA replication, revealed the presence of unit-sized molecules with single-stranded ends and also circular molecules (Jean

and Ben-Porat, 1976; Jacob and Roizman, 1977). Circularisation of the HSV linear genome is thought to occur by direct ligation of the termini (Davison and Wilkie, 1983) rather than by ligation of complementary single-stranded termini as proposed by Jacob and Roizman (1977), and this process can take place in the absence of de novo virus protein synthesis (Poffenberger and Roizman, 1985). This model would also be feasible for PRV DNA which, unlike the HSV genome, does not possess terminally redundant termini (A.J. Davison, personal communication). Circular VZV DNA molecules have also been described (Ecker et al., 1984; Kinchington et al., 1985). At later times in infection, DNA molecules with replicative 'eyes', internal lariats, terminal loops and also Y-shaped molecules can be detected. In addition, molecules which are larger than unit-size with internal eyes and forks, circular molecules with linear tails, and large tangles of DNA are seen (Schlomai et al., 1976; Ben-Porat et al., 1976; Friedmann et al., 1977; Hirsch et al., 1977; Jacob and Roizman, 1977; Jean et al., 1977; Becker et al., 1978). Together with the observation that there is a decrease in the number of terminal fragments detectable in replicating virus DNA compared to virion DNA (Jacob et al., 1979; Jongeneel and Bachenheimer, 1981; Ben-Porat and Rixon, 1979), these data suggest that circularised unit-length molecules are replicated by a rolling-circle mechanism yielding head-to-tail concatemeric DNA.

1.5.1 The origin of replication

Three origins of replication have been identified in the HSV-1 genome, two of which were initially suggested from electron microscopic analysis of replicating DNA; one located in U_L and one in each of the short repeat sequences (Schlomai et al., 1976; Friedmann et al., 1977; Hirsch et al., 1977). Further evidence came from the study of defective HSV-1 genomes which are generated during serial passage of virus stocks

at high multiplicity of infection. Two classes of defective genomes have been described: class I defectives contain the whole of TR_S and also sequences from U_S (Frenkel et al., 1976; Graham et al., 1978; Kaerner et al., 1979; Locker and Frenkel, 1979; Kaerner et al., 1981), whereas class II defectives contain sequences from U_L and also the a sequence from TR_S (Kaerner et al., 1979; Schroder et al., 1975). Three origins of replication were identified from the study of defective genomes; two in the diploid c sequences from TR_S (ori_S), and one in U_L (ori_L) (Vlazny and Frenkel, 1981; Spaete and Frenkel, 1982, 1985). Three comparable origins of replication have been identified in PRV (Jean et al., 1977; Rixon and Ben-Porat, 1979; Ben-Porat and Veach, 1980).

The cis-acting signals in ori_S which are essential for DNA replication were localised to the c sequences in TR_S and IR_S by Stow (1982) and independently by Mocarski and Roizman (1982), and further defined to a 90bp untranscribed region located between the 5' ends of two divergently transcribed mRNAs by Stow and McMonagle (1983). Gray and Kaerner (1984) first determined the sequence of the putative ori_L region in HSV-1 strain Angelotti defective virus DNA. Precise localisation of ori_L in standard HSV-1 DNA was hindered, however, by the inability to clone this origin in an undeleted form in conventional plasmid or lambda vectors. Successful cloning of the region between mu 0.407-0.429 into a yeast vector (Weller et al., 1985) and the analysis of uncloned HSV-1 strain 17 genomic DNA (Quinn and McGeoch, 1985) allowed sequencing across the origin. A palindromic sequence was identified in ori_L which bore great similarity to that in ori_S. This palindrome may well be the functional origin of replication since cotransfection of a deleted ori_L clone with helper virus DNA results in amplification of the clone in which the palindromic sequence had been restored, presumably by recombination with wild-type DNA (Spaete and Frenkel, 1982). Ori_L is situated between two

divergently transcribed early genes which code for the virus DNA polymerase and the major DNA binding protein (Quinn and McGeoch, 1985), a gene layout which is also observed for ori_S. The significance, if any, of the fact that the genes flanking ori_L encode proteins of major importance in HSV DNA replication, is unclear. In addition, it is unknown whether one origin of replication is used preferentially during virus DNA synthesis.

1.5.2 Maturation of viral DNA

Replicating herpesvirus DNA forms a pool of concatemeric molecules from which DNA is withdrawn at random for cleavage into unit length molecules, and packaging (Ben-Porat and Kaplan, 1963; Jacob et al., 1979).

The mature, linear HSV genome has terminally redundant ends which must be generated as a result of site-specific cleavage of concatemeric DNA. Since defective virus DNA, containing sequences from the S region of the HSV genome, (see Section 1.5.1) is cleaved and packaged into capsids, it follows that sequences from this part of standard HSV DNA must contain the signal for cleavage (Vlazny et al., 1982). Subsequently, Stow et al. (1983) localised the cleavage signal to the 400bp a sequence which is common to TR_L and TR_S. Sequence analysis of standard HSV DNA molecules revealed that the a sequence at both the L and S termini ends in a partial copy of the DR1 element (Davison and Wilkie, 1981; Mocarski and Roizman, 1982). Ligation of the L and S termini recreates the complete DR1 sequence. Thus, as shown in figure 7, the termini might arise as a result of cleavage at a target sequence within the single DR1 element located between two adjacent a sequences.

Recent analysis of an HSV-1 strain Justin defective, which has a repeat unit comprising sequences from U_S linked to the short repeat (ca) sequence, suggested, however, that the target sequences for cleavage are

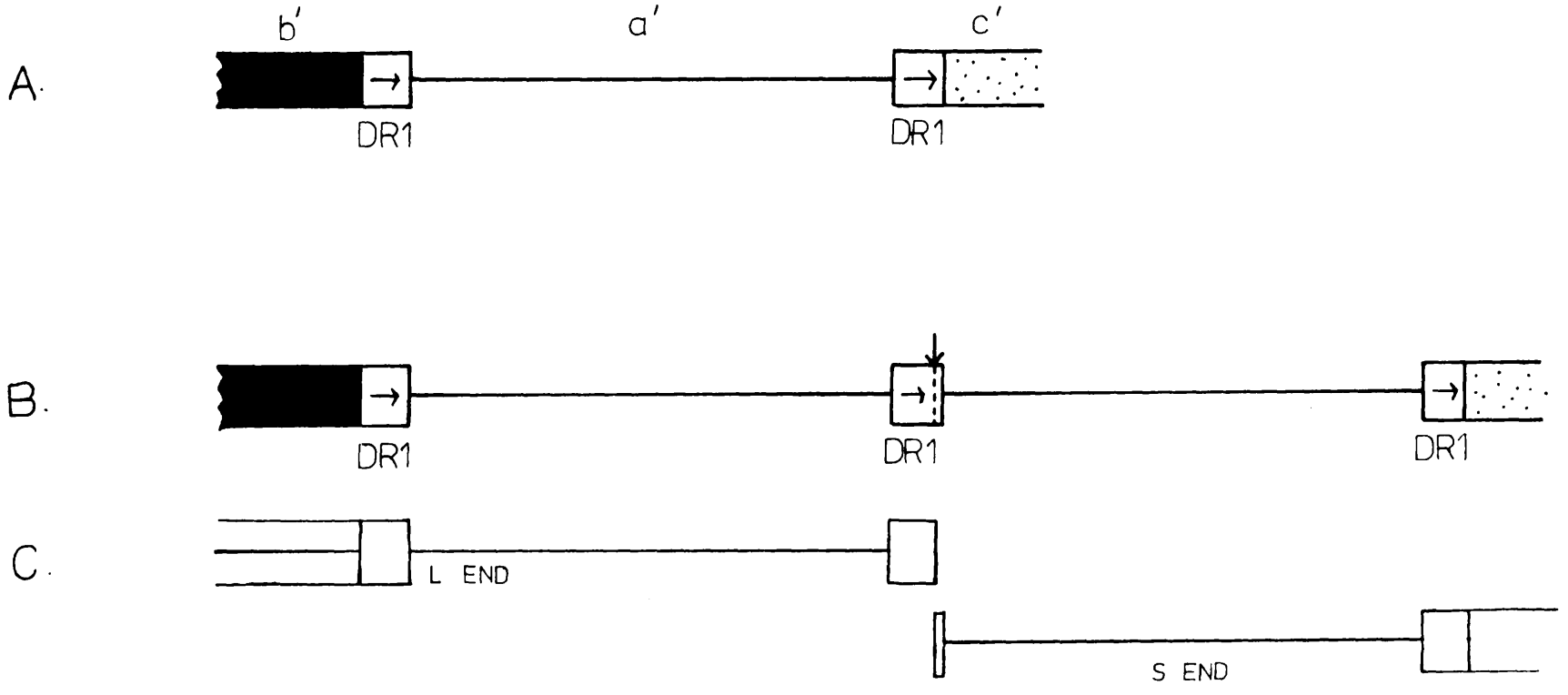

A.
b'
a'
c'
DR1
DR1
B.
DR1
DR1
DR1
C.
L END
S END

FIGURE 7

Generation of the L and S termini of unit length HSV genomes by a single cleavage event within the DR1 element of the a sequence. Taken from Mocarski and Roizman (1982) and Varmuza and Smiley (1985).

(a) Single a sequences are flanked by DR1 elements.

(b) Tandemly reiterated a sequences share a single copy of DR1.

(c) L and S termini may arise as a result of a single cleavage event within a DR1 element separating two adjacent a sequences.

not within DR1. The DNA sequence of the novel U_S-a junction, at which U_S sequences of one repeat unit join the a sequence of the adjacent repeat unit, revealed that only 4bp of the DR1 element was retained (Mocarski et al., 1985). Therefore, since it was unlikely that this 4bp represents the functional cleavage target, these workers suggested that the signal for cleavage must be located elsewhere in the a sequence, and that cleavage takes place at a fixed distance from this signal. Thus, in standard genomes, cleavage within DR1 would be fortuitous. This prediction was supported by work by Varmuza and Smiley (1985). Based on results from a study of recombinant HSV genomes containing various subfragments of the a sequence inserted into the TK locus, these workers proposed two models for generation of the L and S termini of HSV DNA. First, the L and S termini might arise as a result of two distinct cleavage events (Fig. 8), the positions of which are dictated by separate signals within the a sequence, possibly in U_b and U_c (Fig. 3). The introduction of single-stranded nicks is followed by strand-repair synthesis to generate two termini bearing partial copies of DR1. Ligation of these termini would result in tandem reiterations of the a sequence. An additional feature of this model is that junctions bearing two or more tandemly repeated copies of the a sequence might be processed by a ds cleavage, as a result of co-operation between two adjacent recognition signals (Fig. 8). These ds breaks might generate ends bearing a protruding 3' nucleotide, a feature observed in some termini by Mocarski and Roizman (1982). The alternative model proposed by Varmuza and Smiley (1985) postulates that termini are generated by two separate ds cleavage events (Fig. 9). At any given L-S junction, a single cleavage event occurs, creating a terminus bearing an a sequence and a terminus lacking an a sequence. Packaging begins at the a sequence-bearing terminus, and continues until the next L-S junction in the same orientation is encountered. At this point, cleavage occurs such that

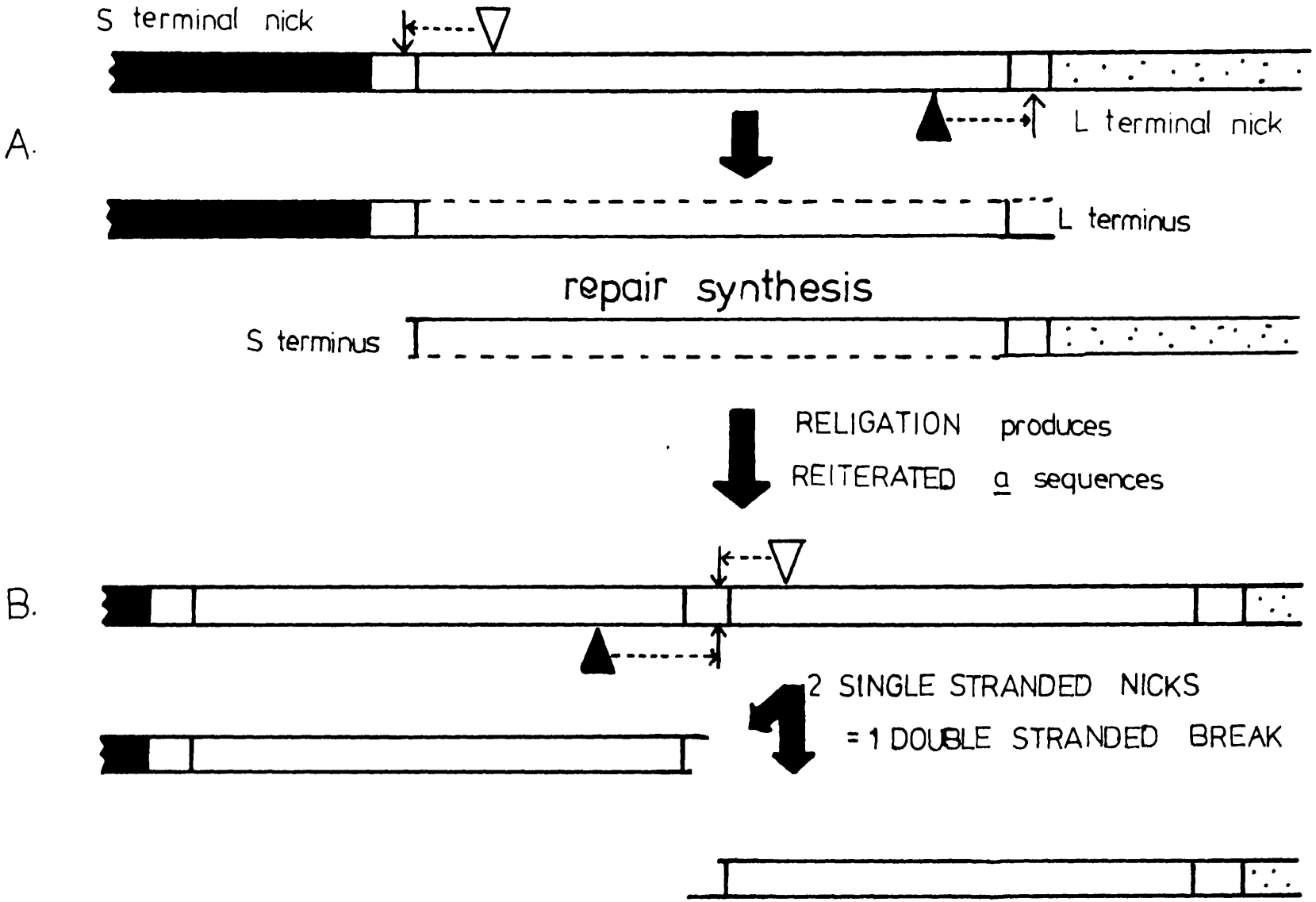

S terminal nick
A.
L terminal nick
L terminus
repair synthesis
S terminus
RELIGATION produces
REITERATED a sequences
B.
2 SINGLE STRANDED NICKS
= 1 DOUBLE STRANDED BREAK

FIGURE 8

(A) The staggered nick-repair model of HSV DNA maturation proposed by Varmuza and Smiley (1985). Open and filled triangles represent the S and L terminus recognition complexes bound to signals located in U_b and U_c respectively.

(B) A model whereby junctions bearing two or more tandemly repeated a sequences give rise to termini, as a result of co-operation between L and S recognition complexes in adjacent a sequences producing a double stranded break.

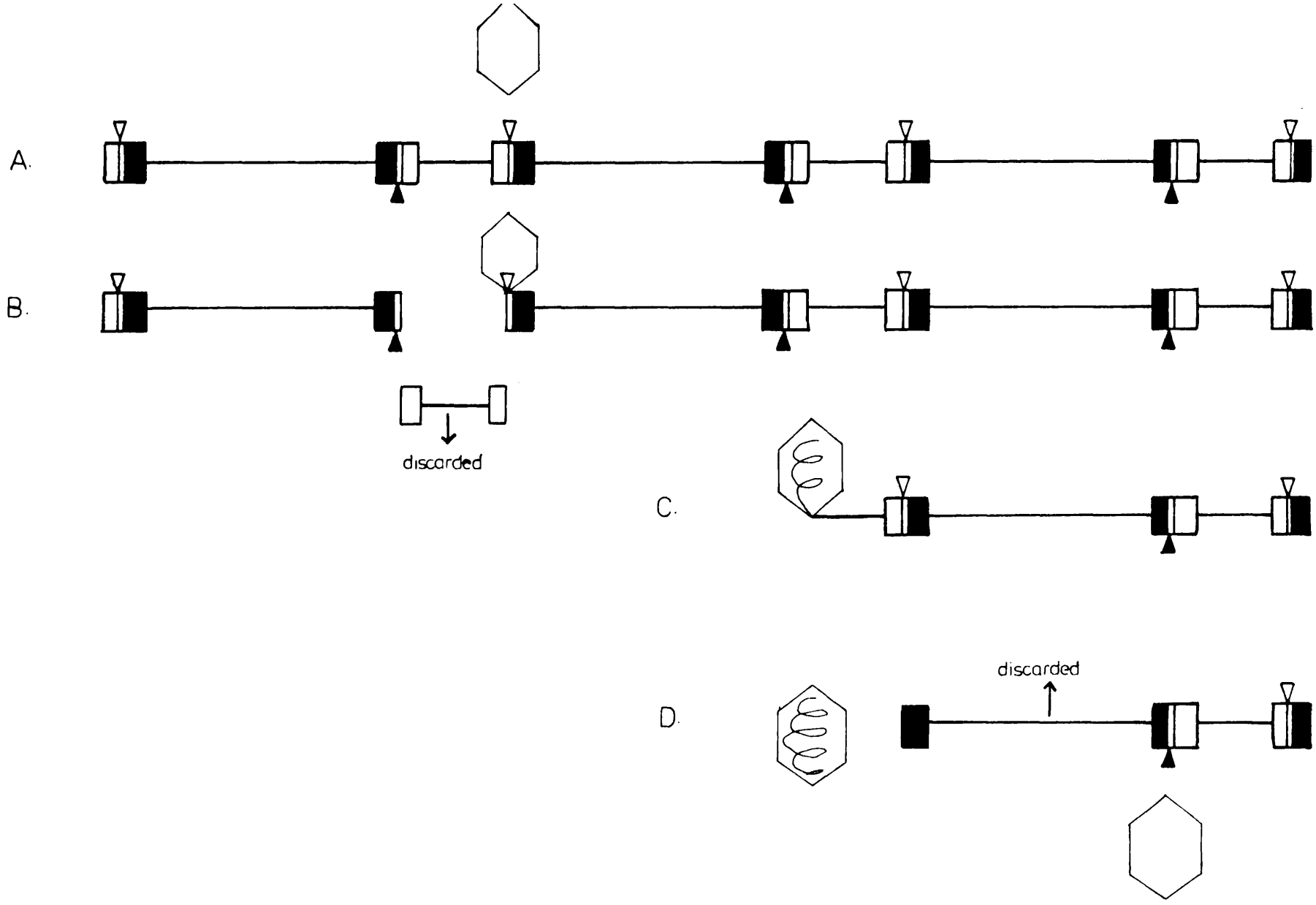

A.
B.
discarded
C.
D.
discarded

FIGURE 9

'Theft' model for cleavage and packaging of HSV DNA proposed by Varmuza and Smiley (1985). Open and filled triangles represent packaging signal(s) in alternate phases. Cleavage/packaging complex is indicated as an empty capsid particle.

(A) Double-stranded cleavage occurs at an L terminus cleavage signal. This step creates an L terminus carrying an a sequence and an S terminus lacking an a sequence.

(B) Packaging proceeds until the next appropriately orientated L-S junction is encountered, at which point,

(C) a double-stranded S terminus cleavage is made, generating an S terminus with an a sequence and an L terminus lacking an a sequence.

(D) The model proposes that ends lacking a sequences are discarded and rapidly degraded.

the packaged genome contains termini which both bear an a sequence. The model predicts that many DNA molecules with L and S termini which lack a sequences are also formed. Such molecules must then be rapidly degraded. Although both these models are feasible proposals for the packaging of HSV DNA into capsids, neither accounts for the observed asymmetric distribution of a sequences in standard HSV genomes, whereby L termini bear varied numbers of a sequences, while S termini have only one (Wagner and Summers, 1978; Locker and Frenkel, 1979).

1.6 HSV POLYPEPTIDES

Approximately fifty HSV-induced polypeptides have been identified by one-dimensional SDS-PAGE (Honess and Roizman, 1973; Powell and Courtney, 1975; Marsden et al., 1976) and over 230 polypeptides have been resolved using two-dimensional PAGE (Haarr and Marsden, 1981). This increased number is probably due in part to the resolution of families of polypeptide species related through post-translational modification, although additional virus-specific polypeptide species have certainly been identified by this method.

1.6.1 Post-translational modifications of HSV polypeptides

The failure of some in vitro synthesized viral polypeptides to comigrate with their in vivo synthesized counterparts on SDS-polyacrylamide gels indicates that many HSV-induced polypeptides undergo some form of post-translational modification (Preston, 1977). Four main types of post-translational modification occur: glycosylation, phosphorylation, sulphation and cleavage, however, other forms of HSV polypeptide modification have been reported, e.g. polyribosylation (Notarianni and Preston, 1983) and fatty acylation of glycoproteins (Johnson and Spear, 1983).

1.6.2 Glycosylation

HSV induces at least four major glycoproteins designated gB, gC, gD and gE (Honess and Roizman, 1975; Spear, 1976; Bauke and Spear, 1979), and several minor species (Palfreyman et al., 1983; Buckmaster et al., 1984; Marsden et al., 1984; Roizman et al., 1984; McGeoch et al., 1985). Processing of HSV glycoproteins occurs in discrete steps, as demonstrated by pulse-chase experiments and two-dimensional PAGE (Haarr and Marsden, 1981; Palfreyman et al., 1983). Processing is inhibited by the drug tunicamycin, indicating that HSV glycoproteins contain N-linked oligosaccharides (Pizer et al., 1980; Hope and Marsden, 1983). Addition of O-linked oligosaccharides also occurs as a late post-translational modification of HSV glycoproteins (Hope and Marsden, 1983; Johnson and Spear, 1983).

1.6.3 Phosphorylation

Approximately sixteen HSV-1 and eighteen HSV-2 polypeptides are phosphorylated (Pereira et al., 1977; Marsden et al., 1978). Wilcox et al. (1980) demonstrated that phosphate cycles on and off several HSV polypeptides, and suggested that these proteins may act as phosphate donors. These workers found that the effect of phosphorylation on the ability of proteins to bind DNA was specific for each polypeptide. Thus, phosphorylation of ICP29 resulted in enhanced DNA-binding activity, perhaps as a result of a change in protein secondary structure. In contrast, a weaker binding activity was observed for the phosphorylated form of ICP6, compared with the unphosphorylated form. This effect probably results from dissociation of the DNA:protein complex as a consequence of the increased negative charge on the phosphoprotein (Wilcox et al., 1980).

1.6.4 Sulphation

At late times in infection, inorganic sulphate is added to the major glycoprotein species (Hope et al., 1982; Hope and Marsden, 1983), in most cases by attachment to the N-linked oligosaccharide moieties. The physiological function of sulphation is not clear. Since it would result in a more polar glycoprotein, this extra modification might influence the orientation of glycoproteins in the membrane.

1.6.5 Proteolytic cleavage

Proteolytic cleavage has been poorly investigated as a form of HSV polypeptide modification. IE polypeptides Vmw175, 136, 110 and 63 induced by tsK (see section 1.4.2) at the NPT, are broken down into lower MW products during an overnight virus infection. Since this degradation did not occur in the presence of tosyl phenylchloromethyl ketone (TPCK), a protease inhibitor, it is likely that a definite cleavage step was involved, rather than non-specific proteolysis. However, the functional significance of this finding is not known. Proteolytic cleavage steps also seem to be involved in the maturation of HSV glycoproteins. The primary amino acid sequences of glycoproteins are altered by removal of a peptide from the N-terminus of the polypeptide. Eisenberg et al. (1984) showed that the first twenty-five amino acids from the predicted sequence of glycoprotein gD were missing from the glycoprotein isolated from infected cells. This stretch of amino acids is largely hydrophobic, and is thought to constitute a signal peptide, which is found at the amino terminus of many transmembrane glycoproteins. Evidence also exists that mature glycoprotein gG (108K) arises from proteolytic cleavage of a precursor polypeptide pgG (120K) (Balachandran and Hutt-Fletcher, 1985).

1.6.6 HSV-induced enzymes

A variety of enzymatic activities are induced by HSV during the course of a normal lytic infection. Most of these are involved in nucleic acid metabolism, and some are associated with the virion particle. They include the following:

(i) The HSV-induced DNA polymerase activity is distinguished from the host cell polymerase both on immunological grounds (Keir and Gold, 1963; Keir et al., 1966) and in its requirement for a high salt concentration for maximum activity. This virus-specific enzyme has a MW of 136,272 in HSV-1 strain 17, as deduced from DNA sequence data (Quinn and McGeoch, 1985). One polypeptide of MW 54,000 is consistently observed in purified DNA polymerase preparations (Powell and Purifoy, 1977; Vaughan et al., 1985). The function of the polypeptide is as yet unclear. The DNA polymerase also has an associated 3'-5' exonuclease activity (Knopf, 1979; Weissbach et al., 1973).

(ii) A pyrimidine deoxyribonucleoside kinase enzyme has been described (Kit and Dubbs, 1963; Cooper, 1973; Jamieson et al., 1974). The enzyme which is commonly referred to as thymidine kinase is non-essential in exponentially growing tissue-culture cells but is indispensible for virus growth in resting (serum-starved) cells (Jamieson et al., 1974). The enzyme has a MW of 40,900, deduced from DNA sequence data (Wagner et al., 1981). In vivo, TK$^-$ viruses are less able to establish a latent infection in nerve cells of experimental animals, and have reduced pathogenicity (Field and Wildy, 1978).

(iii) A deoxyuridine triphosphatase (dUTPase) activity, which catalyses the conversion of dUTP to dUMP and pyrophosphate, has been identified in HSV-1 and HSV-2-infected cells. This enzyme can be distinguished from the host cell species on the basis of activity at 4^{o}, MW and isoelectric focussing point (Wohlrab and Francke, 1980; Wohlrab et al., 1982; Marshall, 1984). The role of the virus dUTPase is unclear. In uninfected cells, the host enzyme reduces incorporation of dUTP into DNA by decreasing the concentration of dUTP in the cell. Provision of a pool of dUMP which can be converted to dTMP by thymidylate synthetase may be another function of this enzyme.

(iv) An HSV-induced ribonucleotide reductase activity has been reported by several workers (Cohen, 1972; Huszar and Bacchetti, 1981; Langelier and Buttin, 1981; Averett et al., 1983). The virus-induced enzyme differs from the host cell enzyme in its insensitivity to dTTP and dATP inhibition and its resistance to high salt concentrations. The enzyme catalyses the synthesis of all four deoxyribonucleotides by reduction of the corresponding ribonucleotides in order to provide a continuous supply of deoxyribonucleotides for DNA synthesis. A ts mutant of HSV-1, ts1207, which induces a thermolabile ribonucleotide reductase activity provided evidence that the enzyme is virus-coded (Dutia, 1983; Preston et al., 1984), and is composed of a complex of two polypeptides of approximately 136,000 and 38,000 MW. These polypeptides can be coprecipitated from extracts of cells infected with wild-type virus, using monoclonal antibodies directed against Vmw136 (Huszar and Bacchetti, 1981; Preston et al., 1984), and using antisera directed against an oligopeptide representing the

carboxy terminal of Vmw38 (Frame et al., 1985). However, the finding that this coprecipitation is abolished in cells infected with ts1207 at the NPT, indicates that the active enzyme is a complex of the two polypeptides (Frame et al., 1985).

(v) An alkaline exonuclease (DNase) activity is induced by HSV-1 (Morrison and Keir, 1968; Strobel-Fidler and Francke, 1980) and HSV-2 (Hay et al., 1971; Hoffman and Cheng, 1977). Characterisation of a ts mutant of HSV-2, ts13, has shown that this virus induces an unstable exonuclease at the NPT. The gene encoding the exonuclease was mapped to a region between 0.12 and 0.21mu on the HSV-2 genome (Francke et al., 1978; Moss et al., 1979) and the enzyme has a MW of approximately 90,000 on SDS-polyacrylamide gels (Strobel-Fidler and Francke, 1980). An associated endonuclease activity has also been described (Hoffman and Cheng, 1979).

(vi) A uracil-DNA glycosylase is induced by HSV but has not as yet been shown to be virus-coded. In uninfected cells, such an enzyme is responsible for removal of uracil residues from DNA, which are produced as a result of deamination of incorporated deoxycytidine monophosphate (dCMP) residues (Caradonna and Cheng, 1981). The enzyme prevents transition mutations occurring by initiation of a base-excision repair process, and thus can be thought of as an antimutator or editing enzyme.

(vii) A DNA topoisomerase activity (type 1) has been described in HSV virions (Muller et al., 1985). These enzymes catalyse the breakage and rejoining of phosphodiester bonds in DNA and may be involved

in processes such as transcription, DNA synthesis, and circularisation or relaxation of the HSV genome.

(viii) A protein kinase activity is reported to be associated with purified virions (Rubenstein et al., 1972; Lemaster and Roizman, 1980). The enzyme is similar in its properties to the host enzyme casein kinase II, and thus may be acquired fortuitously in the virion tegument (Stevely et al., 1985).

1.6.7 HSV DNA binding proteins

Approximately sixteen HSV-1-induced polypeptides and twelve HSV-2-induced polypeptides possess DNA-binding properties (Bayliss et al., 1975; Purifoy and Powell, 1976). Powell et al. (1981) showed that purified preparations of Vmw136, the major HSV-1 DNA binding protein, could bind DNA directly. In contrast, Vmw175, the major HSV-1 immediate-early protein, binds DNA indirectly by association with a host cell DNA binding protein (Freeman and Powell, 1982). Several of these virus proteins have enzymatic activities including Vmw145 (DNA polymerase) and Vmw87 (exonuclease). However, the functions of most DNA binding proteins are still unknown.

1.7 ASSEMBLY OF VIRIONS

1.7.1 Capsid assembly

Herpesvirus capsids are assembled in the cell nucleus (Morgan et al., 1954). Since virus proteins are synthesized in the cytoplasm, there must be a mechanism whereby structural proteins are transported to, or are retained in, the nucleus. The available evidence suggests that this process is linked with that of capsid assembly. In PRV-infected cells which are depleted of arginine, migration of virus structural polypeptides

from cytoplasm to nucleus does not occur (Mark and Kaplan, 1971). Certain arginine-rich virus polypeptides, which are not synthesized under conditions of arginine-starvation, are thought to be involved in the capsid assembly process (Chantler and Stevely, 1973), possibly acting as condensing factors for the aggregation of structural proteins. More recently, a hypothesis was proposed by Ladin et al (1982) based on the analysis of ts mutants of PRV which synthesize DNA at the NPT, but fail to assemble capsids. Since the major structural polypeptides remained in the cytoplasm of cells infected at the restrictive temperature with these mutants, these workers suggested that virus structural proteins are transported or diffuse into the nucleus, and that subsequent assembly of capsids promotes further accumulation of virion proteins in the nucleus by creating a 'sink'.

The site of capsid assembly within the nucleus is thought to be the nuclear matrix or nuclear 'cage'. This is a structure which is defined solely by its extraction procedure,and represents the insoluble material remaining after sequential extraction of purified nuclei with non-ionic detergent, hypotonic low-magnesium buffer, high-salt buffer and treatment with nuclease (Berezney and Coffer, 1974). Electron microscopic examination of these structures revealed a nuclear 'lamina' containing pores, nucleoli and an internal fibrous network (Berezney and Coffer, 1977). Since virus DNA, structural polypeptides and empty capsids are often found associated with a nuclear matrices, it seems likely that this is the site of both DNA replication and capsid assembly (McCready et al., 1980; Pardoll et al., 1980; Bibor-Hardy et al., 1982a, 1982b, 1985).

1.7.2 Encapsidation of virus DNA

Cleavage of concatemeric DNA into unit length genomes is either a prerequisite to, or occurs concomitantly with, the packaging of DNA into capsids (Vlazny et al., 1982). Analysis of nine ts mutants of PRV which

fail to encapsidate DNA at the NPT, showed that this process is complex and under the control of several virus gene products (Ladin et al., 1980). Since the presence of cycloheximide reduces the amount of DNA encapsidation in wild-type PRV-infected cells, it seems probable that the proteins necessary for this process are present in limiting amounts in the virus-infected cell (Ben-Porat et al., 1976; Ladin et al., 1980). As described in Section 1.5.2, the a sequence of HSV is an essential cis-acting signal for cleavage and packaging of DNA into capsids. The search for cleavage/packaging polypeptides led to the identification of two polypeptides (21K and 22K) which appeared to interact specifically with the a sequence of HSV DNA (Dalziel and Marsden, 1984). These polypeptides may be analogous to a 20K polypeptide which remains bound to highly purified HSV DNA (Hyman, 1980). It is also tempting to speculate that the 21/22K polypeptides correspond to proteins visualised by the electron microscope, which are apparently bound to the termini and joint regions of the genome (Wu et al., 1979).

The widely accepted model of DNA encapsidation suggested by Perdue et al. (1976) proposed that viral DNA is inserted into an intermediate form of capsid, in which large cylindrical core structures serve as templates around which the viral DNA is wound. During this stage, condensation of the internal protein cylinder takes place. The final core structure would be the densely staining toroid seen under the electron microscope by Furlong et al. (1972) in thin sections of HSV-infected cells. Similar models have been proposed for packaging of concatemeric DNA into the preformed proheads of bacteriophage T4 (Laemmli and Favre, 1973; Earnshaw and Casjens, 1980) and salmonella phage P22 (King et al., 1973). This model for herpesvirus DNA encapsidation is supported by the observation, drawn from pulse-chase experiments, that there is a precursor-product relationship between intermediate capsids and heavy DNA-

containing capsids. In these experiments, virus structural proteins were pulse-labelled with radioactive amino acids, and after a chase period in unlabelled medium, the types of radioactively labelled particles isolated from the cells were determined. Ladin et al. (1980) in a study of PRV ts mutants which fail to encapsidate DNA at the NPT, demonstrated that structural polypeptides were assembled into empty capsids during the chase period at the restrictive temperature, and that the labelled capsid proteins were transferred to enveloped virions upon subsequent temperature downshift to the PT. In addition, Perdue et al. (1976) demonstrated that EHV capsids containing partial cores disappeared from cells after a chase period, indicating that these particles are precursors to virions, whereas empty capsids which were still detectable following the chase, are not on the pathway of virion assembly. The preliminary characterisation of other ts mutants with defects in encapsidation of DNA at the NPT has supported these findings (Cabral and Schaffer, 1976; Atkinson et al., 1978; Ladin et al., 1980; Ben-Porat et al., 1982; Preston et al., 1983; Dargan and Subak-Sharpe, 1983). The most detailed characterisation has been carried out on ts1201, a ts mutant of HSV-1 which fails both to encapsidate DNA and to process p40 to its lower MW forms at the NPT (Preston et al., 1983). Thus, the correct processing of this polypeptide appears to be an essential step in the packaging of virus DNA into capsids. Intermediate capsids, which accumulate in cells infected with this mutant at the NPT, serve as the substrate for encapsidation of virus DNA, which occurs when the cells are transferred from the NPT to the PT, in the absence of further protein synthesis. Clearly detailed analysis of other mutants which fail to encapsidate DNA is necessary to determine the number, nature and function of the gene products involved in this complex process.

An alternative model for formation of HSV capsids containing DNA has been presented by Pignatti and Cassai (1980), who proposed that newly

replicated viral DNA is packed into nucleoprotein complexes (NPC) which contain capsid structural polypeptides Vmw155 (VP5), 55 (VP19) and 26 (VP24), as well as several minor polypeptide species. These NPC are thought to represent an intermediate stage in capsid assembly, and addition of further structural polypeptides would produce mature nucleocapsids. It should be noted that the possibility that these NPC are degraded nucleocapsids resulting from the extraction procedure has not been ruled out.

DNA molecules which are shorter than standard genomes can be encapsidated, provided they contain the appropriate cleavage/packaging signal. Only nucleocapsids containing unit length DNA, however, are transported to the cytoplasm (Vlazny et al., 1982). The mechanism underlying this selective maturation of nucleocapsids is not known.

1.7.3 Envelopment

The primary site for nucleocapsid envelopment is the inner lamella of the nuclear membrane (Darlington and Moss, 1969; Watson, 1973; Roizman and Furlong, 1974). The mechanism involved appears to be a budding process, whereby nucleocapsids interact with areas of the nuclear membrane which are enriched for viral antigens (glycoproteins) whilst lacking the normal complement of cellular polypeptides (Asher et al., 1969; Ben-Porat and Kaplan, 1971; Spear and Roizman, 1972). These areas may correspond to the distorted or duplicated regions of the nuclear membrane which are a common feature of herpesvirus-infected cells (Nii et al., 1968; Nii, 1971). The intranuclear particles become enveloped by budding outward into the perinuclear space. Gibson (1981) suggested that tegument polypeptides with exposed hydrophobic regions on the nucleocapsid surface facilitate the interaction of capsid and hydrophobic membrane components. It has also been reported that herpesviruses can acquire their envelopes by

budding into cytoplasmic vacuoles (Nii, 1971; Haguenau and Michelson-Fiske, 1975). Treatment of HSV-infected cells with the ionophore monensin, which is thought to block the transit of membrane vesicles from the Golgi apparatus to the cell surface, drastically reduces transport of enveloped virions to the cytoplasmic membrane (Johnson and Spear, 1982). Hence, enveloped particles may move from the perinuclear space to the Golgi apparatus, perhaps via membrane vesicles, and from there to the cell surface and extracellular space. Since HSV particles are retained within the nuclei of cells treated with cytochalasin B, an inhibitor of microfilament-induced cell movement, cytoplasmic streaming may also play a part in transport of virions from the nucleus to the cell surface (Marciano-Cabral et al., 1977).

Virus egress, first seen at approximately 8h pi at 37°, appears to be mediated by a 'reverse phagocytosis' process (Nii et al., 1968; Schwartz and Roizman, 1969; Katsumoto et al., 1981). Some evidence exists that HSV encodes a function which prevents uptake of progeny virus by infected cells. Tognon et al. (1981) reported the isolation of mutant HSV-1 (50B) which exhibited delayed plaque production in Vero cells at 31°, but not at 38.5°. Capsids lacking DNA cores accumulated late in infection at the nuclear pores of cells infected with this mutant at 31°, indicating that progeny virions had re-infected the cells, and had released viral DNA. The lesion in HSV-1 (50B) was mapped to DNA sequences encoding glycoproteins gD and gE (mu 0.9-0.94), and the authors suggest that these glycoproteins, which are incorporated into the cytoplasmic membrane of cells infected with wt HSV, may prevent readsorption of released virus. Obviously, readsorption of progeny virions would account for the delay in plaque production by this mutant at 31°.

1.8 LATENCY

The ability of HSV to establish a latent infection following primary infection of the skin, was first postulated by Goodpasture (1929). Peripheral skin infection normally involves the sensory nerve cells, and virus is transported intra-axonally to the site of latency. This has been demonstrated both in the mouse model (Stevens and Cook, 1971, 1973; Cook et al., 1974; McLennan and Darby, 1980) and in man (Baringer and Swoveland, 1973) to be in the neurons which are found in the sensory ganglia. Latent virus may be reactivated in response to diverse stimuli such as menstruation, fever, UV light and trigeminal nerve root section. Reactivation can result either in recrudescence with the production of a peripheral lesion, or in recurrence with asymptomatic shedding of virus. During the interval between reactivation, the virus genome is thought to be harboured in a non-replicating form in the nervous system, since attempts to detect HSV-specific mRNA have been unsuccessful (Puga et al., 1978). The question remains whether reactivation results in death of the latently infected neuron and re-establishment of latency in neighbouring nerve cells (round-trip theory), or whether latently infected nerve cells survive reactivation and retain the information necessary for renewed recurrences (one-way theory) (Klein, 1976). A low number of HSV-1 genomes (fewer than 0.1 copies per cell) can be detected in samples of latently infected mouse ganglia or brain stem cells, however, the number of copies within a given single cell has not been determined (Cabrera et al., 1980; Rock and Fraser, 1983). The state of the viral DNA within the neuron has been investigated. Rock and Fraser (1983, 1985) demonstrated the absence of genomic termini in latently infected mouse brain stem cells, and instead, detected two copies of the virion DNA joint fragment. This data suggested that the genome was concatemeric or circularised and was either maintained in the neuron in an

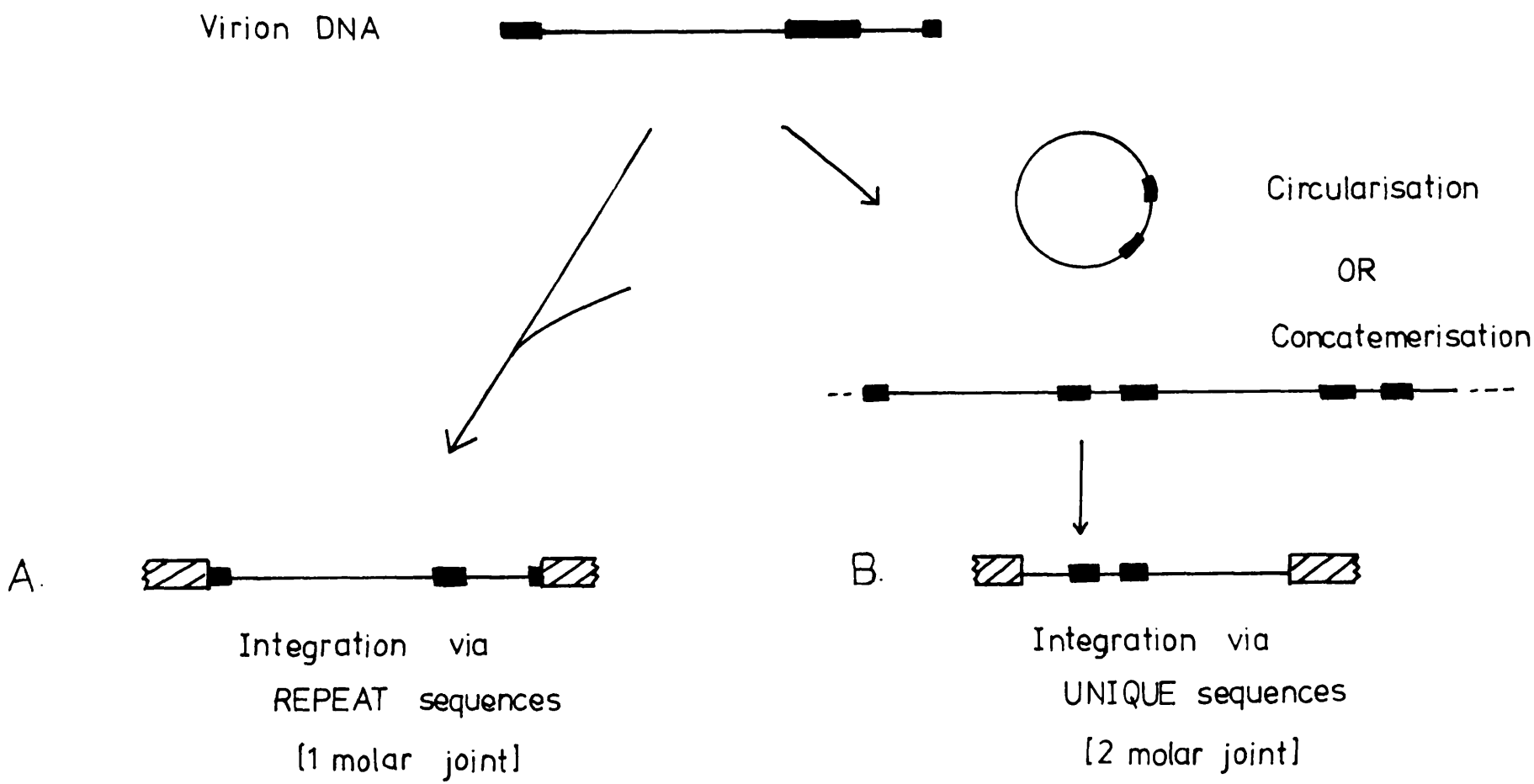

Virion DNA
Circularisation
OR
Concatemerisation
A.
B.
Integration via
REPEAT sequences
[1 molar joint]
Integration via
UNIQUE sequences
[2 molar joint]

FIGURE 10

Alternative forms of latent HSV-1 DNA proposed by Rock and Fraser (1985).

A. Integration via the repeat regions of the genome from linear, circular or concatemeric DNA results in 1 copy of the joint region per integrated genome.

B. Integration via the unique regions of the genome from circular or concatemeric DNA results in 2 copies of the joint region per integrated genome. A 2 molar joint region would also be present if the genome was in the form of non-integrated circles or concatemers.

Filled boxes represent repeat regions of HSV DNA.

Cross-hatched boxes represent cellular DNA.

unintegrated form, or was integrated into the cell genome via a site in the unique regions of the virus DNA (Fig. 10).

In order to understand the molecular processes involved in the establishment and maintenance of HSV latent infections, in vitro latency systems have been developed for HSV-1 and HSV-2. In these systems, virus replication is repressed by treatment of infected cells with drugs such as BVDU, in conjunction with interferon (Wigdahl et al., 1982a, 1982b), or by incubation of infected cells at elevated temperatures (Russell and Preston, 1986). Virus reactivation could be achieved in both systems by superinfection with heterotypic virus or ts mutants. Wigdahl et al. (1984) demonstrated in their system that latently infected HFL cells or rat neurons contained the HSV genome in a non-integrated, linear, non-concatemeric form. Since these results conflict with those of Rock and Frazer (1983, 1985), it is possible that this in vitro latency system does not mimic the in vivo state, and further investigation is required to resolve this difference.

1.9 MORPHOLOGICAL CELL TRANSFORMATION AND ONCOGENESIS

HSV has been considered a potential agent of human cancer, since many epidemiological surveys have established an association between cervical carcinoma and evidence of HSV infection. Investigation of the oncogenic potential of the virus has centred around in vitro cell transformation studies, and the search for HSV-specific macromolecules in neoplastic tissue.

Cell transformation by HSV was first achieved using non-permissive infection conditions such as UV-irradiated virus, sub- or supra- optimal infection temperatures or ts mutants. Subsequently, the observation that transfection of primary rat embryo cells with sheared HSV-2 DNA resulted in morphological transformation (Wilkie et al., 1974), stimulated research to

define the transforming sequence of HSV DNA by transfection with individual restriction endonuclease fragments. These experiments identified at least two transforming sequences in HSV-2 DNA (Reyes et al., 1979; Galloway and McDougall, 1981; Jariwalla et al., 1983), and one in HSV-1 DNA (Camacho and Spear, 1981) (Fig. 11). Detection of HSV-specific antigens in transformed cell lines has been reported (Macnab et al., 1980; Suh et al., 1980), however, no particular antigen has been consistently observed. Indeed, it is possible that an HSV polypeptide may not be involved in transformation process, since Galloway et al. (1984) mapped the transforming potential of the HSV-2 BglII n fragment to a 737bp sequence which bears similarities to an insertion sequence-like element. Reports of the detection of viral nucleic acid sequences in transformed cell lines (Galloway et al., 1980) must also be considered carefully in view of the observation that certain HSV DNA and mammalian DNA sequences share homology (Peden et al., 1982; Puga et al., 1982; Jones and Hyman, 1983). In addition, Galloway and McDougall (1983) and Cameron et al. (1985) have reported that cells transformed by the HSV-2 BglII n fragment do not retain this DNA sequence, and concluded that this transforming element is not required for maintenance of the transformed state.

These studies have been paralleled by attempts to demonstrate the presence of HSV-specific macromolecules in cervical carcinoma tissue. The major DNA-binding protein of HSV-2 has been detected in cervical carcinoma specimens. HSV-2-specific RNA mapping in the left hand end of the genome (mu 0.1-0.4) (Eglin et al., 1981; McDougall et al., 1982), and in the BglII n transforming region of the HVS-2 genome, have also been found in cervical carcinoma samples (McDougall et al., 1982). These results, however, must be investigated further in the light of the evidence that transcripts in rapidly dividing epithelial cells hybridise to several regions of the HSV-2 genome (Maitland et al., 1981). The detection of

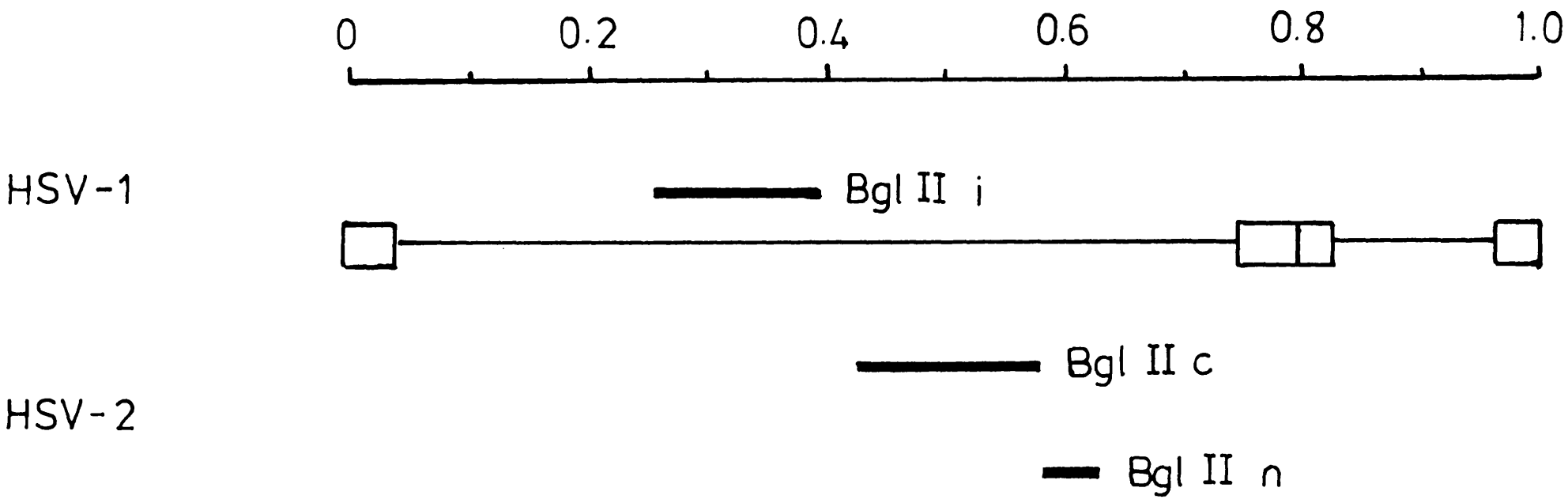

0
0.2
0.4
0.6
0.8
1.0
HSV-1
Bgl II i
Bgl II c
HSV-2
Bgl II n

FIGURE 11

Transforming sequences in HSV DNA. Transforming sequences of HSV-1 and HSV-2 are represented by solid blocks above and below the genome respectively.

HSV DNA sequences in a small percentage of cervical carcinoma specimens has been achieved by Park et al. (1983) and Galloway and McDougall (1983). Thus, in contrast to existing models for virus-induced cell transformation, the consistent presence of an HSV-specific antigen or oncogene in cervical carcinoma tissue or transformed cell lines has not been demonstrated, and attention has turned to more subtle possibilities for a mechanism of HSV oncogenesis.

A number of studies have demonstrated that HSV infection leads to chromosomal rearrangements (O'Neill and Rapp, 1971; Nachtigal et al., 1982). In addition, HSV has been shown to act as a mutagen in mouse cells (Schlehofer and zur Hausen, 1982). It is possible that HSV acts as the initiator of a transforming event through the production of heritable mutational changes. These observations led to the revival of a 'hit and run' mechanism of HSV-induced cell transformation (Galloway and McDougall, 1983).

By far the most exciting data has recently been reported by Macnab et al. (1985), who demonstrated that cellular polypeptides which are upregulated in established cell lines are also upregulated upon HSV infection of normal cells. Clearly these results provide the basis for future investigations into the importance of these polypeptides in the initiation and maintenance of cell transformation by HSV.

1.10 GENETICS OF HSV

One of the most useful approaches in the elucidation of HSV gene function has been the isolation and analysis of mutants of HSV. A wide variety of mutants have been isolated, and these include the following:

1.10.1 Drug-resistant mutants

To date, the majority of HSV drug-resistant mutants have lesions in

either the virus TK or DNA polymerase genes. Analysis of these mutants has made a significant contribution to the understanding of the mechanisms of action of antiviral drugs directed against these enzymes, and has been useful in prediction of the genetic effect of these drugs on virus grown in vivo.

The mechanism of antiviral action of drugs directed against the virus TK depends on the enzyme converting harmless compounds, for example, certain nucleoside analogues, into toxic compounds by phosphorylating them. The phosphorylated nucleosides can either inhibit the virus DNA polymerase directly by binding irreversibly to the active site, or can be incorporated into the virus DNA, interfering with DNA synthesis or inducing mutation. Virus growth in the presence of thymidine analogues 5-bromo-2'-deoxyuridine (BUdR) (Kit and Dubbs, 1963; Dubbs and Kit, 1964), 5-iodo-2'-deoxyuridine (IdU) (Yamamoto et al., 1972) or the deoxycytidine analogue 5-bromo-2'-deoxycytidine (BCdR) (Brown and Jamieson, 1978; Stow et al., 1978) gives rise to TK^- mutants which utilise the de novo pathway of thymidine synthesis, and so avoid the incorporation of lethal quantities of nucleotide analogues into the virus DNA. Mutants resistant to acycloguanosine (ACG), more commonly referred to as the antiviral drug Acyclovir, have been identified. These mutants are mostly TK^-, but TK^+ ACG-resistant viruses specifying a DNA polymerase which fails to recognise the phosphorylated form of ACG have been isolated (Elion et al., 1977; Field et al., 1980; Crumpacker et al., 1980; Darby et al., 1981; Larder and Darby, 1985).

Some antiviral drugs act directly on the virus DNA polymerase, e.g. phosphonoacetic acid (PAA), and mutants resistant to PAA have been isolated (Hay and Subak-Sharpe, 1976; Purifoy and Powell, 1977; Lee et al., 1978). This drug acts by binding to the pyrophosphate-binding site of the virus DNA polymerase (Leinbach et al., 1976).

1.10.2 Plaque morphology mutants

Most HSV-1 strains, e.g. HSV-1 (mP) and HSV-1 strain 17 syn+ cause individual virus-infected cells to round up. Variants have been isolated which, in contrast, cause extensive fusion of virus-infected cells, or produce a mixture of rounded and multinucleate cells in tissue culture. Several loci in the L segment of the HSV-1 genome have been shown to influence the ability of the virus to cause cell fusion. Such loci have been referred to as syn (syncytial) loci (Fig. 5). Ruyechan et al. (1979), on the basis of recombinant virus analysis, identified two syn loci between mu 0.68-0.82 on the HSV-1 (F) genome. This region is now thought to contain one syn locus only, and a marker which affects plaque size (Bond and Person, 1984; Pogue-Geile et al., 1984). DNA sequence analysis of this syn locus (mu 0.732-0.745) revealed a gene specifying a polypeptide which contains extensive hydrophobic domains. It is predicted that this polypeptide may be membrane-associated or may have a trans-membrane location (Debroy et al., 1985). Virus containing extensive deletions in the upstream regulatory sequences of the TK gene, have a syncytial phenotype (Sanders et al., 1982). It is not known, however, in which gene this syn locus maps. Little and Schaffer (1981) mapped the defect in the syncytial mutant HSV-1(KOS)-804, to DNA sequences between mu 0.04-0.64, thereby identifying another syn locus. Since a reduction in the gB dimer was detected in cells infected with this mutant, a role for gB in syncytia formation was suggested. A fourth syn locus was located directly in the gB gene by Ruyechan et al. (1979). In a study of tsB5, a mutant of HSV-1 which fails to incorporate gB into the virus envelope at the NPT, DeLuca et al. (1982) showed that the ts and syn loci in this gene were separable. DNA sequencing studies of the gB gene located the syn locus to the carboxy terminus of the glycoprotein. Since virus-induced

membrane proteins and, in particular, glycoprotein gB are implicated in syncytia formation, it seems reasonable to assume that this fusion process is analogous to the interaction between virus envelope and cell membrane, and the subsequent fusion event which leads to virus penetration. The significance of syncytia formation in vivo is unclear, and indeed, Roizman (1962) reported that the majority of clinical isolates of HSV were non-syncytial in tissue culture.

1.10.3 Immune cytolysis-resistant mutants

Immune cytolysis-resistant (icr) mutants exhibit altered synthesis, processing or incorporation of glycoproteins into infected cell membranes (Machtiger et al., 1980; Glorioso et al., 1980; Pancake et al., 1983). These mutants render virus-infected cells resistant to complement-mediated immune cytolysis with antisera directed against HSV-specific glycoproteins. This approach has identified novel genes controlling the synthesis and processing of glycoproteins. Useful information on the structure of glycoproteins may also be gained, for example, which regions of glycoproteins are exposed on the cell surface and recognised by antisera.

Monoclonal antibody-resistant (mar) mutants possess mutation(s) affecting the antigenic sites of glycoproteins exposed on the virion envelope, and hence are resistant to neutralisation by monoclonal antibodies plus complement (Holland et al., 1983). These mutants should provide useful information on the structure and function of glycoproteins. In addition, it may be possible to identify glycoproteins which would be useful candidates for the production of subunit vaccines against HSV.

1.10.4 Host range mutants

Host range mutants of HSV exist which are able to grow in some cell lines but not in others. Aurelian and Roizman (1964, 1965) observed

that HSV-1 (MP) failed to multiply in dog kidney cells. By passaging the virus several times through this cell line, these workers were able to select a variant which was able to replicate in these cells. Ts host range mutants of HSV-2 have been isolated by Koment and Rapp (1975, 1975a). These mutants were able to replicate in hamster embryo fibroblasts at 33^{o} but not at 39^{o}, whereas virus replication in rabbit kidney cells was unimpaired at either temperature. Interestingly, the in vitro replication characteristics of these mutants correlated with in vivo attenuation of mutant growth in hamsters, but not in mice or rabbits (Koment and Rapp, 1975b).

An important recent development in this field has been the establishment of cell lines which carry stably integrated HSV genes, for example, IE gene Vmw175 (Davison and Stow, 1985; DeLuca et al., 1985). HSV-1 mutants which synthesize inactive Vmw175, as a result of deletions within the gene encoding this polypeptide, are incapable of growth on normal cells. However, infection of the biochemically transformed cell lines with these mutants results in the stimulation of expression of the resident wild-type Vmw175 gene, and the mutant virus can be propagated (DeLuca et al., 1985; N.D. Stow, personal communication). Analysis of such host range mutants should be invaluable in the elucidation of virus-induced polypeptide function.

1.10.5 Mutants lacking restriction endonuclease sites

Restriction endonuclease site deletion mutants of HSV-1 have been isolated (Brown et al., 1984) which lack XbaI recognition sites. A variety of uses are envisaged for these mutants, including analysis of the mechanism and kinetics of HSV recombination, as well as the phenotypic characterisation of the mutants.

1.10.6 Temperature-sensitive (ts) mutants

Ts mutants constitute the majority of currently available conditional-lethal mutants of HSV. Ts mutants with defects in essential genes can replicate at the PT (usually 31^{o}-34^{o}), but not at the NPT (usually 38^{o}-39.5^{o}). Normally, a ts lesion is produced by a missense mutation in a gene, which results in the synthesis of an aberrant gene product. Such a protein is either unstable or is unable to assume a functional conformation at the NPT.

There is a low level of spontaneous ts mutation in wild-type virus stocks. However, most ts mutations have been induced by treatment of replicating virus (DNA) with BUdR, or by mutagenesis of virions with nitrous acid (NA), hydroxylamine (HA), nitrosoguanidine or UV light (Schaffer et al., 1970; Timbury, 1971; Schaffer et al., 1973; Manservigi, 1974; Esparza et al., 1974). One problem with these procedures is the selection of a mutagen dose which increases the frequency of mutation above the background level, whilst minimising the possibility of multiple mutations. Analysis of these mutants revealed the absence of ts mutations in large regions of the HSV genome. In an effort to increase the number of mutated HSV genes, emphasis has turned to the introduction of mutations into specific HSV DNA fragments. In this approach, the DNA is treated in vitro with mutagens such as NA or HA, recombined into the wild-type virus genome, and the progeny are screened for ts virus (Chu et al., 1979; Sandri-Goldin et al., 1981). Although, theoretically, ts mutations can be introduced into any gene, the screening procedure results in selection of ts mutants with defects in essential genes only. Over thirty-five essential genes have been identified by the complementation analysis of HSV ts mutants (Schaffer et al., 1978; Schaffer, 1985) and the genome location of many of these mutations has been determined by the marker rescue technique. In this method, cells are cotransfected with

intact mutant virus DNA, and purified or cloned restriction endonuclease fragments of wild-type virus DNA. After incubation for three days at the PT, the cells are harvested and progeny screened for ts$^+$ virus. Ts$^+$ recombinants will only be present if the mutation in the mutant virus DNA has been replaced by the corresponding wild-type sequence. Marker rescue of HSV-1 mutations was first demonstrated by Wilkie et al. (1974), and subsequently the technique was refined by Stow (1978). Interestingly, the physical map locations of ts mutations of HSV-1 strain 17 did not correlate with the recombinational linkage map (Stow et al., 1978; Stow and Wilkie, 1978; Wilkie et al., 1978). Since marker rescue can localise ts mutations to DNA fragments as small as 320bp (Preston, 1981), this method has enabled mutations to be assigned to specific genes.

The characterisation of ts mutants of HSV has yielded information on widely varying aspects of the HSV replication cycle, from the very early events such as virus penetration (Sarmiento et al., 1979; Little et al., 1980; Addison et al., 1984) and uncoating (Batterson et al., 1983), to regulation of virus gene expression (Preston, 1979b; Sacks et al., 1985) and DNA encapsidation (Preston et al., 1983).

1.10.7 Cold-sensitive (cs) mutants

Cs mutants are able to grow at high temperature (PT) but fail to produce plaques at lower temperatures (NPT). There has only been one published report to date of a cs mutant which was isolated from a HA mutagenesis experiment. This mutant, HSV-1 (50B), exhibited delayed plaque production at 31^0 whilst growing normally at 38.5^0 (Tognon et al., 1981; see Section 1.7.3). Interestingly, the lesion in this mutant maps in the U_S component of the viral genome, a region in which it is particularly difficult to isolate ts mutations.

1.10.8 Complementation

Until recently, most ts mutations have been assigned to different regions on the basis of complementation analysis (Schaffer et al., 1978). Although two main quantitative assays, the progeny yield test and the infectious centre test, have been used (Messer, 1978), the progeny yield test is generally considered more reliable since fewer recombinant viruses are generated by this method. Generally, complementation indices greater than 4 or 2 are considered positive, that is the mutations under test lie in different genes (Timbury, 1971; Brown et al., 1973). Interallelic complementation has been described between TK^- mutants, where it was detected biochemically (Jamieson and Subak-Sharpe, 1978).

1.10.9 Recombination

Recombination between herpesviruses was first shown by Wildy (1955) using lesion morphology on chorio-allantoic membranes and virulence of progeny virus in mice as indicators of recombination. The ability of HSV to recombine was later confirmed by Subak-Sharpe (1969), who reported the detection of ts^+ recombinants among the progeny of crosses between pairs of ts mutants. Using recombination frequencies obtained from crosses of ts mutants, a linear linkage map of the HSV genome was obtained (Brown et al., 1973; Schaffer et al., 1974; Timbury and Calder, 1976). However, later studies, using a variety of markers of known physical map location in addition to ts mutations, argued for a circular linkage map (Honess et al., 1980). This result is most simply explained if intermolecular, generalised recombination takes place between circular molecules or concatemeric DNA. This model was supported by results obtained by Ben-Porat et al. (1982) in a study of the behaviour of labelled PRV DNA in density shift experiments, and in an analysis of the kinetics of appearance of recombinant virus during infection. These workers

reported that recombination in PRV involves mainly parental genomes, and precedes DNA replication. Thus, under these conditions, recombination would presumably occur between circular DNA molecules. In contrast, Ritchie et al. (1977) showed that recombination in HSV-1 increases with time, indicating that both parental and progeny DNA molecules are involved. The production of linkage maps by recombination analysis of mutants has largely been supplanted by the physical location of mutations by marker rescue. Intertypic (HSV-1 x HSV-2) recombination has been of great value, however, in the physical mapping of HSV polypeptides (Timbury and Subak-Sharpe, 1973; Halliburton et al., 1977; Morse et al., 1977; Wilkie et al., 1978; Preston et al., 1978; Marsden et al., 1978).

A remarkable feature of many herpesviruses is that site-specific recombinational events involving reiterated sequences within the genomes can occur. Several lines of evidence have implicated the HSV a sequence in the inversion of L and S segments relative to each other (see Section 1.2.2). First, insertion of DNA fragments containing the a sequence into the TK gene locus results in additional inversion events about the novel junction (Mocarski et al., 1980; Mocarski and Roizman, 1981; Smiley et al., 1981). DNA fragments flanked by a sequences in inverted orientation will invert, whereas those flanked by direct repeats of the a sequence will not (Smiley et al., 1981). Second, deletion of the a sequence at the L-S junction results in mutants which cannot invert (Poffenberger et al., 1983; Poffenberger and Roizman, 1985). Third, intertypic recombinants which possess heterotypic a sequences flanking unique segments will not invert (Davison and Wilkie, 1983). Since all these non-inverting mutants are viable in tissue culture, it remains unclear what function, if any, inversion serves. The mechanism of inversion is not known, although Chou and Roizman (1985), using deleted subclones of the a sequence inserted at the TK locus, proposed that tandemly reiterated

sequences within the a sequence may be involved in site-specific recombination leading to inversion. Varmuza and Smiley (1985), however, using a similar system, failed to detect high-frequency recombination about the a sequence. Discrepancies between these two systems, for example, the number of reiterated sequences within the a sequences under investigation, must be resolved before any conclusions can be drawn.

Evidence for site-specific recombination elsewhere in the HSV genome has been presented. For example, the size variability of DNA fragments containing the intron of the spliced Vmw68 and 12, is probably a reflection of differing copy numbers of reiterated sequences within the intron (Murchie and McGeoch, 1982). It is postulated that these sequences can act as site-specific recombinational 'hot-spots' which, through unequal crossovers, will result in expansion or contraction of the intron, and hence the repeated sequences TR_S and IR_S (Whitton and Clements, 1984).

AIMS OF THE PROJECT

During HSV infection of tissue culture cells, greater than 200 virus-specific polypeptides have been detected by 2D-polyacrylamide gel analysis and these include primary translation products, processed intermediates and mature proteins. However, very few virus-induced polypeptides have so far been assigned specific functions. As described in the Introduction, several enzymes involved in nucleic acid metabolism have been identified while other polypepties are required for the regulation of virus transcription. In addition, approximately thirty polypeptides have been designated as structural proteins due to their presence in purified virions although, again, the functions of only a minority of these proteins are understood. The major HSV glycoproteins are found in the virion envelope, and it is known that glycoprotein gB plays an essential role in virus penetration of the cell membrane. It is thought that glycoproteins

are also involved in the attachment of virions to cell surface receptors. The functions of tegument proteins are largely unknown, although one of the major tegument proteins, Vmw65, is involved in stimulation of IE gene transcription. Seven structural polypeptides are components of the capsid particle. Of these, it is thought that Vmw155 is the major component of the hexamers and that Vmw40 (p40) plays a role in virus DNA encapsidation. However, the functions of the remaining structural proteins are unknown.

One approach to the elucidation of HSV gene function has been the isolation and characterisation of ts mutants. Although greater than thirty-five genes encoded by HSV have so far been defined by ts mutations, few mutants representing these genes have been characterised to any great extent. The aim of this study was to characterise in some detail HSV ts mutants which had potential structural defects. Using this approach, it was hoped to extend the information currently available on the roles of virion polypeptides in the infectious process and in HSV nucleocapsid assembly.

During the course of this study, four ts mutants of HSV-1 which had defects in assembly of functional nucleocapsids at the NPT were characterised. One of these mutants, ts1204, also had a defect in a very early event in infection. For this reason, characterisation of this mutant together with ts1208, a mutant which lies in the same cistron, was placed in a separate section of the thesis.

CHAPTER 2

MATERIALS

VIRUSES

The wild-type HSV-2 strain HG52 was used in this study (Timbury, 1971). In BHK cells, this virus forms plaques which contain a mixture of syncytial and rounded cells. The wild-type HSV-1 was strain 17 syn$^+$ (Brown et al., 1973), which has a non-syncytial plaque morphology. The HSV-1 ts mutants 17tsVP1201, 17tsVP1203, 17tsVP1204 and 17tsVP1208, characterised in this study, were all isolated from this strain. For brevity, these mutants will be referred to as ts1201, ts1203, ts1204 and ts1208 respectively. Ts1201 was derived from the mutant 17tsJC116 (Coates, 1982) which had multiple ts lesions. The DNA fragment EcoRI f from this mutant, which contained one of the ts lesions, was cloned into the plasmid vector pACYC184 and recombined back into wild-type HSV-1. Ts1201 was isolated from this transfection experiment (Preston et al., 1983). Ts1203, ts1204 and ts1208 were all isolated from experiments in which UV-mutagenised HSV-1 DNA fragments were recombined into wild-type HSV-1. Since none of the ts lesions mapped in the mutagenised fragments, they were considered to have arisen spontaneously (Matz et al., 1983; V.G. Preston, unpublished results). Revertants for growth at the NPT, ts1204 rev-1 and ts1203 rev-1, were isolated from mutant stocks which contained low levels of revertant ts$^+$ virus (see Section 2.2.1). The mutant tsK syn$^+$ was derived by Dr. V.G. Preston from a cross between wild-type HSV-1 strain 17 syn$^+$ and tsK syn, which forms syncytial plaques at the PT (Crombie, 1975). Additional ts mutants used in complementation and recombination studies were tsA syn$^+$ (Brown et al., 1973), and tsH (Crombie, 1975; Marsden et al., 1976) obtained from the Institute of Virology, Glasgow; ts656 (Hughes and Munyon, 1975) and tsZ47 (Pancake et al., 1983) which were kindly donated by Professor P.A. Schaffer, and

ts1205 which was supplied by Dr. V.G. Preston.

Intertypic (HSV-1/HSV-2) recombinant viruses used in the receptor mapping studies were isolated in the Institute of Virology and characterised as follows: R12-1, R12-3 (Chartrand et al., 1981) and Dx1 (34-2), Bx1 (31-2) (Marsden et al., 1978).

TISSUE CULTURE CELLS

Two tissue culture cell lines, BHK21 clone 13 and HFL cells were used throughout this study. The BHK cells, a fibroblastic line established by Macpherson and Stoker (1962), were obtained from Dr. V.G. Preston. Low passage HFL cells (Flow 2002) were supplied by Flow Laboratories.

TISSUE CULTURE MEDIA AND SOLUTIONS

Growth media

Cells were cultured in Glasgow modified Eagle's medium (Busby et al., 1964) which was supplied as a 10X concentrate by Gibco Limited. Medium was supplemented with 100units/ml penicillin, 100ug/ml streptomycin and 0.2ug/ml amphotericin. Media are referred to in this study as follows:-

E(T)Cn	Eagle's medium containing (10% tryptose phosphate) n% calf serum.
EFn	Eagle's medium containing n% foetal calf serum.
EHun	Eagle's medium containing n% human serum.
Versene	0.6mM EDTA dissolved in PBS containing 0.002% (w/v) phenol red.
Trypsin	0.25% (w/v) in tris-saline (obtained as a solid from Difco Limited).

Phosphate buffered saline (PBS)	170mM NaCl, 3.4mM KCl, 10mM Na_2HPO_4 and 2mM KH_2PO_4 pH7.2 (Dulbecco and Vogt, 1954).
Tris-saline	140mM NaCl, 30mM KCl, 280mM Na_2HPO_4, 1mg/ml glucose, 0.0015% (w/v) phenol red, 25mM Tris-HCl pH7.4, 100units/ml penicillin, 100ug/ml streptomycin.
Giemsa stain	1.5% (w/v) suspension of Giemsa in glycerol, heated at 56° for 120min and diluted with an equal volume of methanol.
TBE buffer (10X)	8.9mM Tris-HCl pH8.3, 8.9mM boric acid, 0.3mM EDTA containing 0.5ug/ml ethidium bromide.
SDS-polyacrylamide gel buffer (1X)	53mM Tris, 53mM glycine, 0.1% (w/v) SDS.
SSC buffer (10X)	3M NaCl, 0.3M tri-sodium citrate.
TE buffer (1X)	1mM EDTA, 1mM Tris-HCl pH7.4.
HEPES buffered saline (HEBS)	130mM NaCl, 4.9mM KCl, 1.6mM Na_2HPO_4 5.5mM d-glucose, 21mM HEPES (N-2-hydroxyethylpiperazine-N'-2-ethanesulphonic acid) pH7.05.
L-broth	170mM NaCl, 10g/l Difco bactotryptone, 5g/l yeast extract supplemented with 50ug/ml ampicillin.
RIPA buffer	0.1% (w/v) SDS, 1% (w/v) sodium deoxycholate, 1% (v/v) NP40, 1mM EDTA, 150mM NaCl, 10mM Tris-HCl pH7.4.
Washing buffer	600mM LiCl, 100mM Tris-HCl pH7.4, 1% (v/v) -mercaptoethanol.
Disruption buffer	2% (w/v) SDS, 10% (v/v) glycerol, 700mM B-mercaptoethanol, 150mM Tris-HCl pH6.7, 0.004% (w/v) bromophenol blue.

SDS-polyacrylamide gel tank buffer	53mM Tris, 53mM glycine, 0.1% (w/v) SDS.
Dye ficoll	15% (w/v) ficoll, 0.2% (w/v) bromophenol blue, 100mM EDTA.
Elution buffer	5mM sodium acetate, 1mM EDTA, 40mM Tris-HCl pH7.8.
Ligation buffer	20mM Tris-HCl pH7.5, 10mM $MgCl_2$, 10mM DTT and 1mM ATP.
STET buffer	8% (w/v) sucrose, 5% (v/v) NP40, 50mM EDTA, 50mM Tris-HCl pH8.0.
RSB	10mM KCl, 1.5mM $MgCl_2$, 10mM Tris-HCl pH7.5.
NTE	10mM Tris-HCl pH7.5, 10mM NaCl, 10mM EDTA.
Lysis buffer	0.6% SDS, 10mM EDTA, 10mM Tris-HCl pH7.4.
Gel Soak I	200mM NaOH, 600mM NaCl.
Gel Soak II	1M Tris-HCl pH8.0, 0.59M NaCl.
Nick translation buffer	50mM Tris-HCl pH7.5, 5mM $MgCl_2$, 50ug/ml BSA, 1mM DTT.
Denhardt's buffer	0.1% (w/v) Ficoll, 0.1% (w/v) polyvinyl-pyrollidone, 0.1% (w/v) BSA.
Resolving gel buffer	1.5M Tris-HCl pH8.9, 0.4% (w/v) SDS.
Stacking gel buffer	0.49M Tris-HCl pH6.7, 0.4% (w/v) SDS.
T4 DNA polymerase buffer	330mM Tris-acetate pH7.9, 660mM potassium acetate, 100mM magnesium acetate, 5mM dithiothreitol, 1mg/ml BSA.

RADIOCHEMICALS

All radiolabelled compounds were obtained from Amersham International plc.

CHEMICALS

Chemicals were supplied by five companies: BDH Chemicals, Poole, England; Pharmacia Fine Chemicals, Uppsala, Sweden; Koch-Light Laboratories, Suffolk, England; BioRad Laboratories, California, USA and Sigma (London) Limited. Analytical grade chemicals were used wherever available. Reagents for electron microscopy were obtained from two companies: Agar Aids, Stanstead, Essex and Taab Laboratories, Emmer Green, Reading.

ENZYMES

Restriction endonucleases were supplied by Bethesda Research Laboratories (UK) Limited, Cambridge, England.

IMMUNOLOGICAL REAGENTS

Fluorescein isothiocyanate-conjugated rabbit anti-mouse IgG, and rabbit anti-mouse IgG were purchased from Miles-Yeda Laboratories, Israel. Non-immune (pre-immune) rabbit serum was obtained from the Scottish Antibody Production Unit (SAPU), Glasgow, Scotland. A 10% (w/v) suspension of formalin-fixed Staphylococcus aureus strain Cowan I was supplied by Public Health Laboratories and Protein-A Sepharose by Pharmacia Fine Chemicals, Sweden.

MONOCLONAL ANTIBODIES

Monoclonal antibodies were prepared in the Institute of Virology, Glasgow, essentially as described by Palfreyman et al. (1983), except that the SP2/0-Ag14 cell line (Shulman et al., 1978) was used as the parental myeloma cell in cell fusions. MA1147 was specific for the major DNA binding protein, Vmw136 (ICP8) of HSV-1, and the equivalent polypeptide of HSV-2. MA1098 was specific for the IE polypeptide Vmw175 (ICP4) of

HSV-1. Both these antibodies were prepared by Dr. J.W. Palfreyman. Monoclonal antibody 5010B was specific for p40 (ICP35) of HSV-1 and was prepared by Dr. A. Cross.

For immunofluorescence experiments, MA1147 and MA1098 were used at a dilution of 1/40 in PBS, whereas MA5010B was used at a dilution of 1/20 with PBS. For immunoprecipitation experiments, MA5010B was used undiluted.

PLASMIDS

The following plasmids had been previously constructed in this Institute and were made available for use by Dr. V.G. Preston:

pGX38 (contain the HSV-1 EcoRI f fragment),

pGX91 (contains the HSV-1 BamHI u fragment,

pGX37 (contains the HSV-1 BamHI g fragment),

pGX2 (contains the HSV-1 BamHI k fragment),

pGX31 (contains the HSV-1 SalI d fragment),

pGX134 (contains the HSV-1 KpnI m fragment), and

pGX142 (contains the HSV-1 KpnI t fragment).

BACTERIAL STOCKS

Escherichia coli strain DH-1 (F^-, recA1, gyrA96, endA1, supE44) (Hanahan, 1983) was the host bacterium for all the plasmids used.

MISCELLANEOUS

Plastic 850cm^2 roller bottles for cell culture were obtained from Becton Dickinson Ltd., Oxford. Plastic petri dishes were supplied by Nunclon Ltd. Nitrocellulose membranes were purchased from Schleicher and Schull, Dassel, West Germany. Photographic film was supplied by Kodak Limited, London. Noble's Agar was obtained from Difco Limited.

METHODS

2.1 Cell culture

BHK cells were grown at 37° in rotating plastic 850cm^2 culture bottles containing 200ml ETC10 in an atmosphere of 5% CO_2, 95% air. A confluent monolayer of approximately $3x10^8$ cells was sufficient to seed five 850cm^2 bottles. Cells were harvested by washing the monolayer first with 20ml versene followed by 40ml trypsin-versene (1:1, v/v). After the cells had been shaken from the bottle, they were resuspended in tissue culture medium.

HFL cells were grown in the same way as BHK cells except that EF10 was used as tissue culture medium. Non-essential amino acids (1% v/v) were added to this medium every second cell passage. A confluent monolayer of $1x10^8$ HFL cells in a roller bottle was sufficient to seed three 850cm^2 bottles. 50mm and 30mm petri dishes were seeded at a density of $2x10^6$ cells and $1x10^6$ cells per dish respectively, to obtain confluent monolayers in 24h.

2.1.1 Cell storage

Low passage cells were stored at -140°. Briefly, cells were harvested as described in Section 2.1, pelleted and resuspended in EC20 (for BHK cells) or EF20 (for HFL cells) containing 10% (v/v) DMSO, at a concentration of 10^7 cells/ml storage medium. Cells, aliquoted into black cap vials, were frozen slowly to -140°. Recovery of cells was achieved by thawing the cells quickly, diluting them in EC10 or EF10, pelleting the cell suspension to remove the DMSO and finally resuspending them in growth medium.

2.2 Production of virus stocks

Virus stocks were routinely prepared from BHK cells grown in glass 850cm^2 roller bottles. Growth medium was removed and the cells were infected at a moi of 0.003 pfu per cell in 40ml EC5. Virus-infected cells were incubated at 31^o for 3-4 days until extensive CPE had developed. Cells were harvested into the medium and pelleted at 1,500 rpm for 15min at 4^o. Cell-released virus from the supernatant medium was concentrated by centrifugation at 12,000 rpm for 2h at 4^o. The virus pellet from this step was resuspended in supernatant medium, sonicated to disperse virus aggregates and stored at -70^o. Cell-associated virus from HSV-1 and HSV-2-infected cells was prepared by sonicating the cell pellet in a small volume of EC5. Cell debris was removed by low-speed centrifugation and the sonication step repeated if necessary. The supernatants, containing cell-associated virions, were pooled and stored at -70^o. Sterility checks were performed by streaking medium from virus-infected cells onto blood agar plates and incubating the dishes at 31^o for five days.

2.2.1 Isolation of spontaneous ts$^+$ revertant viruses

Spontaneous ts$^+$ revertants were isolated from a low passage stock of mutant ts1203 (obtained from Dr. V.G. Preston) as follows:

Ten-fold dilutions of the ts1203 stock were plated on BHK cells at the NPT. After virus adsorption for 1h, the cells were washed twice with tissue culture medium, overlaid with medium containing 0.6% Noble's agar and 10% calf serum, and were incubated at 38.5^o for two days. Plaques were picked into 0.5ml PBS containing 10% calf serum, using a dissecting microscope. The cells were sonicated to release virus, which was then titrated at the PT and NPT. All the progeny virus tested had an eop NPT/PT similar to that of wild-type virus. This result was taken as an indication that ts1203 contained a single lesion. Two further rounds of

plaque purification at the PT were performed on several ts$^+$ virus isolates. Finally, ts$^+$ virus from one plaque was used to infect a 50mm plate of cells, and virus from this plate harvest was used to grow up a virus stock. The revertant thus obtained was named ts1203-rev 1.

2.2.2 Handling of ts mutant infections

Infections with ts mutant used in this study were at 31^o (PT) and 38.5/39.5^o (NPT). All virus inocula were maintained at 4^o before addition to cells, unless otherwise stated. Cell monolayers were placed at the required temperature for 18-30min prior to infection. All media for washing cell monolayers or overlaying cells were prewarmed to the required temperature before use. Manipulations with virus infected cells were carried out as rapidly as possible, and incubator temperatures were monitored regularly.

2.2.3 Titration of virus stocks

Serial ten-fold dilutions of virus stocks were made in PBS containing 5% calf serum. 0.1ml of each dilution was added to 80% confluent BHK cell monolayers, from which the growth medium had been removed. After adsorption of virus for 1h at the required temperature, the cells were overlaid with EHu5 to neutralise unadsorbed virus and prevent the formation of secondary plaques. Plates were incubated at the PT of 31^o for three days or at the NPT of 38.5^o or 39.5^o (depending on the mutant virus) for two days, after which time the medium was replaced with Giemsa stain. About 15min later, the stain was washed off and the virus plaques were counted under a dissecting microscope.

FIGURE 12

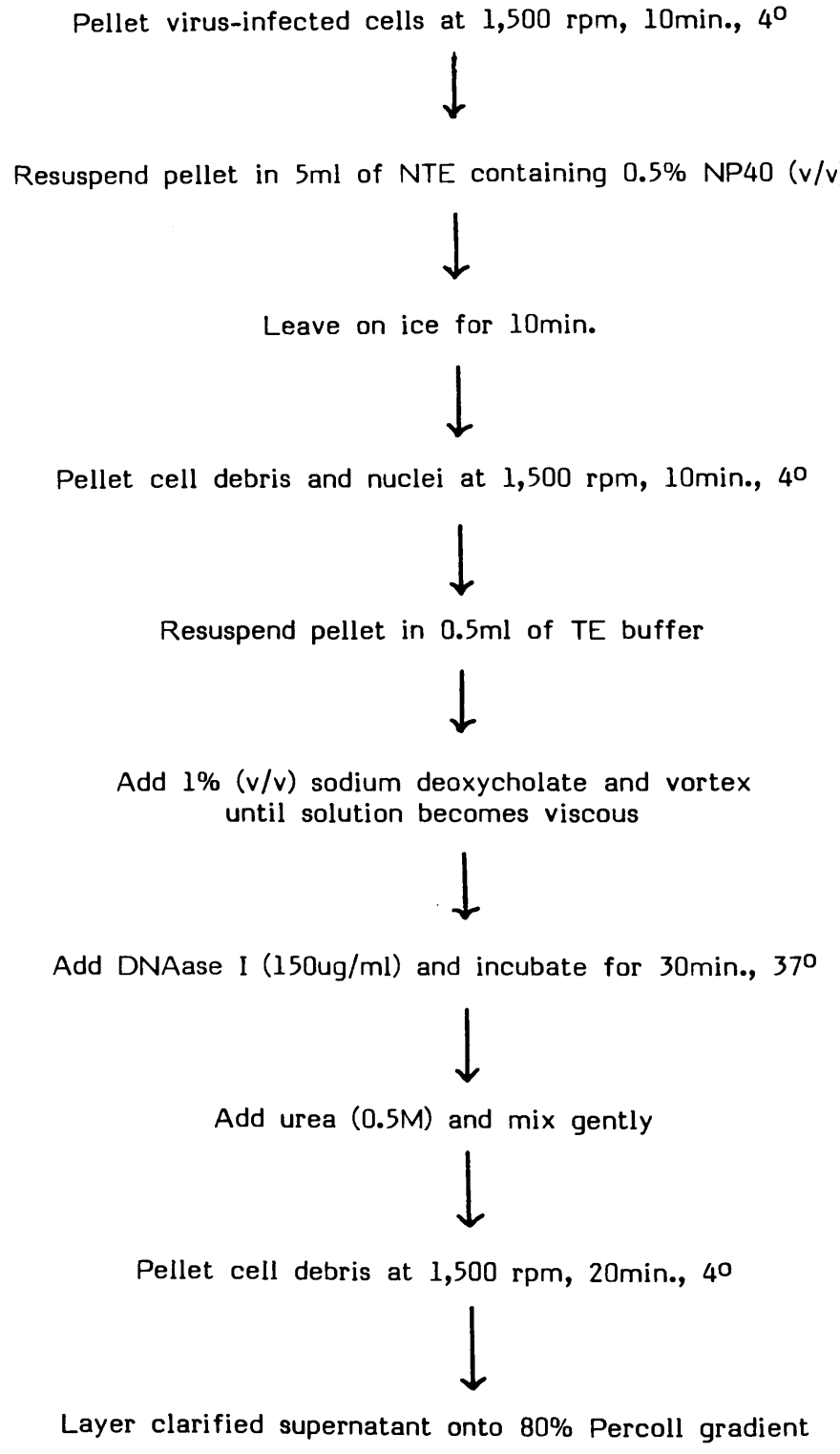

Pellet virus-infected cells at 1,500 rpm, 10min., 4°
Resuspend pellet in 5ml of NTE containing 0.5% NP40 (v/v)
Leave on ice for 10min.
Pellet cell debris and nuclei at 1,500 rpm, 10min., 4°
Resuspend pellet in 0.5ml of TE buffer
Add 1% (v/v) sodium deoxycholate and vortex until solution becomes viscous
Add DNAase I (150ug/ml) and incubate for 30min., 37°
Add urea (0.5M) and mix gently
Pellet cell debris at 1,500 rpm, 20min., 4°
Layer clarified supernatant onto 80% Percoll gradient

FIGURE 12

Flow diagram of the procedure for isolation of HSV nucleocapsids from virus-infected cells. Adapted from a method described by Braun et al. (1984b). All solutions were kept at 4^{o} during the procedure.

2.2.4 Virus adsorption assay

The adsorption assay used was based on a procedure described by DeLuca et al. (1981). Virus inoculum at 4^{o}, 31^{o} and 38.5^{o} was added to cells which had been maintained at each temperature, at a moi of 0.01 pfu per cell in a volume of 0.2ml. At 0, 15, 30, 45 and 60min post addition of virus, 10ul samples were withdrawn into 990ul PBS (containing 10% calf serum), and subsequently titrated in duplicate at 31^{o}.

2.2.5 Virus particle counts

Virus stock with a titre greater than $2x10^{8}$ pfu per ml was mixed with an equal volume of a latex bead suspension of known concentration, and the same volume of phosphotungstic acid. A sample was spotted on a parlodium grid, air dried, and visualised under the electron microscope. The number of virus particles and latex beads were counted in at least ten fields of the grid, and the concentration of particles in the virus stock calculated.

2.2.6 Isolation and purification of HSV nucleocapsids

Isolation of HSV nucleocapsids was performed using a procedure which was based on a method described by Braun et al. (1984b). A summary of this procedure is shown in fig. 12. Briefly, $3x10^{8}$ BHK cells were infected at a moi of 10 pfu virus per cell, and were incubated at the required temperature for 5h in medium containing one-fifth of the normal concentration of methionine. [^{35}S]-methionine was then added to the medium at a concentration of 50uCi/ml, and incubation was continued for a further 12h. As described in fig. 12, infected cells were harvested, and nuclei were isolated by non-ionic detergent treatment. All solutions were maintained at 4^{o} throughout the procedure. Nuclei were lysed with sodium deoxycholate, and the viscosity of the lysate was reduced by incubation

with DNase I for 30min at 37°. The clarified lysate was layered onto a 14ml 80% (starting density 1.25g/ml) Percoll density gradient (Pertoft, 1980; Svennerholm et al., 1980). Centrifugation of these gradients in a Ti65 fixed angle rotor at 40,000 rpm for 30min should have resulted in a well-defined nucleocapsid band approximately two-thirds down the gradient, which could be collected by side puncture of the tube. Capsid bands which were visibly contaminated with cell debris were rebanded on a second 14ml 80% Percoll gradient. Percoll was removed from capsid-containing samples by layering onto a 3ml cushion of 60% (w/v) sucrose in PBS, followed by centrifugation at 40,000 rpm for 60min in the AH650 swing-out rotor. Nucleocapsids formed a tight band at the interface, and were collected with a Pasteur pipette. Samples were used directly for analysis by SDS-PAGE, or were diluted in 10mM Tris-HCl pH7.4, pelleted at 40,000 rpm for 1h, and resuspended in 50ul 10mM Tris-HCl pH7.4 for electron microscopic analysis.

2.2.7 Polyethylene glycol treatment of virus-infected cells

Virus-infected cells were treated with polyethylene glycol (PEG) following a procedure described by Sarmiento et al. (1979). Briefly, sparsely seeded HFL cells on coverslips were infected with 5 pfu virus at the required temperature. After 1h virus adsorption, unbound virus was removed by washing the cell monolayers once with PBS. The virus-infected monolayers were then treated with PBS containing PEG 6000 (50% w/v) for 1min. Subsequent washes containing decreasing amounts of PEG were carried out as described by Sarmiento et al. (1979). In addition, two washes with PBS alone were performed, to remove all traces of the chemical. Following this treatment, the cells were incubated for a further 6h in growth medium before being fixed in methanol-acetone (3:1, v/v)) for immunofluorescence assay.

2.3 Preparation of virion DNA

Virus-infected cells from 5 to 10 850cm^2 glass bottles, exhibiting extensive CPE, were shaken into the medium and pelleted by low-speed centrifugation. Cytoplasmic extracts were prepared from the cell pellet by treatment with 10ml RSB containing 0.5% NP40 (v/v). After incubation on ice for 10min, the sample was centrifuged at 1,500 rpm for 10min at 4^o to remove nuclei and cell debris. The pellet from this step was resuspended in RSB-NP40 and treated as above. The two cytoplasmic supernatants were pooled with the clarified virus-infected cell medium and centrifuged at 12,000 rpm for 2-3h at 4^o. The virus pellet from this step was resuspended in 5ml NTE by sonication and lysed by addition of SDS to a final concentration of 2% (w/v). The virus DNA was extracted sequentially with phenol:chloroform (1:1) and with chloroform. RNA was removed by treatment with RNase I (10ug/ml), overnight at RT. The DNA solution was then deproteinised with proteinase K at 31^o for 2-3h, re-extracted with phenol:chloroform and chloroform, and precipitated with ethanol in the presence of 0.3M sodium acetate.

2.3.1 Small-scale preparation of virus-infected cell DNA

HFL cells (4x10^6 cells per dish) were infected with a moi of 5 pfu of virus per cell and incubated at the appropriate temperature for 18h before extraction of DNA.

2.3.2 Total virus-infected cell DNA

Growth medium was removed and cells were incubated for 4h at 37o in 2ml lysis buffer containing 500ug pronase per ml. After the addition of NaCl to a final concentration of 200mM, the DNA solution was extracted sequentially with phenol:chloroform (1:1) and chloroform, and precipitated in ethanol. DNA was pelleted, lyophilised and resuspended in

200ul H_2O containing 10ug/ml RNAase I.

2.3.3 Encapsidated DNA (DNAase-resistant DNA)

After removal of growth medium, cells were scraped into 0.5ml RSB, containing 0.5% (v/v) NP40 and 100ug/ml DNAase I, and incubated for 2h at 37°. SDS, EDTA and pronase were added to the sample at the same concentrations present in lysis buffer, and digestion continued for a further 2h at 37°. The DNA was extracted as described for total virus-infected cell DNA.

2.3.4 Restriction endonuclease digestion of DNA

Restriction endonuclease digestions were carried out under conditions specified by the suppliers for each enzyme.

2.3.5 Agarose gel electrophoresis

Concentrations of agarose between 0.6% and 1.0% (w/v) in TBE buffer containing 0.5ug/ml ethidium bromide were used in horizontal gels, depending on the size of DNA fragments to be resolved. DNA samples in 10% (v/v) dye ficoll were electrophoresed at $2V/cm^2$ for 16-20h and the separated fragments visualised under UV light.

2.3.6 Purification of DNA fragments from agarose gels

DNA fragments were excised from agarose gels and eluted from the gel slices by high-voltage electrophoresis in elution buffer. DNA was further purified by treatment with phenol:chloroform (1:1, v/v) and chloroform, and precipitated with isopropanol before use.

2.3.7 Polyacrylamide gel electrophoresis

DNA fragments (12-1000bp in size) were separated on

polyacrylamide slab gels containing 10% acrylamide crosslinked with 1 part in 30 (w/w) N-N'-methylene bis acrylamide in TBE buffer. Polymerisation was achieved by the addition of ammonium persulphate (0.006% w/v) and N,N,N'-N'-tetramethylethylenediamine (TEMED) (0.004% v/v) to the gel solution just before pouring. Wells were formed with teflon combs. DNA fragments were separated by electrophoresis at 3V/cm overnight using TBE tank buffer.

2.4 Marker rescue

Marker rescue was performed essentially as described by Stow et al. (1978) with the modifications of Preston (1981). Calcium chloride (final concentration 130mM) was added to a solution containing between 0.2 and 0.7ug intact mutant virion DNA, HSV-1 DNA fragment (usually 5-10 fold molar excess relative to virion DNA) and 10ug/ml calf thymus carrier DNA in HEBS. The samples were left for 5min at RT to allow a fine suspension to develop. This suspension was added to drained BHK cell monolayers in 50mm dishes. After incubation at 37^{o} for 45min, the cells were overlaid with EC5. At 4h pi, the cells were treated for 4min with 25% (w/v) DMSO in HEBS. DMSO was removed from the monolayers by washing the cells three times with EC5 after which incubation was continued at 31^{o} for 3-4 days until extensive CPE had developed. The cells were scraped into the growth medium, disrupted by sonication, and the virus yield at the PT and NPT determined.

2.4.1 Complementation yield test

In this assay, cells and medium were prewarmed to the NPT before use. BHK cells in 30mm dishes were infected at a moi of 10 pfu per cell (5 pfu per cell of each of the ts mutants in the mixed infections). After 1h adsorption at the NPT, the monolayers were washed with EC5 to

remove unadsorbed virus before incubation in this medium for 24h at the NPT. Virus-infected cells were scraped into the growth medium, sonicated and stored until further use at -70°. Virus yield was determined by titrating samples at the PT and NPT. Complementation indices (CI) were calculated as described by Brown et al. (1973) using the following formula:

$$CI = \frac{(X + Y)^{PT} - (X + Y)^{NPT}}{\frac{1}{2}(X^{PT} + Y^{PT}) - (X^{NPT} + Y^{NPT})}$$

where $(X + Y)^{PT}$ and $(X + Y)^{NPT}$ represent the titres of progeny virus from a mixed infection of tsX and tsY titrated at the PT and NPT respectively. The denominator represents the sum of the yields from the control single infections titrated at 31° and 38.5°. The denominator is halved to correct for the control cultures having received twice the number of pfu of a given mutant compared with the mixed infections. The $(X + Y)^{NPT}$ factor corrects for any ts^+ recombinants in the progeny virus. The $(X^{NPT}+Y^{NPT})$ corrects for any reversion or leak-through of mutant virus at the NPT. Normally, complementation indices greater than five were considered positive.

2.4.2 Recombination test

BHK cells in 30mm dishes were infected as for the complementation yield test except that the virus-infected cells were incubated for 24h at the PT instead of the NPT. Virus progeny was titrated at the PT and NPT, and the recombination frequency calculated as described by Brown et al. (1973) using the following formula:

$$RF\ (\%) = 100 \times 2 \times \left[\frac{(X + Y)^{NPT}}{(X + Y)^{PT}} - \frac{1}{2} \times \left(\frac{X^{NPT}}{X^{PT}} + \frac{Y^{NPT}}{Y^{PT}}\right)\right]$$

X and Y represent the ts mutants in the mixed infection. $(X + Y)^{NPT}$

and $(X + Y)^{PT}$ represent titres of progeny virus titrated at the PT and the NPT. The

$$\frac{1}{2}\left(\frac{X^{NPT}}{X^{PT}} + \frac{Y^{NPT}}{Y_{PT}}\right)$$

factor corrects for reversion and leakiness of the parental ts mutants. The equation is doubled on the assumption that the total frequency of recombinants is twice that of the selected ts^+ progeny virus.

2.5 DNA BLOT HYBRIDISATION

2.5.1 Transfer of DNA fragments to nitrocellulose

The procedure followed was essentially that of Southern (1975) in which single-stranded DNA is immobilized on a nitrocellulose membrane. The agarose gel containing the separated DNA fragments was shaken gently in 0.2M HCl for 30min at RT to depurinate the DNA, treated with gel soak I for 1h to denature the DNA and neutralized in gel soak II for 1h. The gel was then transferred onto two sheets of Whatman 3mm filter paper which were in contact with, but not covered, by 10X SSC buffer. A sheet of nitrocellulose moistened with distilled water was placed on top of the gel, followed by four sheets of 3mm paper cut 2mm smaller than gel size. Finally, a weighted stack of cut paper towels was laid on the Whatman paper. The following day, the nitrocellulose sheet was removed, rinsed in 2X SSC buffer, air dried and baked in a vacuum oven at 72^{o}, for 2h. Efficient transfer of DNA fragments from the gel was monitored by visualising blotted gels under UV light.

2.5.2 In vitro ^{32}P-labelling of DNA by nick translation

DNA fragments were labelled internally with ^{32}P essentially as described by Rigby et al. (1977). Plasmid DNA (0.25ug) in a 50ul reaction containing 1X nick translation buffer, 40uM dATP, 40uM dTTP

and $1x10^{-6}$mg/ml DNAase I, was incubated at 37° for 2min. The mixture was then placed on ice and 30uCi each of $-^{32}$P-dGTP and $-^{32}$P-dCTP plus 1 unit of E. coli DNA polymerase I were added. Nick translation was carried out at 15° for 2h. The isotope incorporation was monitored by spotting 2ul samples onto filter discs, precipitating the DNA with 5% TCA (w/v) and measuring the amount of radioactivity by Cherenkov counting. The reaction was terminated by extraction with phenol:chloroform (1:1). Unincorporated deoxyribonucleoside triphosphates were separated from the ^{32}P-labelled DNA by fractionation through a 10ml Sephadex G50 column equilibrated with TE buffer. The nick-translated DNA eluted with the first peak of radioactivity and was used directly in hybridisation experiments. The specific activity of nick translated plasmid DNA was normally $1x10^{7}$-$1x10^{8}$ cpm per ug DNA.

2.5.3 DNA blot hybridisation procedure

DNA hybridisations, in aqueous solution under high stringency conditions, were based on procedures described by Southern (1975) and Denhardt (1966). Nitrocellulose filters were prehybridised for a minimum of 2h in 100ml 6X SSC containing 5X Denhardt's buffer, 0.1% (w/v) SDS and 20ug/ml denatured calf thymus carrier DNA at 72° in sealed plastic bags submerged in a shaking water bath. This solution was replaced by the hybridisation solution which, in addition to the above reagents, contained between $1x10^{7}$ and $5x10^{7}$ cpm of denatured ^{32}P-labelled DNA probe per ml of hybridisation mix. The probe was denatured by incubation in 0.2N NaOH for 10min at RT, and neutralised with 0.2N HCl. After incubation overnight at 72°, nitrocellulose sheets were removed and washed four times in 2X SSC, once in 1X SSC and once in 0.5X SSC, each for 30min at 72°. All washes contained 0.36% SDS. Blots were air dried and placed in contact with Kodak X-Omat XS-1 film in conjunction with a

Du Pont phosphotungstate intensifying screen at -70°. Exposures of 1-2h were required for blots of total virus-infected cell DNA, and 2-4h for blots of encapsidated DNA.

2.6 RECOMBINANT DNA TECHNIQUES

2.6.1 Construction of recombinant plasmids

Linearised plasmid vector pUC9 was treated with calf intestinal phosphatase at a concentration of 5 units per ug plasmid DNA. After incubation at 37° for 1h, the DNA was extracted twice with phenol:chloroform (1:1, v/v), once with chloroform, and precipitated with ethanol in the presence of 0.3M sodium acetate pH6.6. A 10X molar excess of purified HSV DNA fragment relative to phosphatase-treated vector (10ng) was incubated overnight at 15° in a 20ul ligation reaction containing 1 unit of T4 DNA ligase in ligation buffer.

2.6.2 DNA transfection

This procedure was based on a method described by Cohen et al. (1972). E. coli K12 strain DH-1, grown in L-broth to an OD_{590nm} of approximately 0.3, was pelleted at 3,000 rpm for 15min at 4° and resuspended in 1/4 volume ice-cold 100mM $CaCl_2$. After incubation on ice for 1h, the cells were re-pelleted and resuspended in 1/10 volume of 100mM $CaCl_2$. The competence of the bacteria in transfection was tested immediately using uncleaved vector DNA. 0.1ml $CaCl_2$-treated cells were mixed with 10ng plasmid DNA and left on ice for 1h. After heat treatment at 42° for 2min, the bacteria were added to 1.5ml L-broth, and shaken at 37° for 1h before being plated onto L-broth agar containing 100ug per ml ampicillin. Plates were incubated overnight at 37°. Routinely $>1x10^6$ colonies per ug plasmid vector DNA were obtained. Since the competence of DH-1 bacteria is increased by incubation of

$CaCl_2$-treated bacteria at 4° for 16-18h prior to addition of DNA, competent bacteria were added to ligation reactions 16h after the cells had been prepared.

2.6.3 Small-scale preparation of plasmid DNA from bacterial stocks

Bacteria from a single colony were grown overnight in an orbital shaker at 37° in 1.5ml L-broth containing 100ug/ml ampicillin. The cells were harvested by centrifugation at 10,000 rpm for 2min. The pellet was resuspended in 75ul STET buffer and 6ul lysozyme (10mg per ml) was added (Holmes and Quigley, 1981). This mixture was boiled for 1min before centrifugation at 10,000 rpm for 10min at 4°. The supernatant, containing plasmid DNA, was extracted once with phenol:chloroform (1:1, v/v), once with chloroform and the DNA precipitated with an equal volume of isopropanol at RT in the presence of 0.3M sodium acetate. The DNA was subsequently pelleted at 10,000 rpm for 5min, lyophilised and resuspended in 30ul 10mM Tris-HCl pH7.4.

2.6.4 Large-scale preparation of plasmid DNA

The method used for large-scale preparation of plasmid DNA was based on a procedure described by Godson and Vapnek (1973). A flask containing 500ml L-broth, 100ug/ml ampicillin was inoculated with 10ml of an overnight culture of bacteria. This culture was incubated at 37° for 5-6h, at which point chloramphenicol was added to a final concentration of 25ug/ml. After a further 12h incubation at 37°, the bacteria were pelleted by centrifugation at 8,000 rpm for 5min at 4°, resuspended in 12.5ml of 25% sucrose in 50mM Tris-HCl pH7.9, and incubated for 30min on ice in the presence of 1.5mg/ml lysozyme. The mixture was vortexed thoroughly, and left on ice for 30min EDTA (pH7.9) and was added to a final concentration of 50mM, and 5min later, NaCl and SDS were added to

final concentrations of 750mM and 2.5% (w/v) respectively. This viscous solution was incubated at 4° for 2-3h, then centrifuged at 20,000 rpm for 1h at 4° to remove debris and high molecular weight bacterial DNA. The supernatant was extracted 4X with phenol:chloroform (1:1), treated 1X with chloroform and precipitated with an equal volume of isopropanol in the presence of 0.3M sodium acetate. Plasmid DNA was pelleted, washed with 70% ethanol, lyophilized and resuspended in 2ml 10mM Tris-HCl pH7.5 containing 10ug/ml RNAase I. After incubation overnight at RT, plasmid DNA was purified by equilibrium centrifugation on caesium chloride-ethidium bromide gradients.

2.6.5 Caesium chloride-ethidium bromide gradients

Plasmid DNA in a solution containing caesium chloride and ethidium bromide at final concentrations of 1g/ml and 500ug/ml respectively (final density 1.55g/ml), was centrifuged at 45,000 rpm for 18h at 15° in a Beckman TV865 rotor. Plasmid DNA bands were visualised by long-wave UV irradiation (365nm) and the lower band, containing supercoiled DNA, was collected by side puncture of the tube with a syringe. The ethidium bromide was removed from the solution by sequential extractions with isopropanol which had been saturated with caesium chloride. The DNA was dialysed overnight against TE buffer and pelleted after ethanol precipitation.

2.6.6 Storage of recombinant bacterial stocks

For long term storage, concentrated bacteria were kept in 15% glycerol at -20°. Viable bacteria were recovered from these stocks by plating a sterile loopful of bacteria on L-broth agar containing 100ug/ml ampicillin.

2.6.7 ^{32}P-labelling of DNA termini

Plasmid DNA was digested with the desired restriction enzyme in T4 DNA polymerase buffer. Three of the four dNTPs were then added directly to the completed reaction mixture, at a final concentration of 80uM, together with 2uCi of the fourth α-^{32}P-dNTP and 2 units of T4 DNA polymerase. After 5min at 37^{o}, the fourth unlabelled dNTP was added to a final concentration of 80uM and incubation continued for a further 10min. The reaction was terminated by heating at 70^{o} for 5min. A second restriction enzyme could then be added, if the mixture was chilled for 5min. The end-labelled DNA fragments were separated by agarose gel electrophoresis. The fragments were identified by autoradiography of the wet gel covered in cling-film. The fragment of interest was excised and extracted as described in Section 2.3.6.

2.6.8 Partial digestion of ^{32}P-end-labelled DNA

Since a very small amount of ^{32}P-end-labelled DNA fragment is required for partial digestion, 'carrier' DNA is added to the reactions to control the rate at which the labelled fragment is digested (Smith and Birnstiel, 1976). Approximately $1x10^{4}$ cpm of labelled DNA fragment, together with 1ug calf thymus DNA, was digested with 1-2 units of the desired restriction enzyme in a final volume of 20ul. Aliquots (2ul) of the reaction were withdrawn at 2, 5, 10, 15, 30 and 60min into 10ul of dye ficoll and DNA from the combined reactions separated on a 10% polyacrylamide gel. After electrophoresis, the gels were dried under vacuum and placed in contact with X-Omat XS-1 film at -70^{o} to obtain an autoradiographic image of the digest.

2.7 ANALYSIS OF VIRUS-INDUCED POLYPEPTIDES

2.7.1 Preparation of radiolabelled virus-infected cell extracts

Unless otherwise stated, confluent monolayers of HFL cells in 30mm dishes were infected at the required temperature with a moi of 20 pfu virus per cell. After 1h adsorption, the cells were washed once with EF2 to remove unadsorbed virus, and were overlaid with growth medium until the appropriate labelling time.

2.7.2 Induction of virus-induced immediate-early polypeptides

HFL cells were treated with cycloheximide (100ug/ml medium) for 15min prior to infection at the required temperature with 100 pfu virus per cell. The virus inoculum also contained 100ug/ml cycloheximide. After 1h virus adsorption, the cells were washed once with EF2 (containing 100ug/ml cycloheximide) and overlaid with the same medium. At 4.75h pi, actinomycin D was added to the cells at a concentration of 25ug/ml, and the incubation was continued. Finally, at 5h pi, the cells were washed four times with PBS and incubated in EF2, containing the appropriate radioactive isotope if required, for a further 60min before harvesting.

2.7.3 Pulse-labelling polypeptides with [^{35}S]-methionine

At the required time, growth medium was removed from cell monolayers and replaced with PBS containing 100uCi [^{35}S]-methionine per ml. Incubation was continued for 30min. Cells were then washed three times with PBS and either harvested immediately by addition of the appropriate lysis buffer, or after a further period of incubation in EF10.

2.7.4 Long labelling polypeptides with [^{35}S]-methionine

Virus-infected HFL cells incubated in medium containing one-fifth the normal concentration of methionine and 2% foetal calf serum, were

labelled at the required time in the same medium containing ^{35}[S]-methionine at a concentration of 50uCi/ml for periods of between 5h and 12h. The monolayers were then washed three times with PBS and harvested immediately by addition of the appropriate lysis buffer.

2.7.5 Long labelling polypeptides with [^{32}P]-orthophosphate

Virus-infected HFL cells were incubated in phosphate-free medium before incubation in the same medium containing 200uCi of [^{32}P]-orthophosphate per ml for periods of up to 5h.

2.7.6 Harvesting radiolabelled infected cell extracts

Total virus-infected cell extracts were harvested in 300ul disruption buffer per 30mm dish, and prepared for electrophoresis by boiling samples for 5min. Extracts for immunoprecipitation analysis were prepared as described in Section 2.7.7. All radiolabelled infected cell polypeptide samples were stored at -20^{o} prior to analysis.

2.7.7 Immunoprecipitation

Radiolabelled virus-infected cells from 30mm dishes were lysed by addition of 0.2ml RIPA buffer, transferred to glass vials and sonicated to disrupt aggregates. After incubation at 4^{o} for 1h, cell debris was removed from the sample by centrifugation at 10,000 rpm for 15min at 4^{o}. The supernatants were stored at -20^{o} until required. The immunoprecipitation procedure was based on a method described by Kessler (1975). A volume of labelled antigen, equivalent to approximately 1x10^{6} cpm, was pre-incubated with 100ul non-immune rabbit serum and 20ul Staphylococcus A to reduce non-specific antigen-antibody reactions. Bacteria were pelleted at 10,000 rpm for 5min, and the pre-cleared extract was reacted with monoclonal antibody for 1h at 37^{o}. Typically, 50ul acitic fluid (1/40

dilution) or cell culture supernatant (undiluted) was added, usually together with 10ul rabbit anti-mouse immunoglobulin. Immune complexes were bound onto 60ul of Protein-A-Sepharose (33% w/v suspension) at 37° for 30min. Immunoprecipitates were washed 4-6 times by centrifugation at 10,000 rpm for 25sec followed by resuspension in 500ul lithium chloride washing buffer. Samples were finally resuspended in 50ul disruption buffer, and heated at 100° for 5min. Protein-A-Sepharose was removed by centrifugation at 10,000 rpm for 25sec and the supernatant analysed using SDS-PAGE.

2.7.8 SDS polyacrylamide gel electrophoresis

Polypeptides were separated on two types of slab polyacrylamide gels: (i) single concentration gels containing 8% or 9% acrylamide crosslinked with 1 part in 40 (w/w) N-N'-methylenebisacrylamide in resolving gel buffer, and (ii) gradient gels composed of a 5-12% or 5-15% linear gradient of acrylamide crosslinked with 1 part in 20 (w/w) N-N'-methylnebisacrylamide in resolving gel buffer. Gradient gels were formed using a proportioning pump (Technicon Ltd.). Polymerisation was achieved by the addition of ammonium persulphate (0.006% w/v) and TEMED (0.004% v/v) to the gel solution just before pouring. The gel was overlaid carefully with either resolving gel buffer (1/4 strength) or butan-2-ol, to ensure a smooth interface on polymerisation. Stacking gels were prepared shortly before sample loading and contained 5% acrylamide crosslinked with 1 part in 30 (w/w) N,N'-methylenebisacrylamide in stacking gel buffer. Wells were formed with teflon combs. Denatured polypeptides were separated by electrophoresis at either 0.45mA/cm^2 for 3-4h or 0.09mA/cm^2 for 18h, in freshly prepared tank buffer.

2.7.9 Fluorography

Gels were fixed for 1h in methanol:water:acetic acid (50:50:1) followed by several washes in a solution containing 5% methanol, 7% acetic acid. Gels were either dried down immediately, or were soaked in En^{3}hance (New England Nuclear, Boston, USA) for 1h at RT, and re-hydrated by washing in several changes of water for 30min. Finally, gels were dried under vacuum at 80^o onto a sheet of Whatman (3mm) filter paper and placed in contact with Kodak X-Omat XS-1 film. Exposure time was typically 5x10^5 counts per track for 24h.

2.8 IMMUNOFLUORESCENCE ASSAY

HFL cells on 13mm coverslips were infected at a moi of 5 pfu virus per cell. At various times pi, coverslips were removed from the growth medium, and the virus-infected cells were fixed for 10min in a solution of methanol:acetone (3:1) at -20^o and air dried. Virus-infected cells were pre-incubated with 50ul pre-immune rabbit serum for 30min at RT and washed 2X with PBS before the addition of 50ul monoclonal antibody.

After a further 30min at RT, the cells were washed 6X with PBS, treated with 50ul fluorescent isothiocyanate-conjugated rabbit anti-mouse immunoglobulin (1/40 dilution) and incubated as above. The coverslips were finally washed 6X in PBS, 1X in distilled water and mounted in 50% glycerol on glass slides. Fluorescence was visualised under a Leitz UV microscope.

2.9 ELECTRON MICROSCOPY

2.9.1 Preparation of samples for thin sectioning

Growth medium was removed from mock- and virus-infected HFL cells in 30mm dishes and the monolayers washed three times with PBS.

The cells were scraped into 0.5ml PBS, transferred to Beem capsules and centrifuged at 5,000 rpm for 10min. The supernatant from this step was replaced with 500ul 2.5% (v/v) glutaraldehyde in PBS. The pellets were left at 4^{0} overnight after which they were washed three times with PBS and post-fixed in 1% (w/v) osmium tetroxide (OsO_4) for 1h. OsO_4 was removed by three further washes with PBS and the cell pellets were subsequently dehydrated through a series of increasing ethanol concentrations (30%, 50%, 70%, 90% and 100% (v/v) in PBS). The pellets were initially infiltrated with 50% (v/v) epon resin in ethanol followed by two changes of epon resin alone. Finally, the samples were incubated at 65^{0} for three days to polymerise the resin.

2.9.2 Thin sectioning

Thin sections from embedded cell pellets were cut either with glass knives or with a Rawlyer diamond knife set in an LKB Ultratome Type 4, and collected on parlodium-coated grids. Samples were stained for 1h with saturated uranyl acetate in 50% (v/v) ethanol, rinsed in deionized water and counter-stained for a further 1h with lead citrate before being examined at 80KV in a Jeol 100S electron microscope.

CHAPTER 3

3.1 CHARACTERISATION OF A MUTANT OF HSV-1 WITH A TEMPERATURE-SENSITIVE DEFECT IN PENETRATION OF CELLS

Introduction

HSV initiates infection of cells by adsorption of virions onto the cell surface, followed by penetration of the virus particle into the cytoplasm, probably as a result of fusion between the virus envelope and cell membrane. The virion polypeptides which mediate these processes have not yet been unequivocally identified, however, the envelope glycoproteins are most likely to be involved in such interactions with cell membrane receptors.

The following section describes the characterisation of two ts mutants of HSV-1 which fail to complement each other. One of these mutants has a defect in a very early event in the virus replication cycle. Interestingly, the lesion in this mutant does not map in any of the known glycoprotein genes, and appears to represent a novel HSV-1 complementation group.

3.1.1 Ts1204 has a defect in a very early function

Preliminary electron microscopic observations on thin-section preparations of ts1204-infected cells grown at the NPT (38.5^{o}) revealed that more than 60% of the cells showed no evidence of virus infection, for example, nucleocapsid assembly, margination of host chromatin or alterations in nuclear membrane structure. Indirect immunofluorescence studies using a monoclonal antibody MA1147 directed against Vmw136 (ICP8), the major HSV DNA binding protein, confirmed this finding. Whereas greater than 95% of wild-type virus-infected cells gave bright nuclear fluorescence typical with this antibody (Fig. 13b), fewer than 40% of ts1204-infected cells displayed this pattern of fluorescence. To

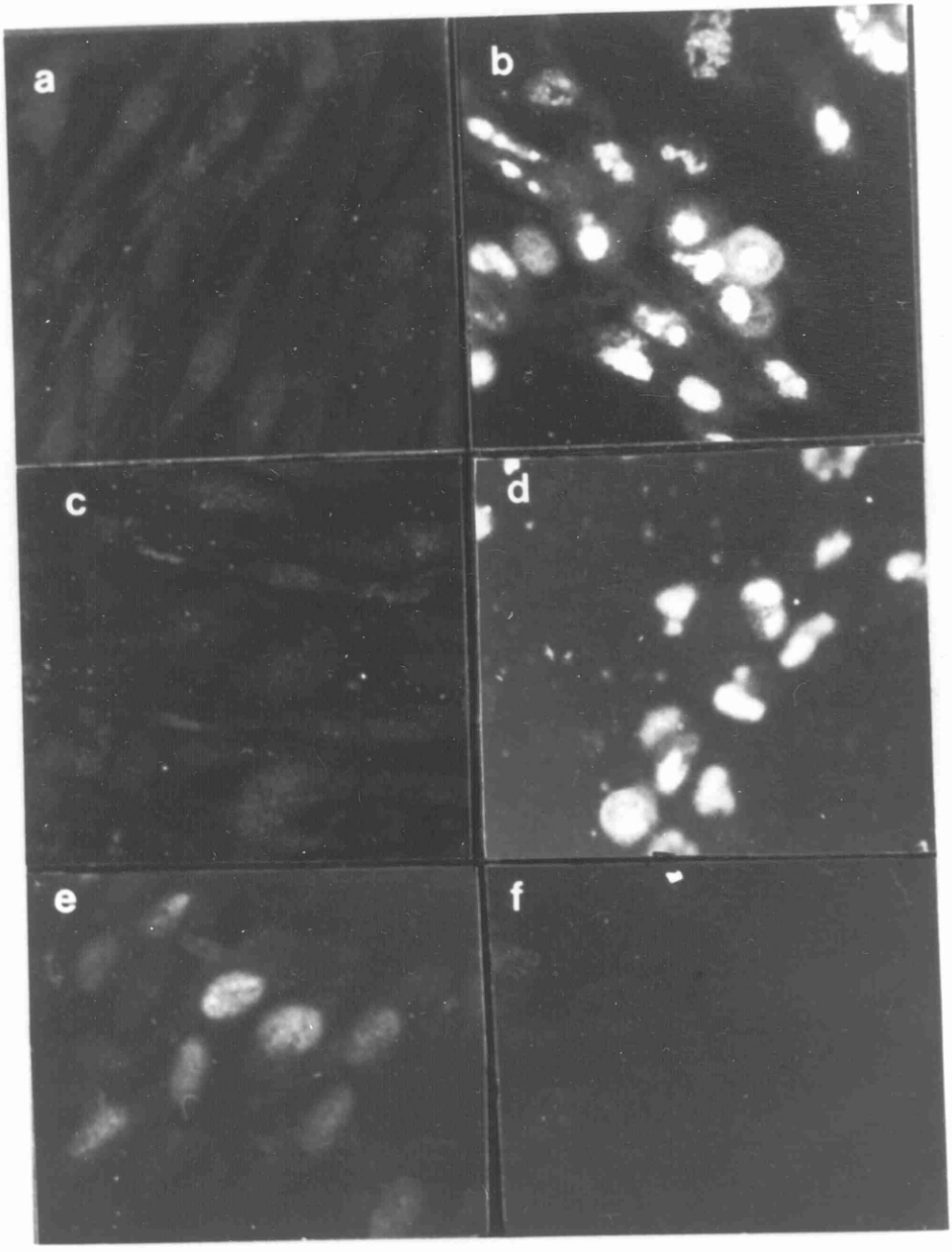

a
b
c
d
e
f

FIGURE 13

Demonstration that tsl204 has an early defect, using indirect immunofluorescence assay. Mock-infected (MI) monolayers of HFL cells and cell monolayers infected at the NPT with 5 pfu of wild-type HSV-1 or tsl204 per cell were harvested at 6h pi, unless otherwise stated, and prepared for indirect immunofluorescence assay:

All samples were incubated at the NPT unless otherwise stated.

(a) Mock-infected cells.

(b) Wild-type HSV-1-infected cells.

(c) Cells infected with tsl204 which was warmed to the NPT prior to infection.

(d) Cells infected with prewarmed tsl204 at the NPT and shifted down to the PT at 2h pi for a further incubation of 6h.

(e) Wild-type HSV-1-infected cells treated with cycloheximide. Cycloheximide was removed at 5h pi, replaced with actinomycin D and incubation was continued for 1h before harvesting.

(f) Tsl204-infected cells treated with cycloheximide. Cycloheximide was removed at 5h pi, replaced with actinomycin D and incubation was continued for 1h before harvesting.

The monoclonal antibody used in (a), (b), (c) and (d) was MA1147 which was specific for Vmw136, the major DNA binding protein of HSV-1. The monoclonal antibody used in (e) and (f) was MA1098 which was specific for Vmw175 of HSV-1.

investigate the possibility that Vmw136, detected in cells infected with ts1204 at the NPT, was synthesized as a result of leak-through of virus which had a defect in a very early function, the mutant inoculum was warmed to 38.5° for 10min prior to addition to cells. This treatment did not result in any significant loss of infectivity of virus when subsequently assayed at 31° (Matz et al., 1983). Figure 13c shows that fewer than 1% of cells infected with prewarmed ts1204 at the NPT exhibited nuclear fluorescence, using MA1147 in the immunofluorescence test. The effect of the mutation was reversible since after downshift of mutant-infected cells from 38.5° to 31° (PT) at 2h pi for a period of 6h, greater than 95% of cells showed nuclear immunofluorescence (Fig. 13d).

Although these experiments clearly showed that ts1204 did not synthesize detectable amounts of Vmw136, an early polypeptide, at the NPT, it was conceivable that virus-specified IE antigens were being synthesized. To investigate this possibility, IE polypeptides were induced in wild-type virus- and ts1204-infected cells at the NPT by cycloheximide treatment. IE mRNAs are synthesized normally in small amounts very early in wild-type virus infection (see Section 1.4.2), however, these transcripts can be induced in large amounts by treatment of virus-infected cells with cycloheximide. This protein synthesis inhibitor, added from the time of infection, prevents the usual transition from IE to E gene expression. Upon removal of the cycloheximide block, these IE mRNAs can be translated in the presence of actinomycin D to prevent further mRNA synthesis (see Section 2.7.2). Under these conditions, IE antigens are easily detected in wild-type virus-infected cells by immunofluorescence assay with a monoclonal antibody directed against an IE polypeptide. Figure 13e shows that >95% of wild-type virus-infected cells treated in this manner gave nuclear fluorescence with MA1098, a monoclonal antibody specific for Vmw175 (ICP4), one of the major IE polypeptides. By

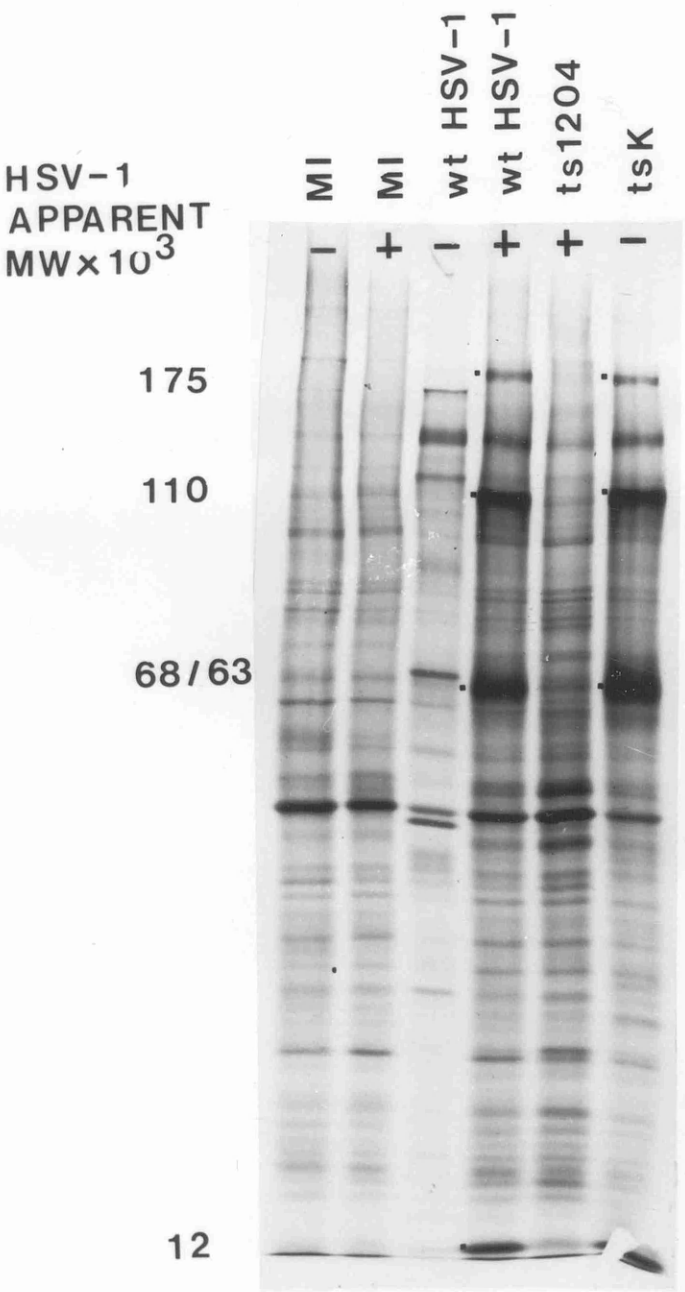

HSV-1
APPARENT
MW x 10^3
175
110
68/63
12
MI
MI
wt HSV-1
wt HSV-1
ts1204
tsK
-
+
-
+
+
-

FIGURE 14

Autoradiogram of polypeptides from mock-infected (MI) cells and cells infected with wild-type HSV-1, tsK or prewarmed ts1204 at the NPT in the presence or absence of cycloheximide treatment at the NPT. After removal of cycloheximide, samples were labelled for 1h at 5h pi with [^{35}S]-methionine in the presence of actinomycin D. Polypeptides were analysed on a 5-12.5% gradient SDS polyacrylamide gel.

Apparent MW of IE polypeptides are indicated.

+ = cycloheximide-treated samples.

- = untreated samples.

contrast, no fluorescence was detected in cells infected with prewarmed ts1204 (Fig. 13f), suggesting that no IE polypeptides were synthesized in mutant-infected cells at the NPT.

These results were confirmed by SDS-polyacrylamide gel analysis of cycloheximide-treated virus-infected cell polypeptides (Fig. 14). TsK, a mutant of HSV-1 which contains a lesion in Vmw175, overproduces IE polypeptides at the NPT, and serves as a control in this experiment. Fig. 14 shows that IE polypeptides were produced in wild-type virus-infected cells which had been treated with cycloheximide, and in tsK-infected cells. However, the polypeptide profile seen in cycloheximide-treated cells which had been infected with prewarmed ts1204 resembled a mock-infected cell profile.

3.1.2 Ts1204 fails to induce virus-specific polypeptides at the NPT

Virus-infected HFL cells, incubated at 38.5°, were pulse-labelled with [^{35}S]-methionine for 30min at 6h pi, harvested, and the radiolabelled polypeptides were analysed by SDS-PAGE (Fig. 15). When cold (4°) ts1204 inoculum was added to cells at the NPT, the polypeptide profile was very similar to that seen in wild-type virus-infected cells. Reduced shut-off of host polypeptide synthesis was noted in ts1204-infected cells compared to wild-type virus-infected cells, although this varied between experiments. Since immunofluorescence studies had shown that approximately 60% of cells remained uninfected when cold ts1204 inoculum was used, this finding was not unexpected. However, when the mutant stock was warmed to the NPT prior to addition to cells, the polypeptide profile resembled that of mock-infected cells, although there was some inhibition of host polypeptide synthesis, which again was variable from experiment to experiment. Some differences were evident in the intensity of several host polypeptide bands in ts1204-infected and mock-infected cell extracts. For example, bands of

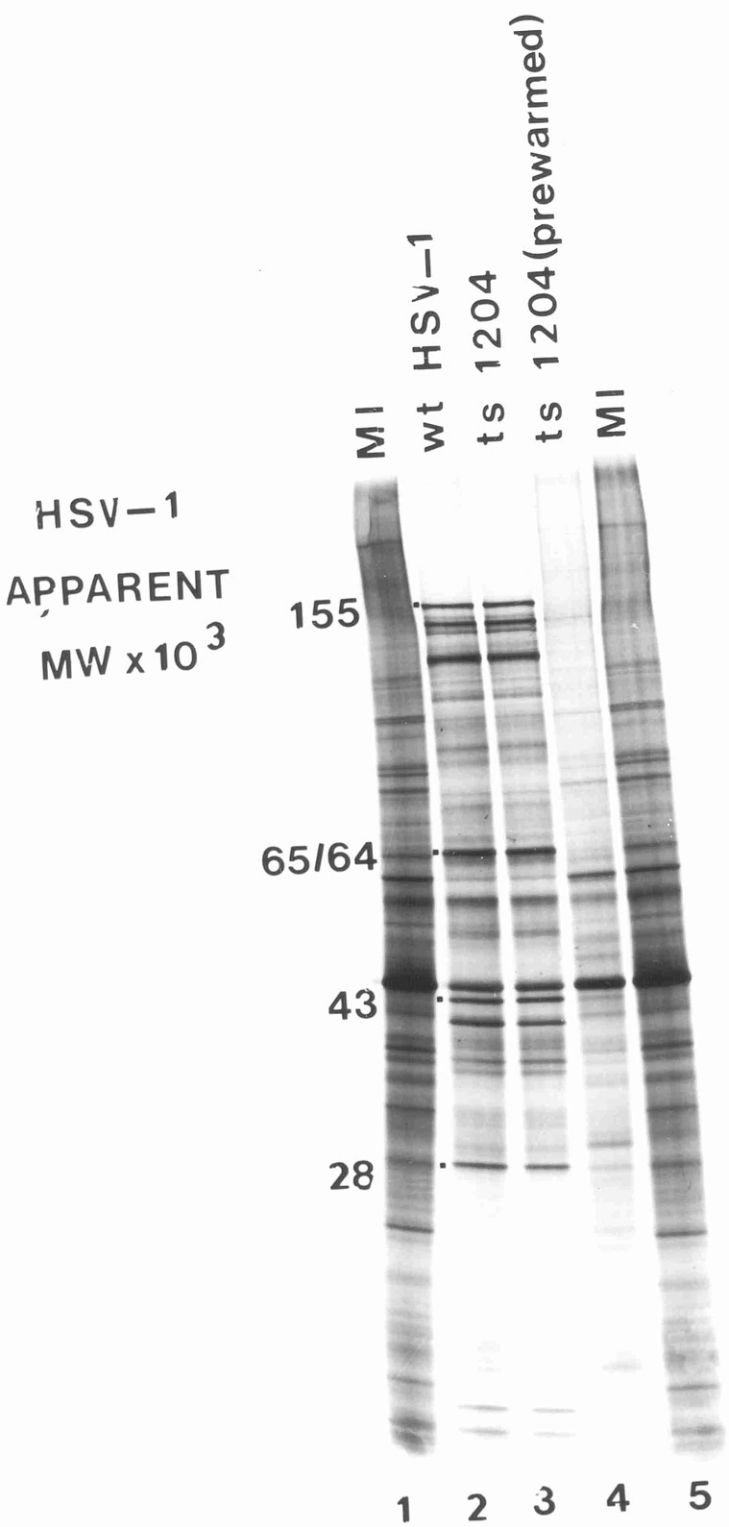

HSV-1
APPARENT
MW x 10 3
155
65/64
43
28
MI
wt HSV-1
ts 1204
ts 1204(prewarmed)
MI
1
2
3
4
5

FIGURE 15

Autoradiogram of polypeptides induced in wild-type HSV-1, ts1204 and mock-infected (MI) HFL cells at 38.5°. Virus-infected cells were labelled for 30min at 6h pi with [^{35}S]-methionine. Protein samples were analysed on a 5-15% gradient SDS polyacrylamide gel. Wt HSV-1 and ts1204 inocula were maintained at 4° prior to addition to cells. Ts1204 (prewarmed) inoculum was warmed to 38.5° for 10min prior to addition to cells.

approximately 57,000, 33,000 and 15,000 MW were among those which appeared to be increased in intensity in the ts1204-infected cell polypeptide profile, compared to the mock-infected cell profile. The possible significance of these observations will be discussed later.

3.1.3 Ts1204 has a defect in penetration of cells

Since previous experiments suggested that ts1204-infected cells failed to synthesize any virus-specific polypeptides at the NPT, it seemed feasible that the mutant had a defect in either uncoating, penetration or adsorption. In order to investigate these possibilities, electron microscopic studies were carried out on thin-section preparations of cells infected at a high moi (200 pfu per cell) of prewarmed ts1204 or wild-type virus at the NPT. Cells were harvested at 6h pi and prepared for electron microscopy as described in Section 2.9. No evidence of infection could be detected in the mutant virus-infected cells, however numerous enveloped particles containing DNA were observed around the cell surfaces (Fig. 16a). Many of these particles were in close association with the cell membrane (Fig. 16b). In contrast, no virions were seen on the surfaces of cells infected with 200 pfu per cell of wild-type virus (Fig. 16c).

These observations indicated that ts1204 was defective either in adsorption or penetration of cells at the NPT. In order to distinguish between these two possibilities, a series of experiments was designed to compare the abilities of ts1204 and wild-type virus to adsorb efficiently to cells at 4^{o}, 31^{o} and 38.5^{o}, based on an adsorption assay for wild-type virus described by DeLuca et al. (1981) (see Section 2.2.4). The mean results from two such experiments are shown in Fig. 17, where the unadsorbed virus has been expressed as a percentage of the initial virus inoculum at time zero. The 4^{o} serves as a control temperature at which wild-type virus can absorb to cells (a temperature-independent process), but cannot

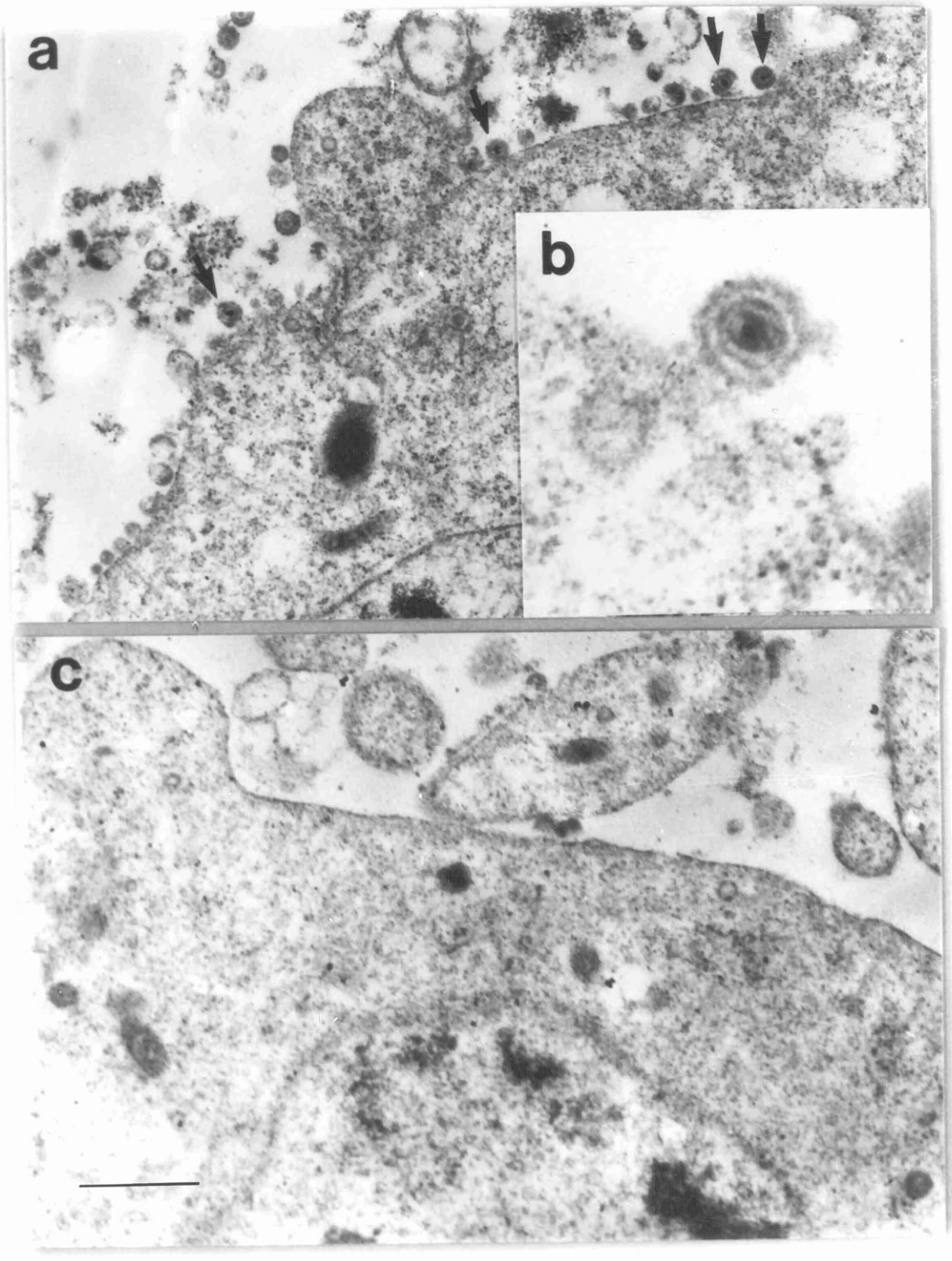

a
b
c

FIGURE 16

Electron micrographs of thin section preparations of HFL cells infected with 200 pfu of prewarmed ts1204 or wild-type HSV-1 per cell. Samples were harvested after incubation for 6h at the NPT.

(a) Enveloped ts1204 virions on the surface of a cell.

(b) Enveloped ts1204 virion in close association with the cell membrane.

(c) Surface and interior of a cell infected with wild-type HSV-1.

Arrows indicate enveloped virions containing DNA.

The bar represents 0.5uM.

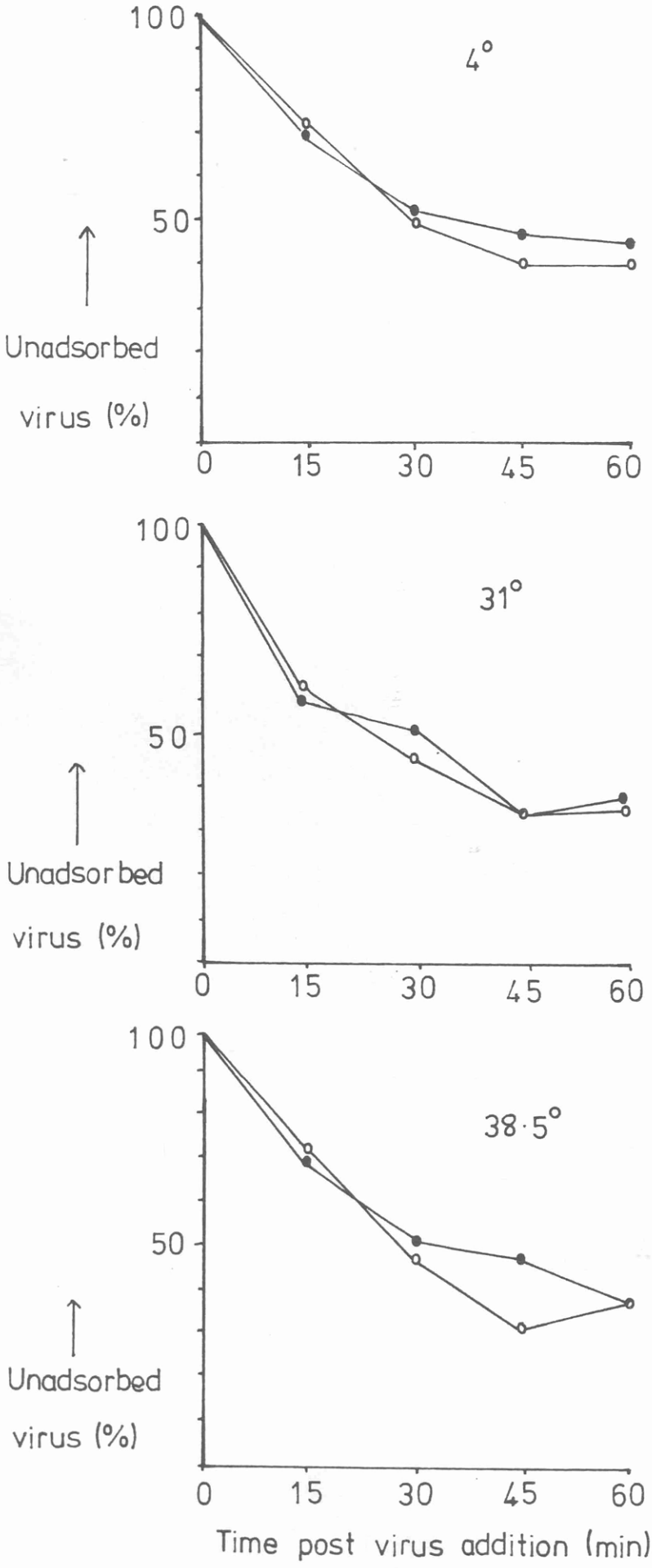

100
50
4°
Unadsorbed
virus (%)
0
15
30
45
60
100
50
31°
Unadsorbed
virus (%)
0
15
30
45
60
100
50
38·5°
Unadsorbed
virus (%)
0
15
30
45
60
Time post virus addition (min).

FIGURE 17

Kinetics of adsorption of wild-type HSV-1 and ts1204. HFL cell monolayers were infected with 0.01 pfu of wild-type HSV-1 or ts1204 per cell at 4^{o}, 31^{o} and 38.5^{o}. Samples (10ul) were withdrawn at 0, 15, 30, 45 and 60min post addition of virus to cells and subsequently titrated at 31^{o}. Unadsorbed virus is expressed as a percentage of the initial virus inoculum at time zero. Open circles represent the percentage of unadsorbed ts1204 virions. Filled circles represent the percentage unadsorbed wild-type HSV-1 virions. Figures represent the mean values from two experiments.

penetrate (a temperature-dependent process). At all three temperatures, wild-type virus and ts1204 exhibited very similar adsorption kinetics (Fig. 17). Between 40% and 50% of the initial wild-type virus and ts1204 inocula remained unadsorbed after 60min at 4°, between 35% and 40% at 31°, and approximately 35% for both viruses at 38.5°. Taken together with electron microscopic observations, these results indicate that at the NPT, ts1204 adsorbs normally to the cell surface but fails to penetrate.

3.1.4 Enhancement of infection by ts1204 at the NPT using PEG

As an additional confirmation that ts1204 virions attach to cellular receptors and remain bound, but fail to penetrate the cell surface, an experiment was performed following a procedure described by Sarmiento et al. (1979) (see Section 2.2.7). In this experiment, wild-type virus and ts1204-infected cells grown at the NPT were treated with polyethylene glycol (PEG), a membrane fusion promoter, in an attempt to facilitate the entry of extracellular bound virions. Control wild-type virus and ts1204-infected cell monolayers were treated in the same manner, except that PEG was omitted from all solutions. Immunofluorescence assay was performed using MA1147. As a consequence of PEG treatment, approximately 90% of ts1204-infected cells exhibited typical bright nuclear fluorescence (Fig. 18d). A similar percentage of wild-type virus-infected cells treated with PEG also exhibited this pattern of fluorescence (Fig. 18b). In the absence of PEG, ts1204-infected cells resembled mock-infected cells (Fig. 18c). These results confirm the suggestion that ts1204 binds to, but does not penetrate host cells at the NPT.

3.1.5 Nature of ts1204 binding to cells

Since ts1204 remained on the cell surface at the NPT, it was of interest to determine whether ts1204 bound to specific cellular receptors

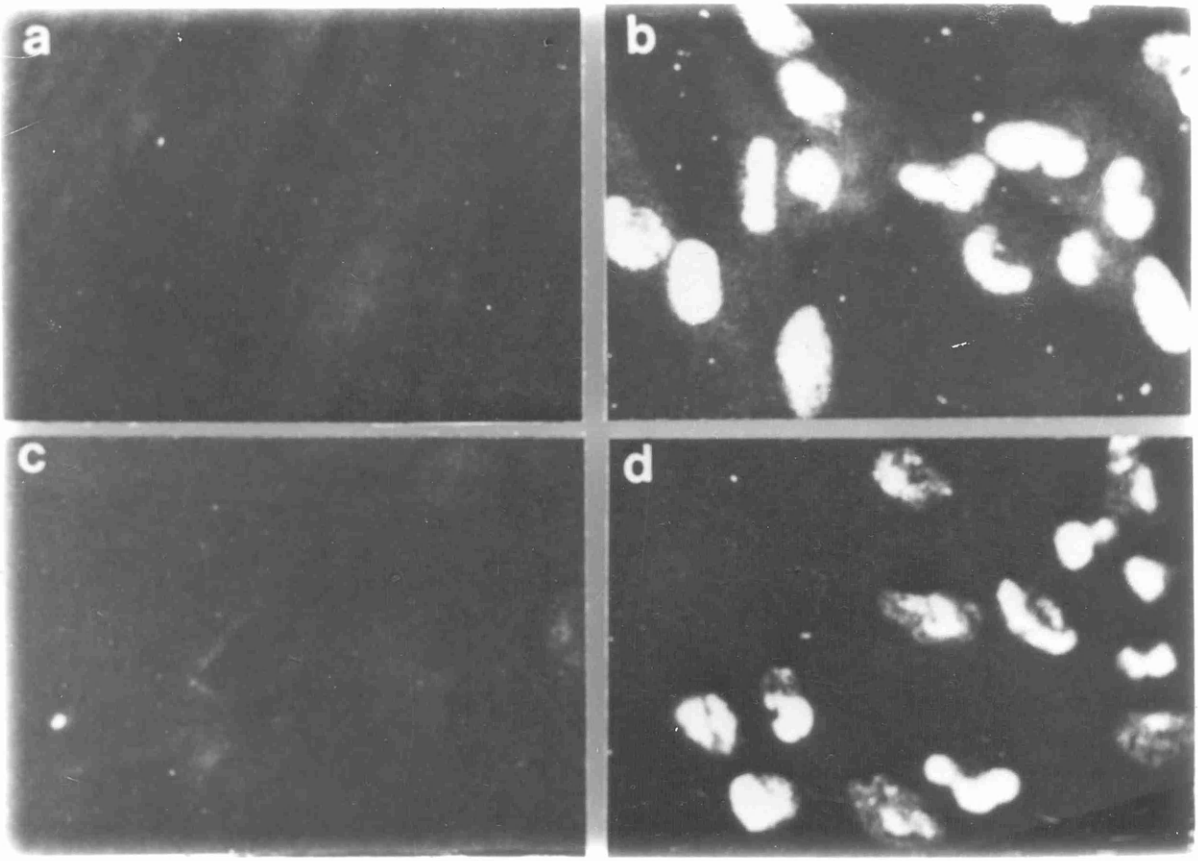

a
b
c
d

FIGURE 18

Demonstration that PEG enhances infection of cells by ts1204 at the NPT, using indirect immunofluorescence assay. Monolayers of HFL cells were infected at the NPT with 5 pfu wild-type HSV-1 or ts1204 per cell, harvested at 6h pi and prepared for immunofluorescence assay.

(a) Mock-infected cells.

(b) Wild-type HSV-1-infected cells treated with PEG.

(c) Cells infected with ts1204 which was prewarmed to the NPT prior to infection.

(d) Ts1204-infected cells treated with PEG.

The monoclonal antibody used in all cases was MA1147 which was specific for Vmw136, the major DNA binding protein of HSV-1.

*Wild-type virus-infected cells, in the absence of PEG treatment, gave bright nuclear fluorescence in 95% of cells.

for HSV, or bound non-specifically to the cell membrane. To this end, experiments were conducted in which wild-type HSV-1 or HSV-2 were used to superinfect cells which had been previously infected at the NPT with prewarmed ts1204 or tsK at a moi of 200 pfu per cell. TsK has a mutation in Vmw175 (Preston, 1979; Preston, 1981) and serves as a control. After 1h adsorption at the NPT, unattached ts1204 or tsK virions were removed by washing the cell monolayers twice with PBS. The cells were immediately superinfected with 10 pfu per cell of wild-type HSV-1 or HSV-2. After a further 1h adsorption time, the cells were washed once with PBS, overlaid with growth medium and incubation was continued for 6h at 38.5°, after which time the cells were fixed and prepared for indirect immunofluorescence. Again, MA1147 was used in the assay and the presence of bright nuclear fluorescence was taken as evidence that penetration by wild-type virus had occurred. TsK, which penetrates cells normally at the NPT but induces only a limited number of virus-specific polypeptides, did not synthesize Vmw136. Cells infected with either this mutant or prewarmed ts1204 resembled mock-infected cells in the immunofluorescence assay (Figs. 19a and 19d). After superinfection with HSV-1 or HSV-2, bright nuclear fluorescence was observed in tsK-infected cells (Figs. 19e and 19f). By contrast, no specific Vmw136 nuclear antigen was detected in ts1204-infected cells which had been superinfected with wild-type HSV-1 (Fig. 19b). If, on the other hand, the superinfecting virus was wild-type HSV-2, nuclear fluorescence was detected in >90% of ts1204-infected cells (Fig. 19c). These results were confirmed by analysis of radiolabelled virus-infected cell polypeptides on SDS polyacrylamide gels (Fig. 20). The pattern of polypeptides from ts1204-infected, HSV-1-superinfected cells resembled that of mock-infected cells, whereas the pattern of mutant virus-infected cells superinfected with HSV-2 was indistinguishable from that of HSV-2 (Fig. 20). These results indicated that

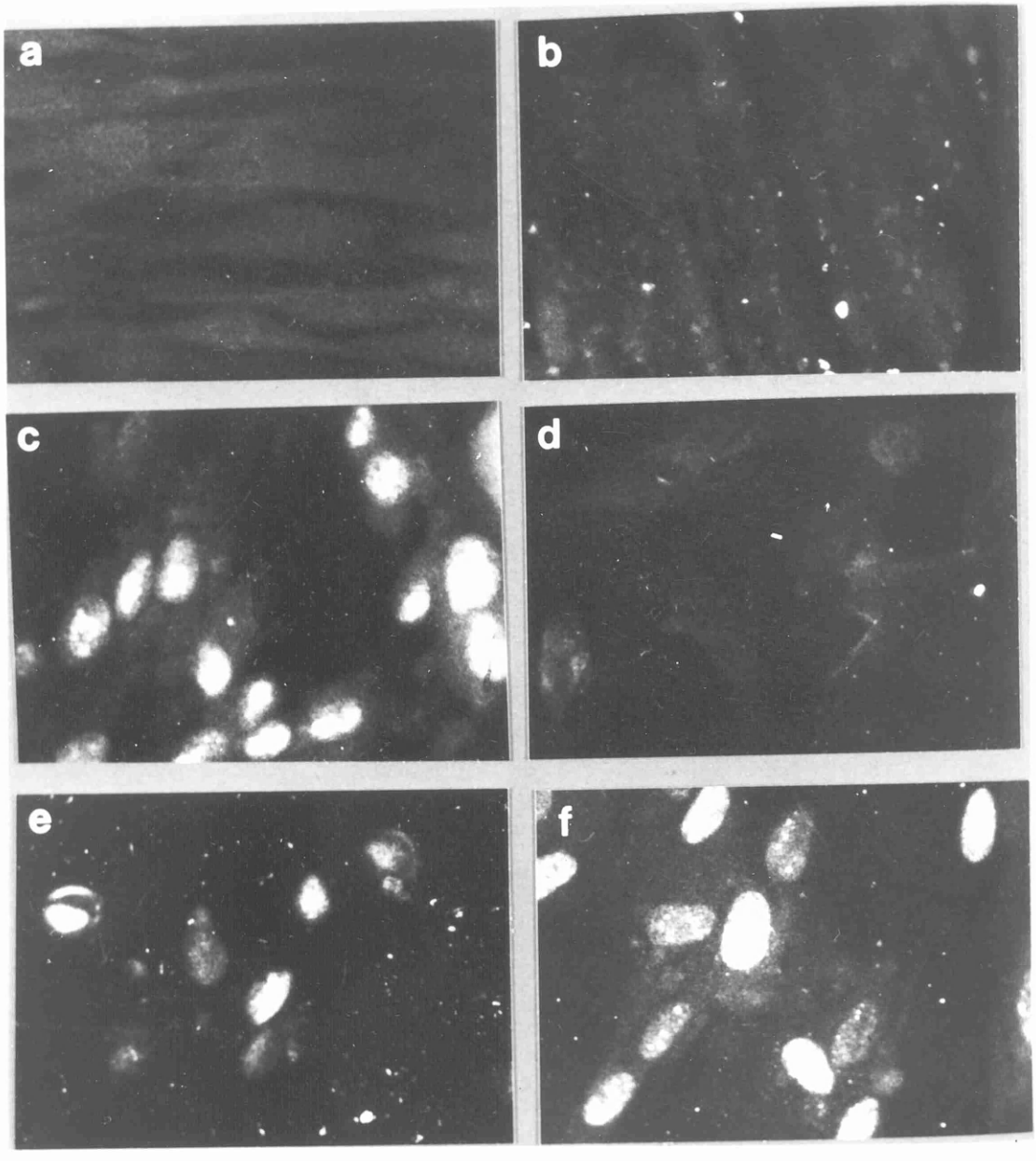

a
b
c
d
e
f

FIGURE 19

Demonstration that ts1204 binds to HSV-1-specific cellular receptors, using indirect immunofluorescence assay. Monolayers of HFL cells infected with 200 pfu of prewarmed ts1204 or tsK per cell were harvested at 6h pi and prepared for indirect immunofluorescence assay. Cells were superinfected with 5 pfu of wild-type HSV-1 or HSV-2 per cell 1h after the addition of ts1204.

(a) Cells infected with 200 pfu of ts1204 per cell.

(b) Cells infected with ts1204 and superinfected with wild-type HSV-1.

(c) Cells infected with ts1204 and superinfected with wild-type HSV-2.

(d) Cells infected with 200 pfu of tsK per cell.

(e) Cells infected with tsK and superinfected with wild-type HSV-1.

(f) Cells infected with tsK and superinfected with wild-type HSV-2.

The monoclonal antibody used in all cases was MA1147 which was specific for Vmw136, the major DNA binding protein of HSV-1 and the equivalent HSV-2 polypeptide.

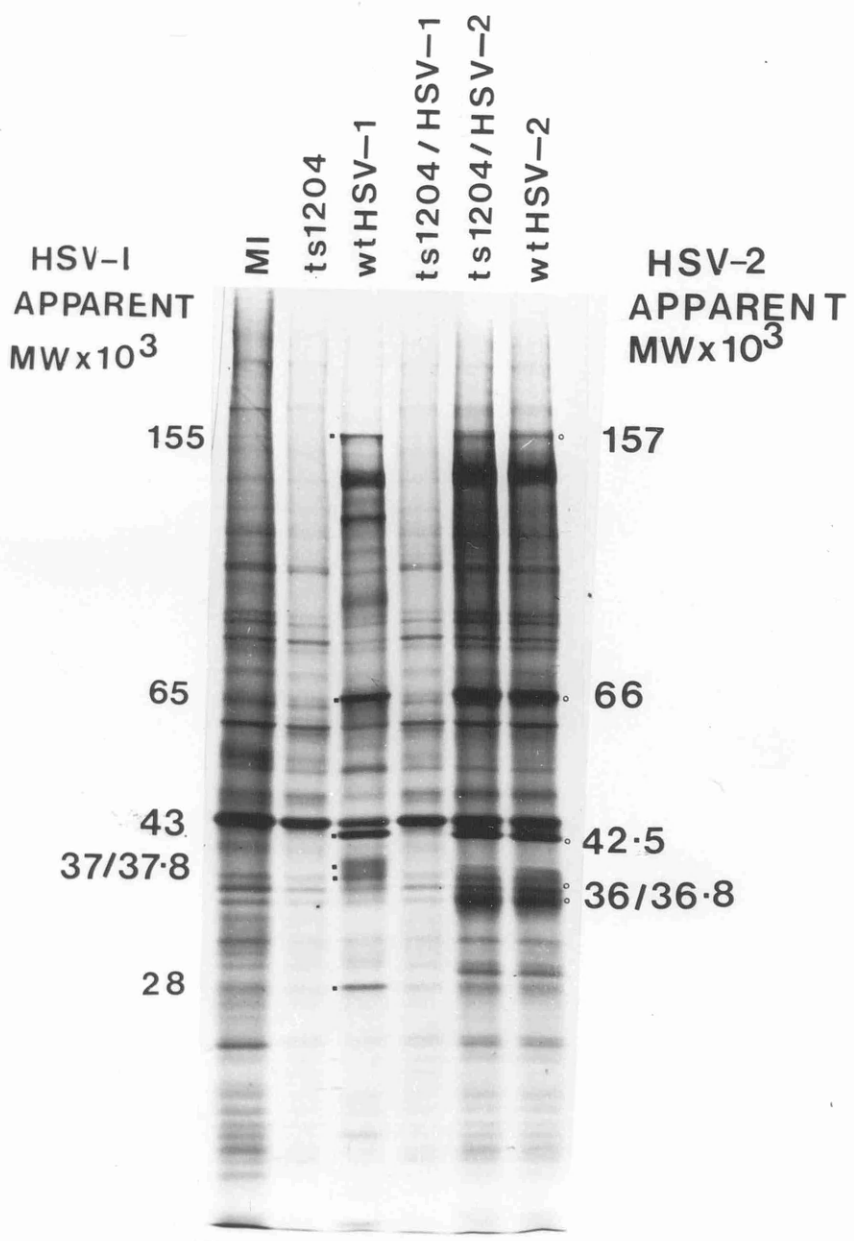

MI
ts1204
wtHSV–1
ts1204/HSV–1
ts1204/HSV–2
wtHSV–2
HSV–1
APPARENT
MW x 10³
155
65
43
37/37·8
28
HSV–2
APPARENT
MW x 10³
157
66
42·5
36/36·8

FIGURE 20

Autoradiogram of polypeptides induced in HFL cells infected with 200 pfu of prewarmed ts1204 per cell at the NPT. After incubation for 1h at 38.5°, cells were superinfected with 5 pfu of wild-type HSV-1 or HSV-2 per cell or were mock-superinfected. Samples were labelled for 30min with [^{35}S]-methionine 7h after infection with ts1204. Polypeptides from mock-infected cells and cells infected with wild-type HSV-1 or HSV-2 alone were also labelled for 30min with [^{35}S]-methionine at 7h pi. Samples were then harvested and analysed on a 5-15% gradient SDS polyacrylamide gel.

The apparent MW of HSV-1 (filled squares) and HSV-2 (open circles) polypeptide markers are indicated.

ts1204 binds tightly to cellular receptors specific for HSV-1, and that HSV-1 and HSV-2 utilize different cellular receptors.

Experiments to determine if ts1204 can enter cells co-operatively with other viruses were also carried out. HFL cells on coverslips were co-infected with ts1204 together with ts1213, which has a defect in uncoating (V.G. Preston, unpublished result) or tsK. Since both these mutants are defective in stages of the replication cycle prior to E gene expression, the detection of Vmw136 in co-infected cells could be taken as evidence that ts1204 virions were able to penetrate cells co-operatively with other virions and initiate a normal infection. However, no nuclear Vmw136 was observed in immunofluorescence tests on co-infected cells using MA1147 (data not shown). Therefore, this result indicated that ts1204 cannot utilize cellular receptors in conjunction with other viruses which are capable of penetration.

3.1.6 Physical map location of the ts1204 lesion

The ts1204 lesion had been previously mapped to DNA sequences within fragment KpnI t (map units 0.322-0.344) by Matz et al. (1983). Using separated HpaI fragments from cloned HSV-1 EcoRI f (plasmid pGX38) in marker rescue experiments, the map co-ordinates of ts1204 were refined. The mutation mapped within a 400bp region of BamHI u, shared by HpaI i and KpnI t (Table 2, Fig. 21).

3.2 FURTHER CHARACTERISATION OF TS1204 AND COMPARISON WITH TS1208, A MUTANT WHICH FAILS TO COMPLEMENT TS1204

3.2.1 Ts1204 has a second phenotypic defect

Preliminary electron microscopic studies on ts1204-infected cells at the NPT revealed that when the mutant inoculum was not warmed to 38.5^{o}

TABLE 2

Summary of marker rescue results for ts1204 and ts1208.

Cloned EcoRI f, digested with HpaI, gave two fragments: a which contained sequences from HSV-1 HpaI i, b and pACYC184, and b which contained sequences from HSV-1 HpaI t only. These fragments were purified and used in marker rescue experiments. The other DNA fragments were available as cloned DNAs and were digested with the appropriate restriction endonuclease prior to transfection.

The relative efficiency of plating (eop $^{NPT/PT}$) was calculated from the yield of progeny virus at the PT and NPT from the transfected cells.

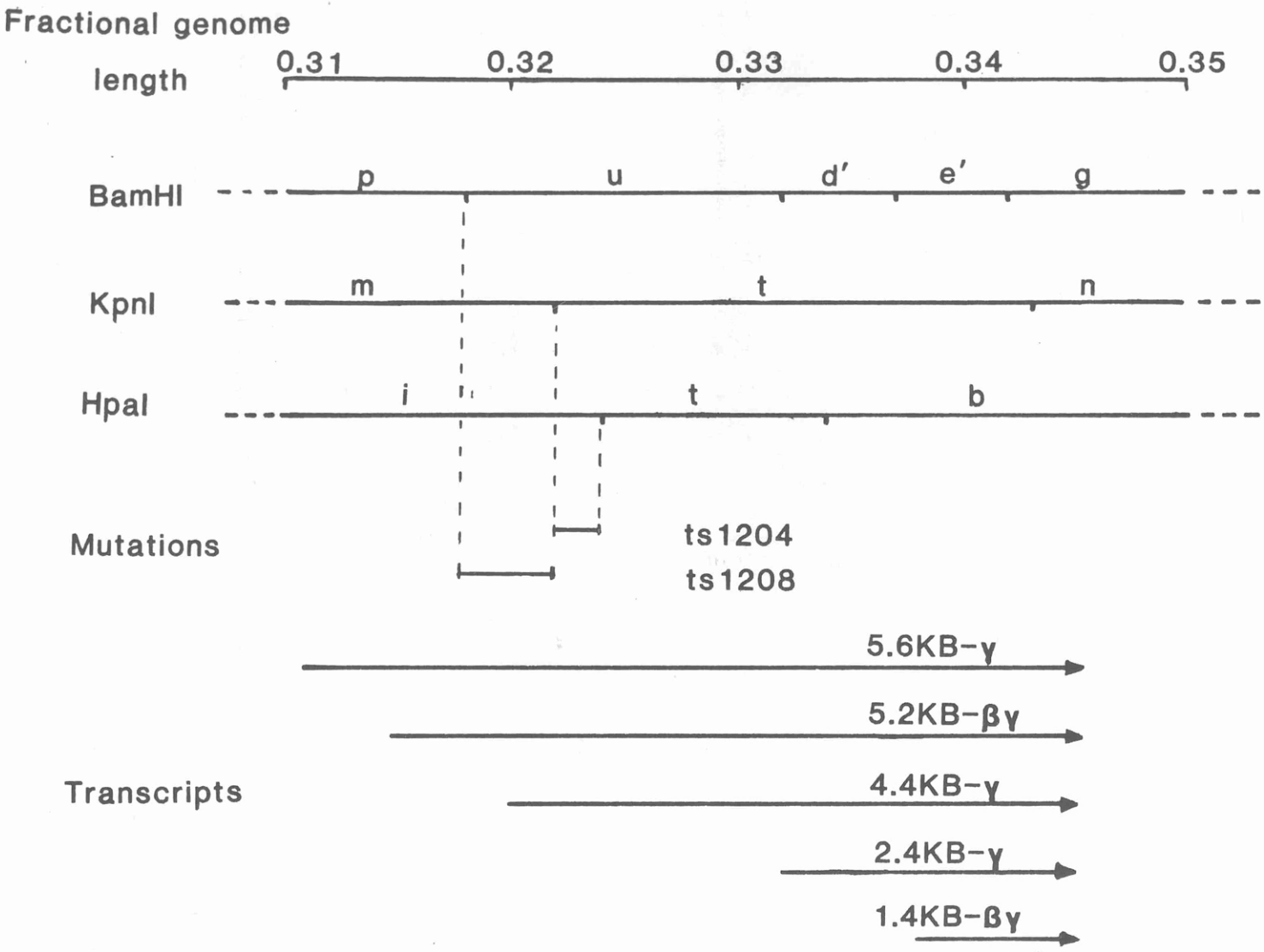

Fractional genome
length
0.31
0.32
0.33
0.34
0.35
BamHI
p
u
d'
e'
g
KpnI
m
t
n
HpaI
i
t
b
Mutations
ts1204
ts1208
Transcripts
5.6KB-γ
5.2KB-βγ
4.4KB-γ
2.4KB-γ
1.4KB-βγ

FIGURE 21

Marker rescue of ts1204 and ts1208. The solid lines represent the regions of HSV-1 DNA which rescue the mutants. Arrows indicate transcripts identified by Holland et al. (1984) which map in this region of the HSV-1 genome.

before addition to cells, about 40% of cells showed signs of infection such as margination of host chromatin and production of capsids (data not shown). A striking feature of these cells was that low numbers of capsids were seen, none of which contained DNA. To investigate the possibility that ts1204 had a second phenotypic defect, mutant virus was adsorbed for 1h at the PT instead of 38.5° to allow the virus to enter the cells. After this time the cells were transferred to 38.5° and incubation was continued for a further 9h. Electron microscopic studies of these cells showed that ts1204 appears to have a defect in assembly of functional nucleocapsids since very few capsids were produced at the NPT, and none of these capsids contained DNA (Fig. 22b).

3.2.2 Ts1204 contains a single lesion

One explanation for the finding that ts1204 had defects both in penetration of cells and in assembly of functional capsids could be that the virus contains multiple mutations. Since the ts lesion in ts1204 was rescued by a single HSV-1 DNA fragment (see Section 3.1.6) this explanation seemed unlikely, but to further exclude this possibility, ts$^+$ progeny virus was isolated from marker rescue experiments in which cells were co-infected at 31° with BamHI u and ts1204. This virus, and a ts$^+$ revertant of ts1204 (ts1204 rev-1), were tested for the ability to infect cells at the NPT. Both types of virus gave similar results to wild-type virus in indirect immunofluorescence assay using MA1147 (data not shown). In addition, the relative efficiency of plaque formation (eop) NPT/PT of the marker rescue ts$^+$ progeny virus was comparable to wild-type virus (data not shown). On the basis of these experiments, it was concluded that ts1204 contains only a single ts mutation.

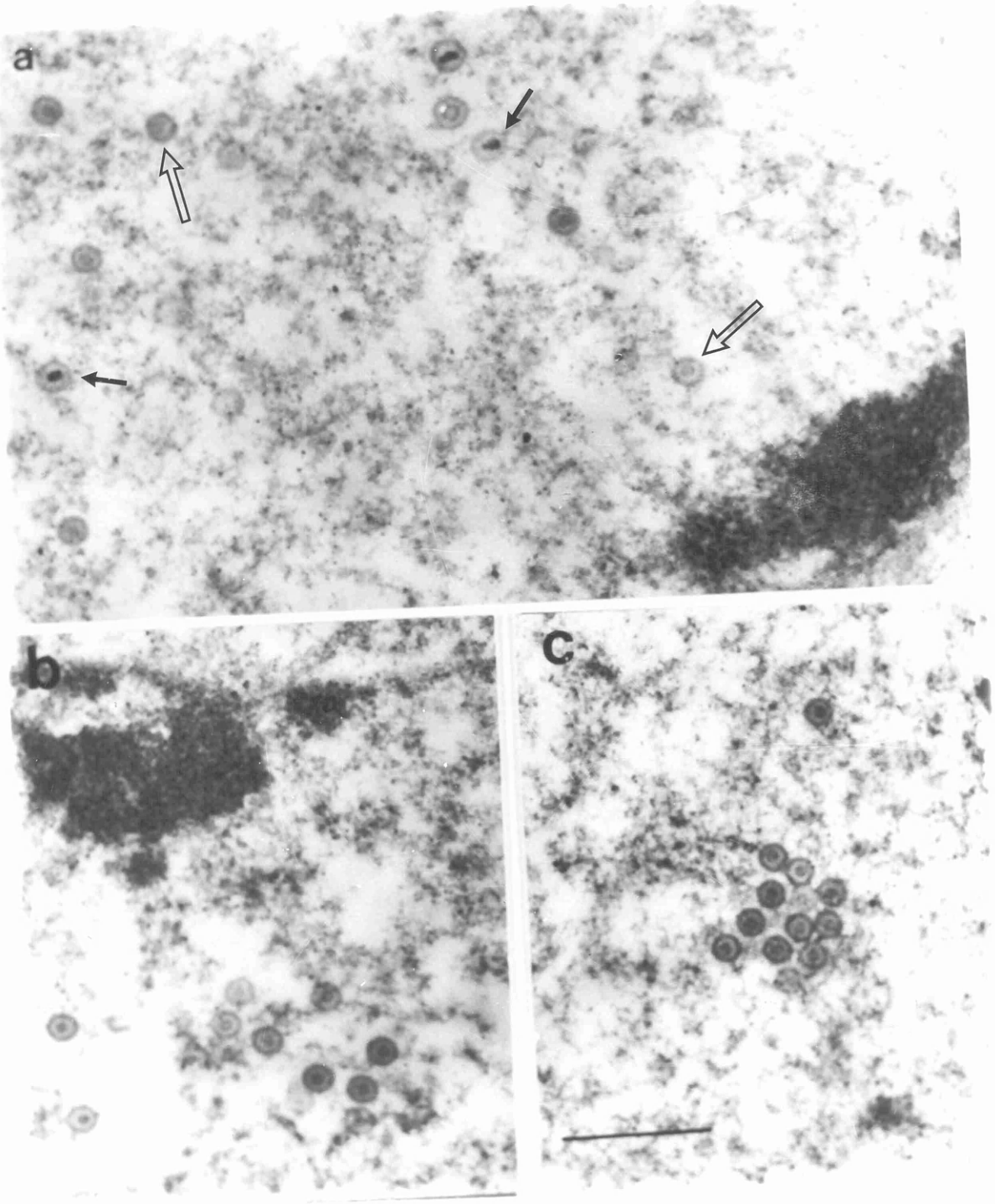

a
b
c

FIGURE 22

Electron micrographs of thin-section preparations of HFL cells infected with 5 pfu of ts1204, ts1208 or wild-type HSV-1 per cell. The NPT in these experiments was 39.5°.

(a) Nucleus of a cell infected with wild-type HSV-1. Cells were harvested after a 9h incubation at the NPT.

(b) Nucleus of a cell infected with ts1204. After 1h incubation at the PT, cells were transferred to the NPT and harvested at 9h pi.

(c) Nucleus of a cell infected with ts1208 at the NPT. Cells were harvested after 9h incubation at the NPT.

Filled arrows indicate full capsids.

Open arrows indicate empty capsids.

The bar represents 0.5uM.

TABLE 3

Complementation studies with tsl204, tsl208, tsl201 and tsA

Mutant	tsl204	tsl208	tsl201	tsA
tsl204	1	1.56	24.32	101.6
tsl208		1	42.7	88
tsl201			1	98.4
tsA				1

TABLE 3

Complementation studies with ts1204, ts1208, ts1201 and tsA.

Complementation yield tests were performed as described in Section 2.4.1. The numbers in the table represent complementation indices calculated from the formula used by Brown et al. (1973). Values greater than 4 were considered positive.

Ts1201 has a temperature-sensitive lesion which maps within DNA fragment BamHI u.

TsA has a temperature-sensitive lesion which maps in the glycoprotein gB gene (V.G. Preston, personal communication).

TABLE 4

Recombination studies with tsl204, tsl208, tsl201 and tsA

Mutant	tsl204	tsl208	tsl201	tsA
tsl204	<0.001	0.1	13.58	52.4
tsl208		<0.001	11.9	85.3
tsl201			<0.001	16.1
tsA				<0.001

TABLE 4

Recombination studies with ts1204, ts1208, ts1201 and tsA.

Recombination tests were performed as described in Section 2.4.2. The numbers in the table represent recombination frequencies which were calculated as described in Section 2.4.2.

3.2.3 Ts1208 has a lesion within the same gene as ts1204

Providing ts1204 was not prewarmed to the NPT, complementation and recombination tests could be used to determine whether the mutant represented a new complementation group within EcoRI f. Previous work by Matz et al. (1983) showed that ts1204 would complement mutants in other cistrons, including those which mapped in the glycoprotein gB gene. In this study, complementation tests revealed that a spontaneous mutant, ts1208, failed to complement ts1204, and recombined at low frequency with this mutant (Tables 3 and 4). On the basis of these results, ts1208 was placed in the same cistron as ts1204.

3.2.4 The phenotypes of ts1208 and ts1204 differ

Although ts1208 failed to complement ts1204, preliminary characterisation using indirect immunofluorescence assay revealed that, in contrast to ts1204, ts1208 did not have a penetration defect since virus prewarmed to 39.5^{o} infected all susceptible cells at the NPT (data not shown). 39.5^{o} was used as the restrictive temperature in these experiments, because ts1208 was leaky at 38.5^{o} with respect to plaque formation. The observation that the mutant virus-infected cell polypeptide profile at 39.5^{o} resembled wild-type virus-infected cell polypeptide (Fig. 23) confirmed that ts1208 was able to infect cells at the NPT. Finally, electron microscopic studies of thin-section preparations of cells infected with ts1208 at the NPT revealed the presence of capsids which had some internal structure but lacked DNA (Fig. 22). Although approximately 15% more capsids were seen in ts1208-infected cells than in ts1204-infected cells at 39.5^{o}, there were still fewer capsids than seen in wild-type virus-infected cells. Therefore, although ts1208 lies in the same cistron as ts1204, only the latter mutant is unable to penetrate cells at the NPT. Both mutants, however, fail to assemble functional nucleocapsids at this temperature.

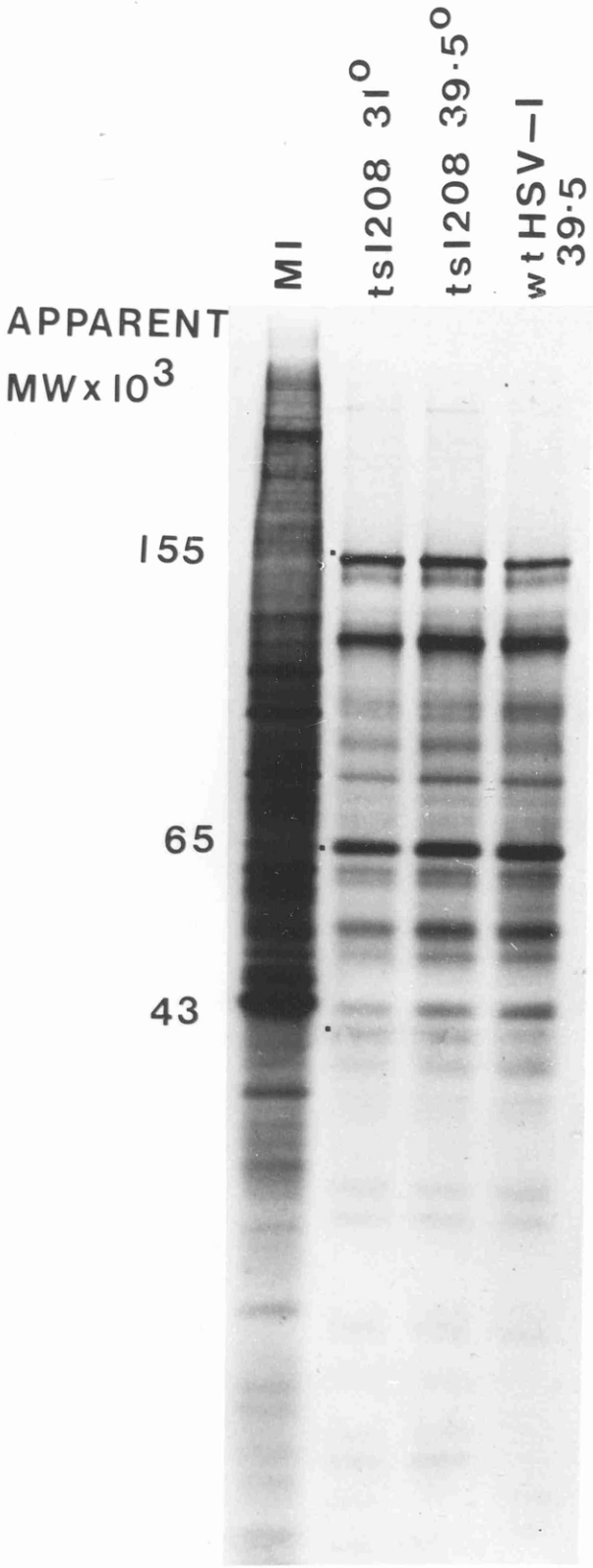

MI
tsI208 31°
tsI208 39·5°
wt HSV–I 39·5
APPARENT MW x 10³
155
65
43

FIGURE 23

Autoradiogram of polypeptides induced at the PT (31^{o}) or NPT (39.5^{o}) in HFL cells infected with wild-type HSV-1 or ts1208 or in mock-infected cells. Virus-infected cells were labelled for 30min at 6h pi with [^{35}S]-methionine and harvested. Protein samples were analysed on a 9% single concentration SDS-polyacrylamide gel.

3.2.5 Physical map location of the ts1208 mutation

The lesion in ts1208 had previously been mapped within HSV-1 DNA fragment BamHI u (V.G. Preston, personal communication). Cloned HSV-1 KpnI fragments t (pGX142), in which the ts1204 lesion had been mapped, and m (pGX134) were screened for ability to rescue the ts1208 mutation (Table 2). The lesion was found to map within fragment KpnI m. The ts1208 lesion is therefore situated to the left of the ts1204 mutation within BamHI u (Fig. 21).

3.3 CHARACTERISATION OF VIRUS POLYPEPTIDES WHICH FORM THE HSV ATTACHMENT COMPLEX

3.3.1 Ts1204 provides a novel means of determining the receptor binding (attachment) polypeptides

The ability of ts1204 to bind specifically to cellular receptors for HSV-1, thereby preventing penetration of cells by HSV-1 but not HSV-2, was utilized to determine the map location of HSV polypeptides involved in the recognition of these receptors. Several HSV-1/HSV-2 intertypic recombinants with crossovers at various points throughout the genome (Fig. 24) were used in superinfection experiments. HFL cells grown on coverslips were infected with ts1204 or tsK at 200 pfu per cell at the NPT. After 1h adsorption, the cell sheets were washed with prewarmed PBS to remove unadsorbed virus and immediately superinfected with wild-type viruses HSV-1 strain 17 or HSV-2 strain HG52, or one of four intertypic recombinants, Bx1(31-2), Dx1(34-2), R12-3 and R12-1 (Fig. 24). After a 6h incubation at the NPT, the coverslips were washed, the cells were fixed in methanol:acetone (3:1) and screened for the presence of Vmw136 using immunofluorescence assay with monoclonal antibody MA1147. Under these conditions, the ability of a recombinant virus to penetrate cells and synthesize Vmw136 will be dependent on the presence of HSV-2 envelope attachment polypeptides capable of recognising HSV-2-specific

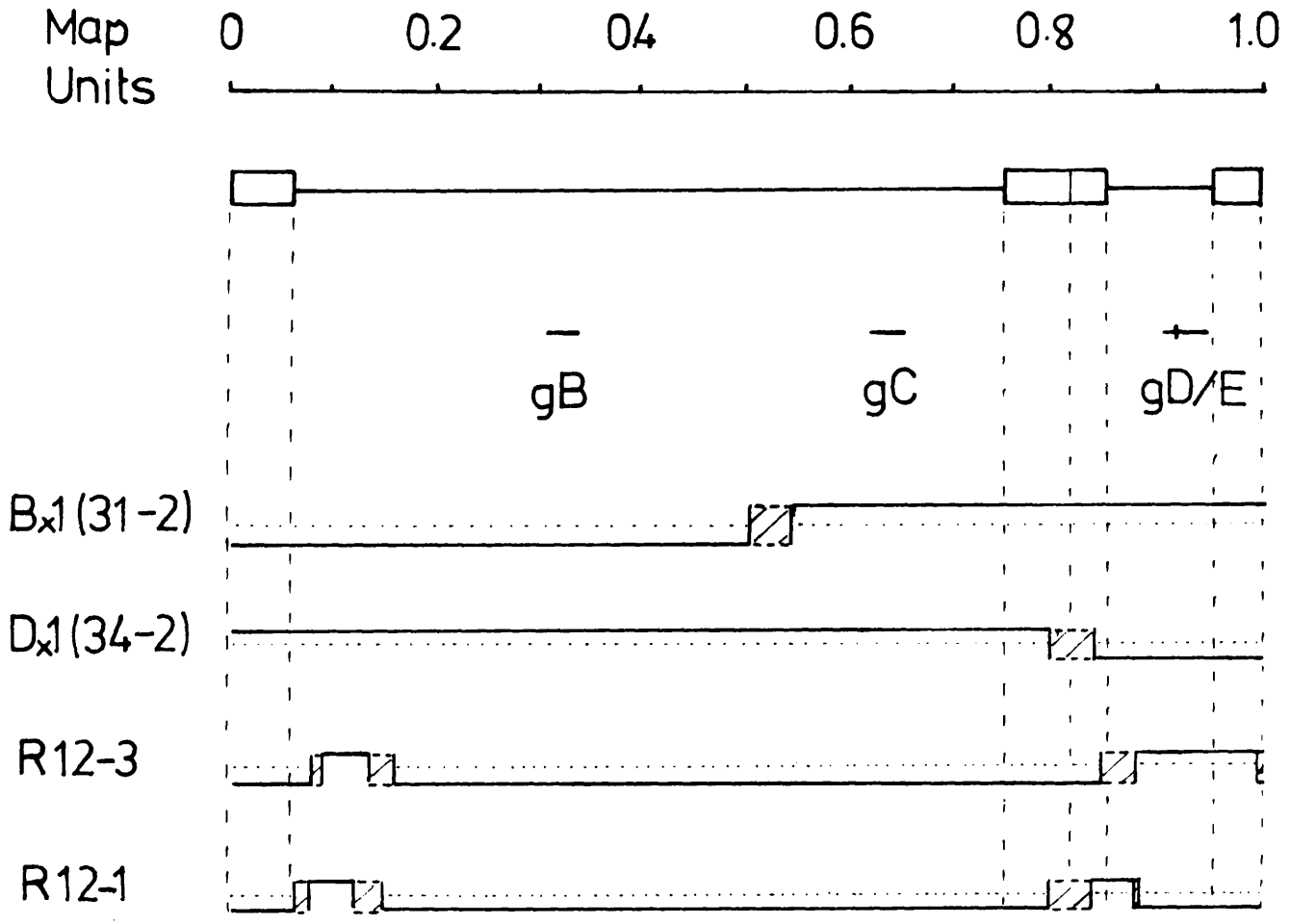

Map Units
0
0.2
0.4
0.6
0.8
1.0
gB
gC
gD/E
Bx1 (31-2)
Dx1 (34-2)
R12-3
R12-1

FIGURE 24

Summary of the genome structures of the four HSV-1/HSV-2 recombinants used in this study. The genome arrangement of HSV DNA is shown at the top of the figure. Map locations of the major HSV glycoproteins are also indicated. Vertical dotted lines correspond to the ends of the long and short repeat sequences of the HSV genome. The sequences of the recombinants derived from the HSV-1 and HSV-2 parents are represented by continuous lines above and below the dotted line respectively. Crossover regions are indicated by cross-hatched boxes.

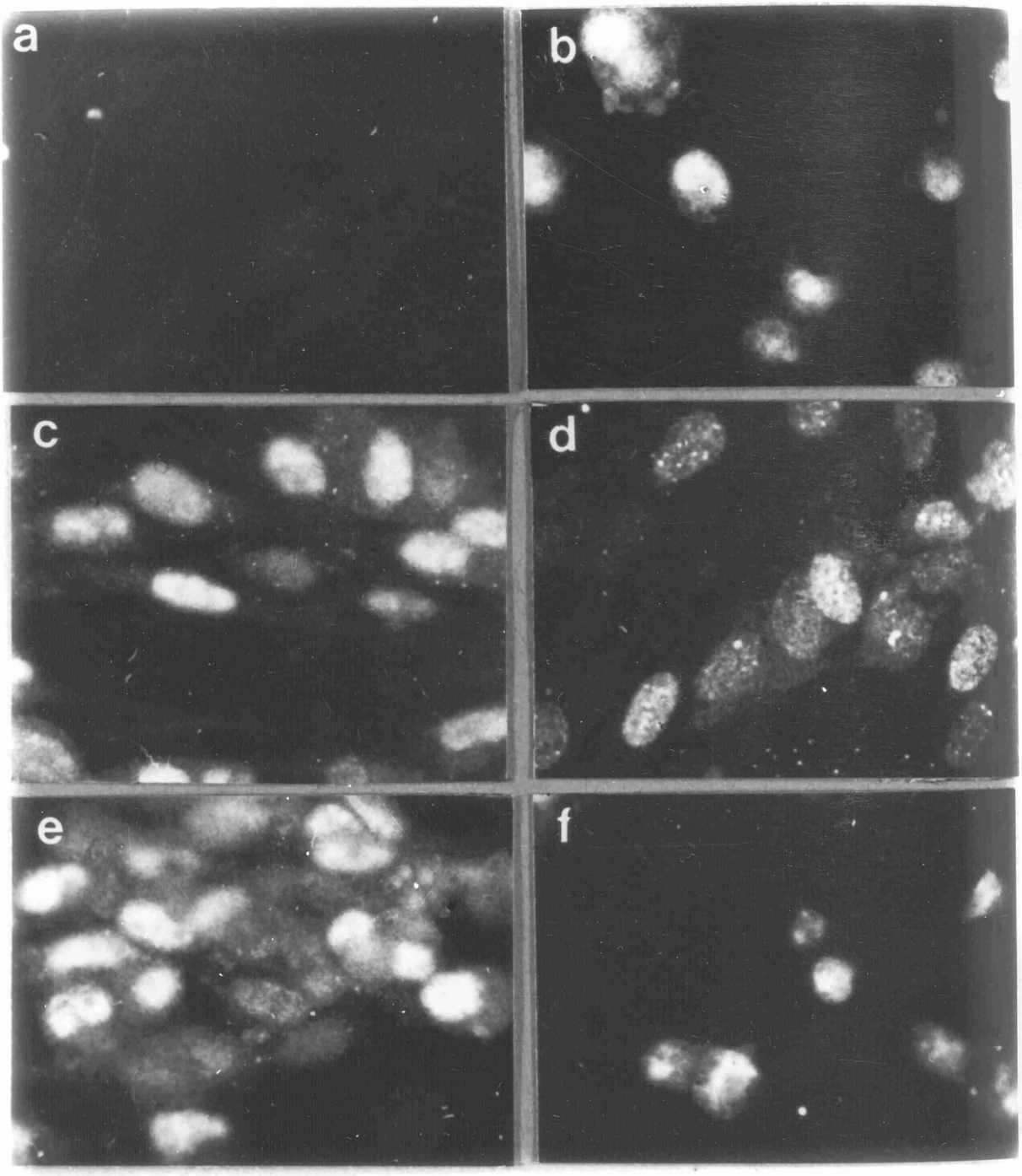

a
b
c
d
e
f

FIGURE 25

Investigation of virus attachment polypeptides, using indirect immunofluorescence assay. Monolayers of HFL cells were infected with 200 pfu prewarmed ts1204 per cell, superinfected at 1h pi with 5 pfu wild-type HSV-1, wild-type HSV-2, Bx1 (31-2), Dx1 (34-2), R12-3 or R12-1 per cell at the NPT (38.5°), harvested at 6h post superinfection and prepared for indirect immunofluorescence assay.

(a) Cells infected with ts1204 and superinfected with wild-type HSV-1.

(b) Cells infected with ts1204 and superinfected with wild-type HSV-2.

(c) Cells infected with ts1204 and superinfected with Bx1 (31-2).

(d) Cells infected with ts1204 and superinfected with Dx1 (34-2).

(e) Cells infected with ts1204 and superinfected with R12-3.

(f) Cells infected with ts1204 and superinfected with R12-1.

The monoclonal antibody used in all cases was MA1147, which was specific for the major DNA binding protein of HSV-1 and the equivalent HSV-2 polypeptide.

cellular receptors. In a series of repeat experiments, it was found that all the recombinant viruses tested had the ability to penetrate cells previously infected with 200 pfu per cell of ts1204 at the NPT, and synthesize Vmw136 (Fig. 25 c, d, e and f). Control virus, wild-type HSV-1, failed to penetrate cells previously infected with saturating amounts of ts1204 at the NPT (Fig. 25a).

3.4 DISCUSSION

The previous section describes the identification of a mutant, ts1204, which fails to penetrate tissue culture cells. Electron microscopic observations of cells infected at the NPT with a moi of 200 pfu per cell of prewarmed ts1204 revealed large numbers of enveloped particles in close association with the outside surface of the cell membrane. Since the particle:pfu ratio of this mutant is similar to that of wild-type HSV-1 (20:1), it was unlikely that the ts1204 stock contained many defective particles which were unable to penetrate the cells. Treatment of mutant virus-infected cells with PEG enabled the virus to infect cells at the NPT, indicating that ts1204 had a defect in fusion of the virus envelope with the cell membrane.

Cellular receptors for herpesviruses

The failure of ts1204 to penetrate cells appears to be independent of the cell type used since the same phenotype was observed in BHK clone 13 cells as well as in HFL cells. In addition, on the basis of superinfection experiments, ts1204 binds to cellullar receptors which are specific for HSV-1. These results are of interest when considering the idea that virus attachment to cells is mediated by plasma membrane receptors which are otherwise engaged in the normal homeostatic functions of the cell. The wide *in vitro* and *in vivo* host range of HSV indicates

either that this virus binds to a receptor which is common to many different cell types originating from different organisms, or that HSV can bind to more than one type of receptor. Interestingly, the results from this and previous studies (Vahlne et al., 1979) show that HSV-1 and HSV-2 utilize different cellular receptors, at least in the cell lines tested. It is probable that the interaction of viruses with specific cellular receptors plays an important role in determining the localisation of virus infection (tissue tropism). Support for this theory also comes from work by Vahlne et al. (1978) who showed that neuronal perikarya from rabbit, rat or mouse brain tissues contained fewer receptors for HSV than did synaptosomes. The authors speculate that the presence of receptors at the nerve terminals may facilitate initiation of HSV infection in the CNS, and that scarcity of receptors on the neurons may prevent the spread of infection between neurons, and contribute to the development of a latent infection.

Further support for the idea that tissue tropism is determined by the availability of specific cellular receptors has come from the identification of the cell surface receptor for EBV, a gammaherpesvirus. The EBV receptor is the human B-cell surface receptor CR2 which binds a cleavage product of the third component of complement, C3d (Takada and Osato, 1981; Jonsonn et al., 1982; Fingeroth et al., 1984; Wells et al., 1984). This receptor is expressed on mature human B lymophocytes, which are one of the in vivo target cells for EBV, and on certain B-cell lines which support the growth of EBV in vitro. Identification of the normal function of cellular receptors for viruses may provide clues about the mechanisms underlying different virus-cell interactions.

Saturation binding of ts1204 to HSV-1-specific cell receptors on HFL cell monolayers was obtained using a moi of 200 pfu per cell (approximately 4×10^3 particles per cell). Therefore, there appears to be roughly greater than 10^3 receptors for HSV-1 on an HFL cell in monolayer.

This figure is similar to estimates of receptors for HSV-1 and HSV-2 on several cell lines by Vahlne et al. (1979). Saturatable binding sites for other enveloped viruses, such as vesicular stomatitis virus (VSV) on Vero cells (Schlegel et al., 1982), and rabies virus on cultured cells of both neural and non-neural origin (Wunner et al., 1984), have been demonstrated (see also Lonberg-Holm et al., 1976). The demonstration that binding sites for a virus can be saturated provides additional evidence that specific cellular receptors mediate attachment of that virus to the cell surface. One interesting finding obtained during work on ts1204 was that saturating amounts of HSV-1 mutant tsK, which penetrates cells normally, did not prevent subsequent infection by wild-type HSV-1, 1h later at the NPT. This result indicates that cell surface receptors are either re-usable, or rapidly replaced. In this respect, it is noteworthy that an area of membrane equivalent to the whole cell surface can be recycled by L-cells every 125min, and by macrophages every 33min, during pinocytosis (Steinman et al., 1976).

Virus attachment proteins

Adsorption of HSV onto the cell surface is a temperature-independent process (Farnham and Newton, 1959; Duyckinck-Smith and de Harven, 1974). The adsorption profiles for HSV-1 and ts1204 in this study support the above findings since the kinetics of adsorption for these viruses were similar at 4^{o} and 38.5^{o}. The HSV envelope glycoproteins are thought to represent virus attachment proteins. Such proteins which mediate recognition of the cellular receptor and subsequent binding of the virus to the cell surface have been the subject of extensive investigation. Fuller and Spear (1985) demonstrated that polyclonal and monoclonal antibodies, specific for gD and gC of HSV-1, were effective in the inhibition of virus adsorption to HEp-2 cells. In addition, Johnson et al. (1984) showed that

binding of lipid vesicles containing HSV-1 glycoproteins (virosomes) to cells was inhibited by polyclonal and monoclonal antibodies to gB. Taken together, these results indicate that HSV glycoproteins form an attachment complex in the virus envelope, and suggest that stable attachment of the virus to the cell surface may be a result of multiple interactions of several glycoproteins with the cellular receptor(s).

This idea was supported by the results obtained from receptor mapping experiments in this thesis. These experiments were designed on the basis that the ability of a virus to penetrate cells previously infected with saturating amounts of ts1204 at the NPT will depend on the presence of HSV-2 attachment proteins in the virus envelope, such that HSV-2-specific cellular receptors can be utilized.

As shown in figure 24, each of the recombinants induced at least one HSV-2 glycoprotein. R12-3 contained sequences encoding gB-2, gC-2, gD-1 and gE-1. Bx1(31-2) contained sequences encoding gB-2, gC-1, gD-1 and gE-1. Dx1(34-2) contained sequences encoding gD-2, gB-2, gC-1 and gE-1. R12-1 contained sequences encoding gB-2, gC-2, gD-2 and gE-2. Thus, no HSV-2 DNA sequences were common to all four recombinant viruses, although the possibility that any of the recombinants contained undetected crossovers cannot be ruled out. All the recombinant viruses were capable of penetrating cells previously infected with ts1204 at the NPT. Provided the recombinants did not contain undetected crossovers, this result indicates that recognition of, and successful binding to, a cellular receptor can be achieved by a virion attachment complex which is composed of a mixture of HSV-1 and HSV-2 glycoproteins. This preliminary conclusion could be unequivocally confirmed by recombining cloned HSV-2 glycoprotein genes, singly and in combination, into wild-type HSV-1, thus eliminating the possibility of undetected crossovers at other glycoprotein loci. The resulting recombinants could then be tested for the

ability to utilize HSV-2-specific cellular receptors.

Membrane fusion

Glycoprotein gB plays a centrol role in the fusion of the HSV envelope and cell membrane. Ts mutants of HSV-1, tsB5 and tsJ12, which have lesions in the gB gene, produce progeny virions at the NPT which possess gB-deficient envelopes. These progeny virions are unable to penetrate cells (Sarmiento et al., 1979; Little et al., 1981). Glycoprotein gD may also be involved in the fusion process. Fuller and Spear (1985) reported that polyclonal or monoclonal anti-gD antibodies which exhibited potent virus neutralising activity were ineffective at blocking virus adsorption. However, these antibodies inhibited HSV-induced cell-cell fusion (Noble et al., 1983; Fuller and Spear, 1985). It is possible, therefore, that some anti-gD antibodies affect virus neutralisation by preventing membrane fusion required for virus penetration.

In this study, all the intertypic recombinants tested for ability to utilize HSV-2-specific cellular receptors contained either HSV-2 glycoprotein gB or gD in their envelopes, and all the recombinants were successful in penetrating the cell surface. On the basis of this data, it can be postulated that the virus polypeptides involved in the fusion process can act independently of the cellular receptor being used. In this case, an HSV-2 envelope glycoprotein could mediate fusion at an HSV-1-specific cellular receptor and vice versa. Alternatively, if the virus fusion polypeptides can act only at a homologous cell receptor, then this data suggests that either gD and gB alone can mediate the fusion process, provided that these glycoproteins play a direct role in penetration.

Glycoproteins have been identified as the fusion proteins in other enveloped viruses, for example, the F protein of paramyxoviruses and the HA protein of orthomyxoviruses. Analyses of these glycoproteins have

given clues about the mechanism of the virus envelope-cell membrane fusion process. Briefly, cleavage of the fusion glycoprotein exposes hydrophobic regions (Hsu et al., 1981; Skehel et al., 1982) which are likely to be important in the fusion process. Maeda et al. (1981) showed that influenza virus can fuse with artificial liposomes which are devoid of proteins or sialic acid-bearing cellular receptors. Therefore, it seems that the fusion process is a direct result of interactions between the fusion peptide and cell membrane lipids. Enveloped viruses require such fusion proteins in order to elevate the low fusion incidence normally found between membranes. This is because there is a repulsive force which opposes the close approach of hydrated polar surfaces (Parsegian et al., 1979). Doms et al. (1985) suggest that the influenza virus HA and presumably other virus fusion glycoproteins overcome this repulsion by integrating into the target membrane, and mechanically bringing the virus envelope and cell membrane close enough so that direct interaction between lipid molecules in both membranes becomes possible.

Alterations in cellular macromolecular synthesis

Analysis of virus-induced polypeptides in ts1204-infected cells grown at the NPT showed that the pattern of polypeptide synthesis in these cells resembled that of mock-infected cells, except for two observations: First, in ts1204-infected cells, some inhibition of host polypeptide synthesis was seen. Although a virion polypeptide has been implicated in this process (Fenwick and Walker, 1978; see Section 1.3.3), it is unclear whether in this case the inhibition was caused by a structural component of the virion, or was induced as a consequence of alterations to the cellular membrane brought about by binding of ts1204 virions to cell surface receptors. Attachment of HSV to the cell membrane results in a decrease in cell surface protein mobility, an event which is presumably a

consequence of crosslinking of receptor proteins which interact with the cytoskeleton (Gall and Edelman, 1981; Rosenthal et al., 1984). It is not known whether such disturbance of the cytoskeleton could affect cellular protein synthesis.

Secondly, several host polypeptide bands are increased in intensity in ts1204-infected cell polypeptide profiles compared to the mock-infected cell profile, the most major one being a protein of approximately 57,000 MW. This observation indicated that the expression of some cellular genes had been upregulated as a consequence of virions binding to the cell surface. Increases in the synthesis of specific cellular proteins in response to herpesvirus infection have previously been described and it is possible that the proteins induced in ts1204-infected HFL cells at the NPT may correspond to some of these. Infection of chick embryo fibroblast cells at the NPT with HSV-1 mutant tsK results in the overproduction of IE polypeptides. In addition, four major cellular stress proteins are synthesized, having apparent MWs of 90K, 70K, 35K and 25K (Notarianni and Preston, 1982). However, since synthesis of these proteins requires both an inactive Vmw175 and overproduction of the other IE polypeptides (C.M. Preston, personal communication), it is unlikely that they are analogous to any of the upregulated cellular polypeptides seen in ts1204-infected cells. Perhaps of greater interest is the observation that the synthesis of several cellular polypeptides is increased following HSV-2 infection of HFL cells (LaThangue et al., 1984). Monoclonal antibodies specific for some of these polypeptides are available, and obviously provide a means whereby any homologous polypeptides induced in ts1204-infected cells could be detected. Superinfection of lymphoblastoid cells with EBV also results in enhanced production of cellular proteins and, interestingly, one of these has an apparent MW of 53,000 (Casareale et al., 1984). Again, antisera raised against this polypeptide is available and it should be

possible to investigate whether this protein is analogous to the 57,000 MW polypeptide induced in ts1204-infected HFL cells. Finally, it is noteworthy that treatment of cells with interferon results in the upregulation of cellular genes (Friedman et al., 1984; Faltynek et al., 1985), one of which codes for a polypeptide of apparent MW 56,000 (Chebath et al., 1983; Faltynek et al., 1985). It would be of great interest to determine whether the synthesis of this interferon-induced polypeptide is also upregulated following binding of HSV virions to the cell membrane. Alternative approaches to the investigation of upregulated genes in ts1204-infected cells are discussed later.

Capsid assembly defect of ts1204 and ts1208

Provided ts1204 was allowed to enter cells by a brief incubation at the PT before temperature shift-up to the NPT, a second phenotype defect became evident. In comparison with wild-type HSV-1-infected cells, fewer capsids were produced and none of these contained DNA. Another mutant, ts1208, which failed to complement ts1204, penetrated cells normally at the NPT. However, this mutant also assembled fewer capsids than wild-type HSV-1 at the NPT and, like ts1204, failed to package DNA. In this respect, ts1204 and ts1208 differed from unrelated mutants ts1201 and ts1203 (see Section 4) which made considerably more capsids at the restrictive temperature than wild-type virus.

Analyses of ts^+ virus, generated by marker rescue of ts1204 with cloned DNA fragment BamHI u from wild-type virus, and a ts^+ revertant of ts1204, suggest that ts1204 has a single mutation that affects the ability of the mutant both to penetrate cells and assemble functional nucleocapsids at the NPT. Marker rescue and recombination experiments indicated that the ts1204 and ts1208 lesions are in close proximity. Thus, both mutations might lie in a multifunctional gene, a possibility which

poses the question of how one polypeptide might be involved in two apparently unrelated events in the virus replication cycle. The defect in penetration of cells indicates that the ts polypeptide is a component of the infecting virion, for example, an envelope fusion glycoprotein. However, it is difficult to envisage how such a polypeptide would be required for assembly of nucleocapsids. Therefore, it might be postulated that the ts gene product is a component of the tegument which interacts both with the virus envelope and the nucleocapsid in a manner analogous to the M (matrix) protein of influenza virus (Compans et al., 1970; Gregoriades, 1980). In the infecting virion this polypeptide might function to anchor envelope glycoproteins in the correct receptor-binding complex formation. In this case, in ts1204 virions at the NPT, the altered configuration of this polypeptide would result in a disturbance in the envelope attachment complex, such that the correct interaction between virus envelope proteins and cell membrane components is prevented and fusion cannot occur. The mechanism whereby a tegument polypeptide is involved in capsid assembly requires further consideration. The site of HSV capsid assembly is thought to be the nuclear matrix (see Section 1.7.1). A similar situation exists for the assembly of icosahedral bacteriophage T4 capsids at the bacterial cell inner membrane. A T4 gene product has been described which is involved in the initiation of capsid assembly at the bacterial membrane, and appears to anchor the maturing capsids to the membrane (Onorato et al., 1978; Showe and Onarato, 1978; Black and Showe, 1983). A similar role might be envisaged for the ts gene product in ts1204 and ts1208-infected cells. Assuming that this polypeptide controls a rate-limiting capsid assembly initiation step, the process might be slowed at the NPT, thereby accounting for the low numbers of capsids seen. Alternatively, the affected gene product might be a tegument polypeptide involved in maintenance of capsid stability. In this case, at the NPT in ts1204- and

ts1208-infected cells capsid structures may be assembled but be unstable, such that few complete shells are seen at any time.

It is also feasible that ts1204 might contain a single mutation within the coding regions of two overlapping genes and, as a consequence, have ts defects in two gene products. In this situation, ts1208 would have a mutation affecting the function of one of these genes only. It is noteworthy that genes with overlapping coding regions have been described in the U_S region of the HSV-1 genome (McGeoch et al., 1985).

Holland et al. (1984) have mapped five mRNAs within the DNA fragment BamHI u. Three of these, 5.6-, 5.2- and 4.4- kb mRNAs hybridise to the region of BamHI u where the ts1204 and ts1208 lesions lie (Fig. 21). Further investigation is required to determine the mRNA encoding the polypeptide(s) which is ts in ts1204- and ts1208- infected cells. On the basis of results from in vitro translation experiments, Millette and Clevland (personal communication) have identified several polypeptides in this region with apparent MWs of 62,000, 51,000, 37,000 and 27,000. As previously stated, it is probable that the ts1204 mutation lies within a structural gene. However, the possibility that incorrect processing of polypeptides at 31^{o} caused a structural polypeptide to become thermolabile cannot be excluded.

It is of interest to note that several polypeptides with known or suspected roles in the process of membrane fusion map near to the ts1204 mutation. Two glycoproteins, gB and gH (see Section 1.2.2) map to the right and left of the ts1204 lesion respectively. Although the involvement of gB in fusion of virus envelope and cell membrane has been clearly documented, the role of gH in the adsorption or penetration processes is purely speculative. Mutants of HSV-1 strain 17 syn^{+} which have deletions in the 5' regulatory and coding regions of the TK gene have a syncytial plaque morphology (see Section 1.10.2). These deletions map within DNA

sequences encoding the 5' regions of the 5.6- and possibly the 5.2- kb mRNAs identified by Holland et al. (1984) (Fig. 21). The significance, if any, of this grouping of genes involved in membrane fusion events is at present obscure.

3.5 FUTURE PROSPECTS

Unequivocal demonstration that the ts1204 phenotype results from a single mutation can be achieved by cloning the DNA sequence which contains the ts1204 lesion, and recombining this fragment into wild-type virus. Any ts progeny virus isolated from such an experiment should be phenotypically identical to ts1204. An altered phenotype, i.e. loss of the penetration defect or the capsid assembly defect, would indicate that ts1204 contained more than one lesion.

It would be of great interest to identify the gene product which is ts in ts1204-infected cells. Marker rescue experiments have localised the ts1204 mutation to a 400bp region in DNA fragment BamHI u. Cloning and sequencing of this fragment will enable the precise bp change of the ts1204 mutation to be determined. Assignment of the mutation to a specific gene should be possible from DNA sequence analysis, and the amino acid sequence of the polypeptide which is ts in ts1204-infected cells can then be deduced. Analysis of the amino acid sequence may provide clues as to the nature of the protein and its possible location in the virion. The next step would be the synthesis of oligopeptides based on the amino acid sequence of the polypeptide. Antisera raised against these peptides can be used to immunoprecipitate the inferred gene product from wild-type virus-infected cell extracts. Successful use of this technique has already enabled Frame et al. (1985a, 1985b) to identify several gene products whose presence was inferred by the DNA sequence of the U_S region of the HSV-1 genome.

Using antisera specific to the polypeptide, it should be possible to locate the polypeptide in the virus-infected cell and to determine whether it is a component of the virion.

Investigation of the upregulated cellular genes in ts1204-infected cells is already underway. Synthesis of the 57,000 MW cellular protein may be cell-dependent since the polypeptide is detectable in several human fibroblast cell lines infected with ts1204 at the NPT, but is undetectable in ts1204-infected BHK cells or CV-1 cells (V.G. Preston, personal communication). Investigation of whether any of the upregulated cellular genes in ts1204-infected cells correspond to those seen in HSV-2-infected HFL cells or interferon-treated cells will be of great interest.

CHAPTER 4

4.1 THE CHARACTERISATION OF TS1201 AND TS1203, TWO TS MUTANTS WITH DEFECTS IN ENCAPSIDATION OF VIRUS DNA

4.1.1 Introduction

The pathway of herpesvirus DNA encapsidation has not yet been characterised in any great detail. The genetic and biochemical analyses of PRV mutants have provided valuable information about virus DNA packaging. Ts mutants of PRV which synthesize normal amounts of virus DNA at the NPT have been assigned to nine complementation groups (Ladin et al., 1980). Members of six of these cistrons produced capsids at the NPT, but failed to package DNA. Viruses in the remaining three complementation groups did not assemble capsids or cleave concatemeric virus DNA into unit size. These results indicated that the process of DNA encapsidation was complex, and that capsid assembly and DNA maturation were genetically linked (Ladin et al., 1980).

At the start of this study, only one HSV-1 strain 17 ts mutant, ts1201, which produced capsids and virus DNA but was unable to encapsidate DNA at the NPT, had been identified (Preston et al., 1983). The following section describes the characterisation of a new HSV-1 strain 17 mutant, ts1203, which, like ts1201, has a defect in packaging virus DNA into capsids at the restrictive temperature. In addition, observations made on ts1201 have been extended, and comparisons between the two mutants have been made wherever possible. The study was undertaken in an attempt to elucidate the mechanism of HSV DNA encapsidation.

4.1.2 Phenotypic analysis of ts1203 using electron microscopy

Electron microscopic examination of thin-section preparations of ts1203-infected cells grown at the NPT revealed that the mutant assembled large numbers of capsids which contained some internal structure but

lacked the dense cores present in capsids containing mature virion DNA (compare Figs. 26a and 26c). In this respect, ts1203 resembled ts1201 which also assembled many intermediate-type capsids at the restrictive temperature (Preston et al., 1983). However, whereas ts1203 capsids were spread randomly throughout the nuclei (Fig. 26c), ts1201 capsids were grouped in large crystalline arrays, usually positioned at the nuclear membrane (Fig. 26b). It was estimated that both ts1201 and ts1203 assembled 20% more capsids than wild-type HSV-1 at this temperature. A difference in core structure was also apparent. The cores in ts1201 capsids appeared to be less compact than those in ts1203 capsids (compare Figs. 26b and 26c). Since both these types of electron-translucent core structure are seen in wild-type virus capsids during a normal infection (Fig. 26a), it seemed likely that the ts1201 and ts1203 lesions affected different steps in the pathway of DNA encapsidation. Electron microscopic observations suggested that the lesion carried by ts1203 also had some effect on the efficiency of DNA encapsidation by this mutant at the PT. Ts1203 assembled similar numbers of capsids to wild-type virus at the PT, however, only approximately 12% of ts1203 capsids contained DNA compared with 30% of wild-type virus capsids. No significant differences in the numbers of full capsids in ts1201- and wild-type virus-infected cells could be detected at the PT.

The defect in DNA encapsidation observed in ts1203-infected cells was irreversible upon temperature downshift at 9h pi, from the NPT to the PT, in the presence of cycloheximide to prevent further protein synthesis. After a 4h incubation at the PT, no full capsids could be detected (Fig. 26d). In contrast, the ts1201 defect was reversible under the same temperature shiftdown conditions: the large crystalline arrays of ts1201 capsids disaggregated, the uniform core structure was lost, virus DNA was packaged and enveloped virions were produced (Preston et al., 1983; see

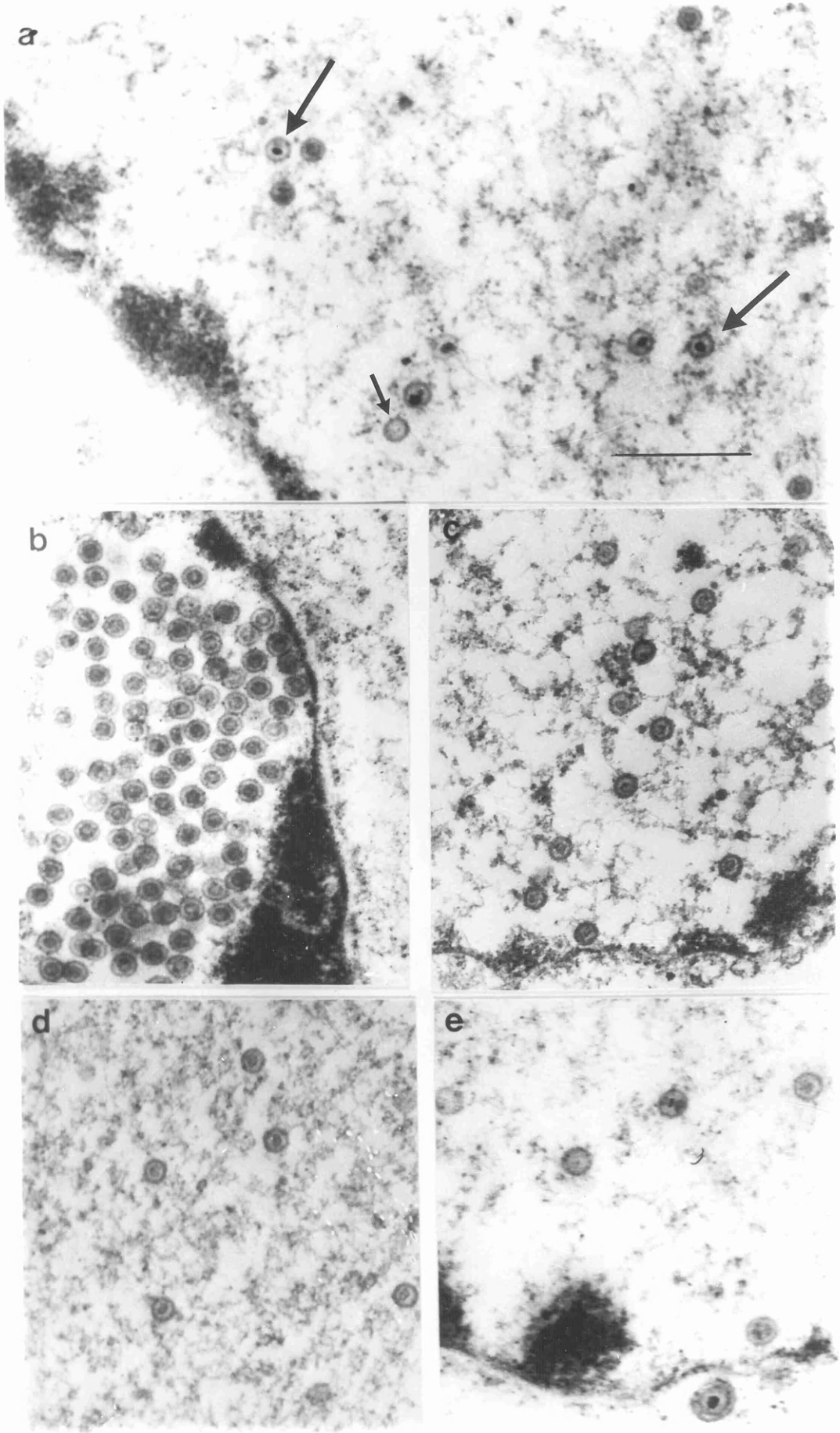

a
b
c
d
e

FIGURE 26

Electron micrographs of thin-section preparations of HFL cells infected with 10 pfu of wild-type HSV-1, ts1201 or ts1203 per cell at the NPT (38.5^{o}).

(a) Nucleus of a cell infected with wild-type HSV-1. Cells were harvested after a 10h incubation at the NPT.

(b) Nucleus of a cell infected with ts1201. Cells were harvested after a 10h incubation at the NPT.

(c) Nucleus of a cell infected with ts1203. Cells were harvested after a 10h incubation at the NPT.

(d) Nucleus of a cell infected with ts1203 at the NPT. After 9h incubation at this temperature, cycloheximide was added, cells were transferred to the PT and harvested at 12h pi.

(e) Nucleus of a cell infected with ts1201 at the NPT. After 9h incubation at the NPT, cycloheximide was added, cells were transferred to the PT and harvested at 12h pi.

Long arrows indicate full capsids. Short arrows represent empty or intermediate capsids. The bar represents 0.5uM.

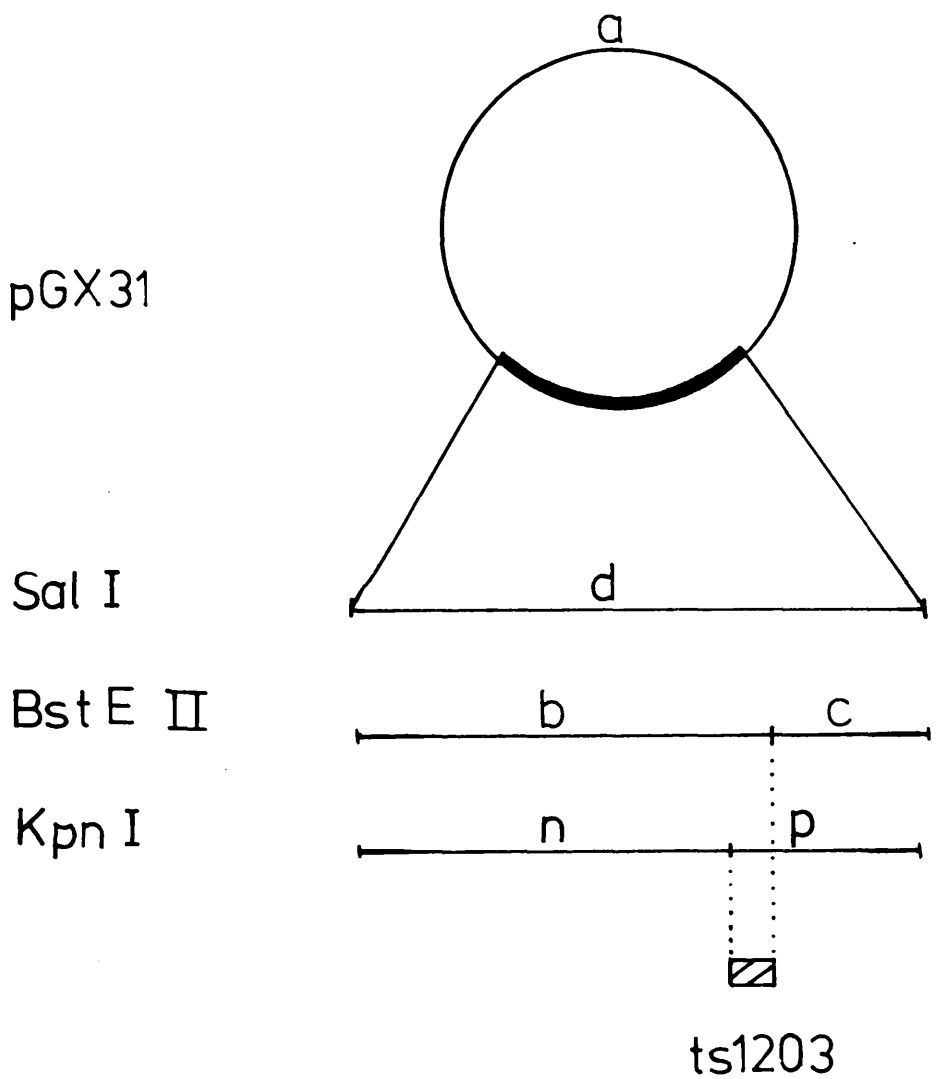

pGX31
a
Sal I
d
BstE II
b
c
Kpn I
n
p
ts1203

FIGURE 27

Position of the ts1203 mutation within HSV-1 DNA fragment SalI d, defined by marker rescue experiments. Fragments SalI d, KpnI n and KpnI p are indicated by the letters d, n and p respectively. Plasmid sequences (pAT153) are indicated by the letter a. The large (3200bp) SalI-BstEII fragment and the small (800bp) SalI-BstEII fragment are indicated by the letters b and c respectively. The position of the ts1203 mutation is represented by the hatched box.

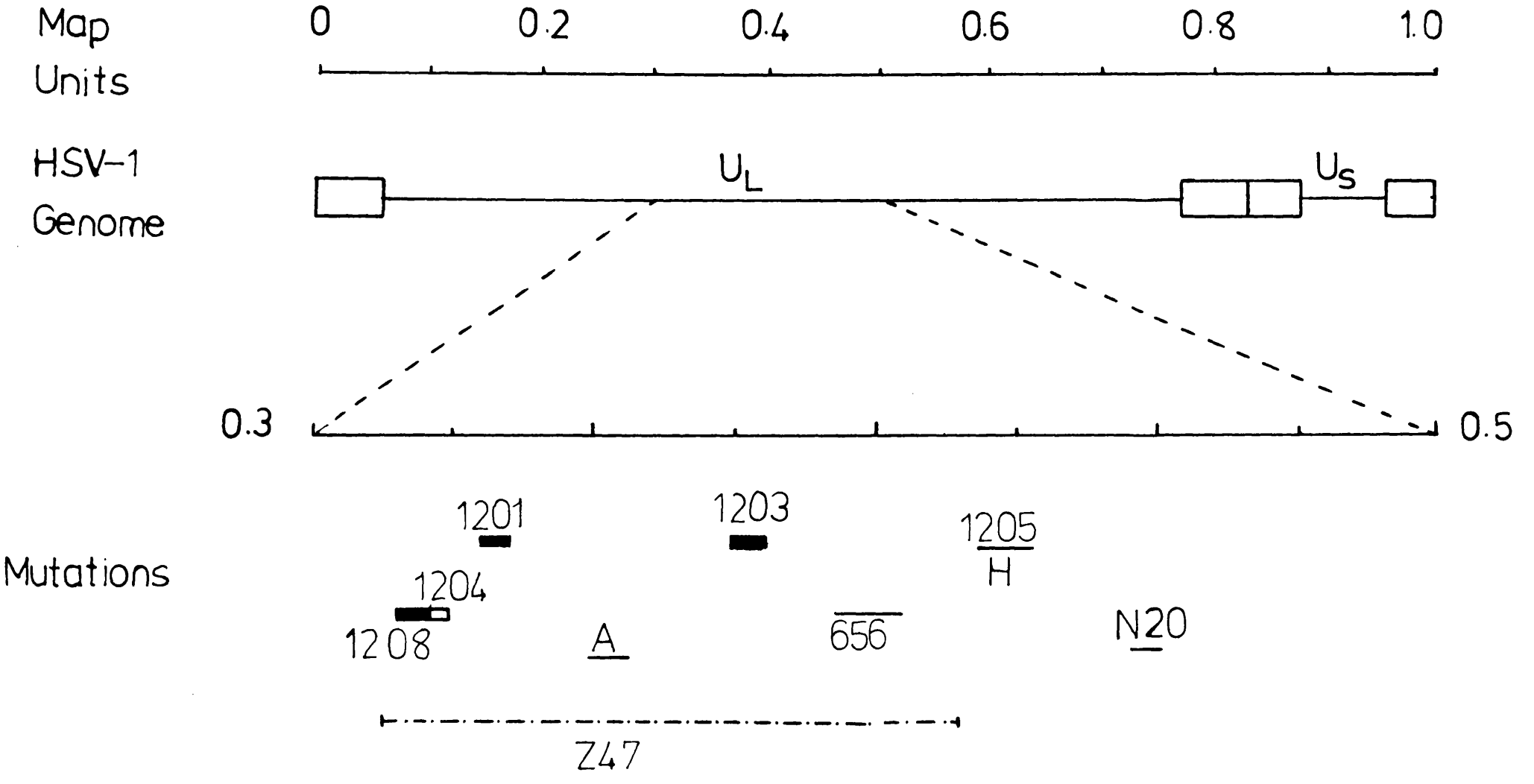

Map Units
0
0.2
0.4
0.6
0.8
1.0
HSV-1 Genome
U_L
U_S
0.3
0.5
Mutations
1201
1203
1205
H
1204
1208
A
656
N20
Z47

FIGURE 28

Genome locations of the mutations of ts1201, ts1203, ts1204, ts1208 and other relevant HSV-1 ts mutants. Details of the genes represented by these mutants, and citations for their isolation and mapping, are given in the text and in Table 7.

TABLE 5

Summary of marker rescue results for ts1203

HSV-1 DNA fragment	eop NPT/PT ($\times 10^{-3}$)	Rescue
None	<0.001	-
BamHI g	0.57	+
KpnI (m + a)	<0.001	-
n	<0.001	-
p	3.2	+
t	<0.001	-
v	<0.001	-
SalI d	1.0	+
SalI a	<0.001	-
b	3.4	+
c	<0.001	-

TABLE 5

Summary of marker rescue results for ts1203.

Cloned EcoRI f, digested with KpnI, gave five fragments: KpnI (m + a) which contained sequences from KpnI m, a and pACYC184, and KpnI n, p, t and v. Cloned SalI d, digested with SalI plus BstEII, gave three fragments: a which contained sequences from pAT153, b which contained sequences from the large (3200bp) SalI-BstEII fragment, and c which contained sequences from the small (800bp) SalI-BstEII fragment. All these fragments were purified and used in marker rescue experiments. Plasmids containing the other DNA fragments were digested with the appropriate restriction endonuclease prior to transfection.

The relative efficiency of plating (eop NPT/PT) was calculated from the yield of progeny virus at the PT and NPT from the transfected cells.

Fig. 26e).

4.1.3 Physical map location of the ts1203 lesion

The ts1203 lesion was mapped by intratypic marker rescue. Experiments using separated KpnI fragments from cloned EcoRI f (plasmid pGX38) showed that the ts1203 lesion mapped within fragment KpnI p (Table 5). This map location was further refined using a cloned SalI fragment (plasmid pGX31), designated in this study SalI d, which mapped entirely within EcoRI f and rescued the ts1203 lesion (Dr. V.G. Preston, personal communication). SalI d was cleaved with restriction endonucleases SalI and BstEII. The resulting fragments of 3556bp (plasmid vector pAT153), 3200bp (large SalI-BstEII fragment), and 800bp (small SalI-BstEII fragment) were screened for the ability to rescue the ts1203 mutation (Fig. 27, Table 5). Since the mutation was rescued by the large (3200bp) SalI-BstEII fragment, the ts1203 lesion must map within a region of approximately 450bp defined by KpnI and BstEII sites at mu 0.377-0.380 respectively (Fig. 27). The ts1201 lesion had previously been mapped to a region of approximately 640bp within the HSV-1 DNA fragment BamHI u (0.330mu) (Preston et al., 1983). Thus, the ts1203 lesion maps to the right of the ts1201 lesion within DNA fragment EcoRI f. The map positions of these two mutations are shown in Fig. 28.

4.1.4 Ts1203 represents a new complementation group within DNA fragment EcoRI f

Complementation experiments between ts1203 and other ts mutants which map in DNA fragment EcoRI f were performed to determine whether ts1203 belonged to an existing complementation group. Details of the mutants used are given in Table 6, and Table 7 shows the results from a representative complementation experiment. Taking complementation indices of greater than 4 as positive, mutant ts1203 is complemented by

TABLE 6

Mutant	Complementation Group	Gene represented	Reference
tsZ47	1-33	Unknown Defect in insertion of glycoproteins into cell membrane (mu 0.315-0.415)	Pancake et al. (1983) Mutant obtained from P.A. Schaffer
ts656	1-1	Major DNA binding protein (Vmw136)	Hughes and Munyon (1975) Mutant obtained from P.A. Schaffer
tsH	1-3* 1-4	DNA polymerase	Stow and Wilkie (1978)
ts1205	1-3* 1-4	DNA polymerase	V.G. Preston, personal communication
tsA	1-9	Glycoproteins gB (mu 0.35-0.40)	Stow and Wilkie (1978) V.G. Preston, personal communication

TABLE 6

Details of ts mutants used in complementation experiments with ts1203. Complementation groups are as defined by P.A. Schaffer (personal communication).

*Ts DNA polymerase mutants of HSV-1 strain 17 fail to complement mutants in either group 1-3 or 1-4 and in our laboratory mutants in groups 1-3 and 1-4 fail to complement each other (V.G. Preston, personal communication).

TABLE 7

Complementation between ts1203 and other ts mutants with lesions in HSV-1 DNA fragment EcoRI f

Mutant	1201	1203	Z47	656	H	A	1205
1201	1	2116	89	402	90.4	33	14.19
1203		1	22.4	111.8	39.6	10.1	24.7
Z47			1	80.36	36.4	27.1	20
656				1	13.6	15.9	21.4
H					1	11.5	1.27
A						1	13.8
1205							1

TABLE 7

Complementation studies with ts1203 and other ts mutants with lesions which map in HSV-1 DNA fragment EcoRI f. Details of the ts mutants used in this test are given in Table 6. Complementation yield tests were performed as described in Section 2.4.1. The values in the table represent complementation indices calculated from the formula used by Brown et al. (1973). Values greater than four were considered positive.

TsH and ts1205 each contain a mutation within the virus DNA polymerase gene (complementation group 1-3/1-4) and serve as negative controls in this experiment.

The results are representative of two such complementation experiments.

all the other mutants tested. Thus, ts1203 appears to represent a novel complementation group within EcoRI f.

4.1.5 DNA processing by ts1203 and ts1201

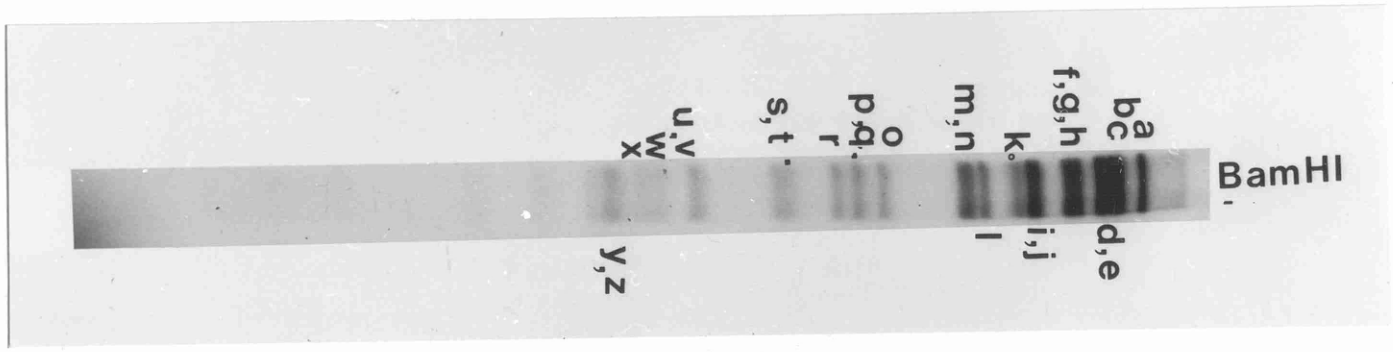
Electron microscopic observations on thin-section preparations of ts1203- and ts1201-infected cells revealed that mutant virus-infected cells grown at the restrictive temperature did not contain dense cored capsids or mature virions. In order to confirm that virus DNA was not encapsidated at this temperature, and to examine whether the unpackaged DNA was cleaved or not, virus DNA processing in ts1203- and ts1201-infected cells was investigated using the technique of Southern blotting as described below.

Total virus-infected cell DNA and encapsidated DNA (DNase-resistant DNA) were prepared from HFL cells infected with wild-type virus, ts1203 or ts1201 at the required temperatures (see Sections 2.3.2 and 2.3.3; Stow et al., 1983). These DNAs were cleaved with BamHI which cleaves within both the short and long repeat sequences of the HSV genome (Fig. 29b). The resulting fragments were separated on an agarose gel (Fig. 29a) and transferred to a nitrocellulose membrane. The terminal (s and q) and joint-spanning (k) fragments in the samples were identified by hybridisation to a [^{32}P]-labelled BamHI k fragment probe (pGX2). The amounts of virus DNA cleavage and encapsidation were determined by densitometric analysis of autoradiographs of the [^{32}P]-labelled bands representing joint-spanning and terminal fragments.

4.1.5a Rationale for the densitometric quantitation of cleavage of virus DNA

Quantitation of the joint-spanning fragment, BamHI k, gives a representation of the total amount of virus DNA in the infected cell, i.e. both concatemeric and unit-length DNA, whereas quantitation of the

A.


BamHI
a
b,c
f,g,h
k
m,n
o
p,q
r
s,t
u,v
w
x
y,z
l
i,j
d,e

B.

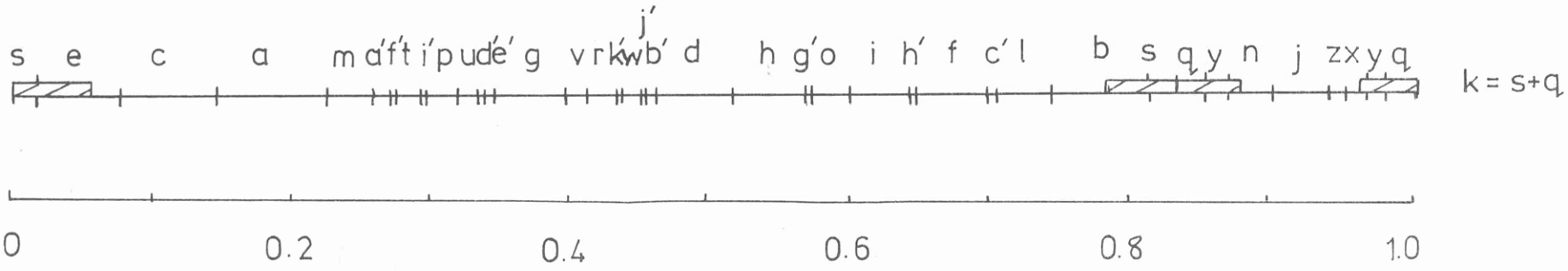

s e c a m a' f' t i' p u d é' g v r ḱ w b' j' d h g' o i h' f c' l b s q y n j z x y q
k = s+q
0
0.2
0.4
0.6
0.8
1.0

FIGURE 29

The physical map of HSV-1 for restriction endonuclease BamHI, and an autoradiogram of the corresponding BamHI restriction digest pattern of [^{32}P]-labelled HSV-1 DNA.

(a) [^{32}P]-labelled DNA samples were digested with BamHI and separated on 1.0% agarose gels. The letters refer to specific HSV-1 DNA fragments, the physical map locations of which are shown in (b). Autoradiogram was supplied by Dr. V.G. Preston.

(b) Physical map of HSV-1 DNA for restriction endonuclease BamHI was taken from Davison (1981). The composition of the joint fragment is given beside the map.

terminal fragments, BamHI q and s, gives an estimate of unit-length DNA only. Theoretically, since BamHI cleaves within the repeat sequences of the HSV genome, fragments k, q and s should each be 1 molar in a BamHI digest of unit-length (encapsidated) DNA (Fig. 29). However, in this study, the BamHI k probe fragment was treated with DNAase I prior to nick translation, and therefore the amount of hybridisation of the probe to restriction endonuclease digest fragments should be proportional to both the size and molarity of the fragment.

Analysis of wild-type virus encapsidated DNA revealed that the formula k = q + s, where k, q and s represent the intensity of bands BamHI k, q and s respectively, was valid. It follows that the proportion of 'endless' DNA which was cleaved to unit lengths can be calculated from the following formula:

$$\text{\% total virus DNA cleaved} = \frac{q + s}{k} \times 100$$

It should be noted that, for the purpose of these quantitations, it has been assumed that all the terminal fragments detected have arisen as a result of site-specific cleavage of concatemeric DNA prior to or during DNA encapsidation.

4.1.5b Analysis of total virus-infected cell DNA

The proportion of 'endless' virus DNA which had been cleaved into genome length DNA was estimated from the analysis of total virus-infected cell DNA. A representative autoradiograph of a Southern blot of BamHI digested total virus DNA is shown in Fig. 30. Densitometric analysis of the proportion of BamHI k, q and s present in each sample is shown in Table 8. The results show that at the PT, although wild-type virus, ts1201 and ts1203 all synthesized similar amounts of DNA, the amount of DNA processed differed for each virus. Approximately 48% of wild-type virus

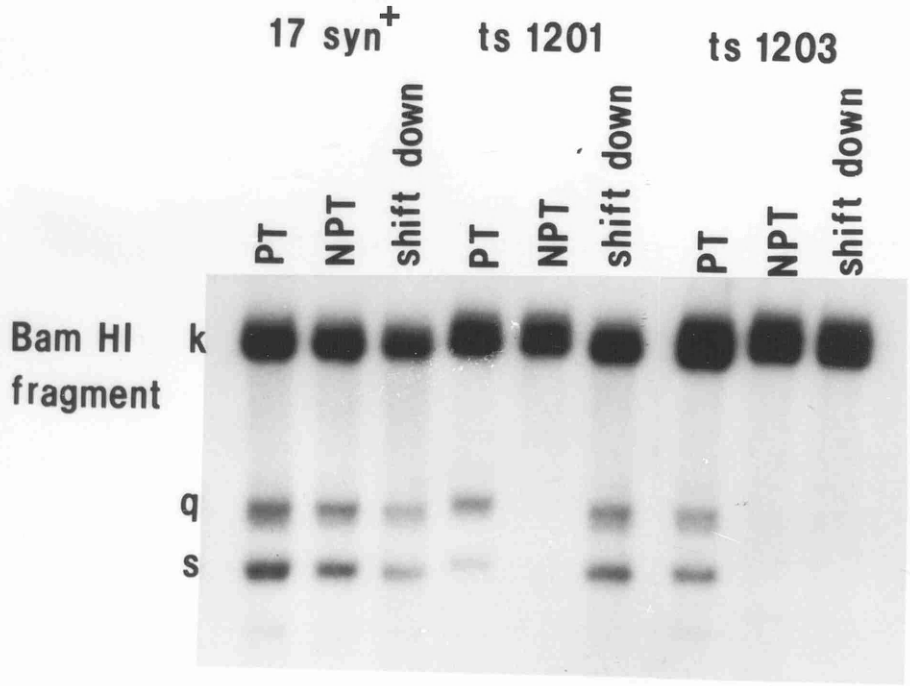

17 syn+
ts 1201
ts 1203
PT
NPT
shift down
PT
NPT
shift down
PT
NPT
shift down
Bam HI fragment
k
q
s

FIGURE 30

Autoradiogram of the analysis of total virus-infected cell DNA. Unlabelled DNA was prepared from HFL cells which had been infected with a moi of 5 pfu of wild-type HSV-1, ts1201 or ts1203 per cell as described in Section 2.3.2. Virus-infected cells were incubated at the PT (31^{o}) or NPT (38.5^{o}) or were shifted down from the NPT to the PT in the presence of cycloheximide at 10h pi prior to harvesting at 18h pi. One-tenth of the sample from a 50mm petri dish was cleaved with BamHI and the resulting fragments were separated by electrophoresis through a 0.8% agarose gel. The fragments were transferred to a nitrocellulose sheet and hybridised to *in vitro* labelled [^{32}P]-pGX2 DNA (contains HSV-1 fragment BamHI *k*).

TABLE 8

Densitometric analysis of total virus-infected cell DNA

(a) Permissive temperature

Virus	wt HSV-1	ts1201	ts1203
Amount of DNA synthesized	100.00*	110.27*	100.32*
	100.00	80.27	119.77
	100.00	194.52	99.40
Proportion of 'endless' DNA cleaved to unit-length genomes	48.63*	39.90*	9.92*
	58.97	35.91	18.63
	31.41	29.91	13.28

(b) Non-permissive temperature

Virus	wt HSV-1	ts1201	ts1203
Amount of DNA synthesized	100.00*	124.44*	72.21*
	100.00	99.30	129.32
	100.00	115.40	121.20
Proportion of 'endless' DNA cleaved to unit-length genomes	36.00*	<1.00*	<1.00*
	40.50	2.25	<1.00
	20.06	<1.00	<1.00

(c) Temperature downshift

Virus	wt HSV-1	ts1201	ts1203
Amount of DNA synthesized	100.00	142.82	159.10
	100.00 (100)	121.01 (131.91)	97.59 (128.45)
Proportion of 'endless' DNA cleaved to unit-length genomes	44.73	22.71	<1.00
	43.54 (44.13)	39.88 (31.29)	<1.00 (<1.00)

TABLE 8

Densitometric analysis of Southern blots of total virus-infected cell DNA. The amount of DNA synthesized by each mutant (represented by the amount of [^{32}P]-labelled probe which hybridised to the joint fragment BamHI k) was expressed as a percentage of the amount of DNA synthesized by wt HSV-1. The proportion of 'endless' DNA which had been cleaved to unit-length genomes by each virus was calculated as described in Section 4.1.5a. In the case of samples incubated at the PT or NPT, the results of three experiments are given and the case of temperature downshift samples, the results of two experiments are given. The figures in brackets represent the average of these results. Cells were harvested at 18h pi.

* These samples were not treated with cycloheximide from 10-18h pi.

(a) Analysis of total virus-infected cell DNA at the PT.

(b) Analysis of total virus-infected cell DNA at the NPT.

(c) Analysis of total virus-infected cell DNA after temperature downshift from the NPT to the PT at 10h pi.

DNA and 39% of ts1201 DNA had been cleaved into unit length genomes by 18h pi, whereas only 9% of ts1203 DNA had been processed under the same conditions. These results were consistent in repeated experiments.

At the NPT, all three viruses again synthesized similar amounts of DNA. Cleavage of wild-type DNA was less efficient at 38.5° than at 31°, with approximately 36% of total virus DNA in the form of unit length genomes at the NPT, compared with 48% at the PT (Fig. 30, Table 8). However, <1% of total ts1201 and ts1203 DNA was cleaved at the NPT. This result indicates that the mutant DNAs were 'frozen' in the form of circles or concatemers, but does not distinguish between the two possibilities. On longer exposure of the blots to film, terminal BamHI fragments q and s could be detected, and these bands may represent a background level of cleavage of both mutant DNAs.

Experiments in which virus-infected cells were shifted from the NPT to the PT at 10h pi in the presence of cycloheximide and incubated for a further 8h revealed that approximately 44% of wild-type virus DNA and 31% of ts1201 DNA was cleaved. However, a corresponding increase in the cleavage of total ts1203-infected cell DNA was not seen. These results confirm electron microscopic observations that the ts1203 defect is irreversible in the absence of protein synthesis, in contrast to the defect of ts1201 which is fully reversible under these conditions.

4.1.5c Analysis of encapsidated (DNase-resistant) DNA

Hybridisation of the probe to BamHI digested DNase-resistant DNA gives an estimate of the amount of genomic DNA which was encapsidated. Figure 31 shows a representative autoradiogram of a Southern blot of BamHI digested encapsidated DNA. Table 9 shows the densitometric analysis of BamHI k, q and s in each sample. These results confirm the findings from the analysis of total virus-infected cell DNA.

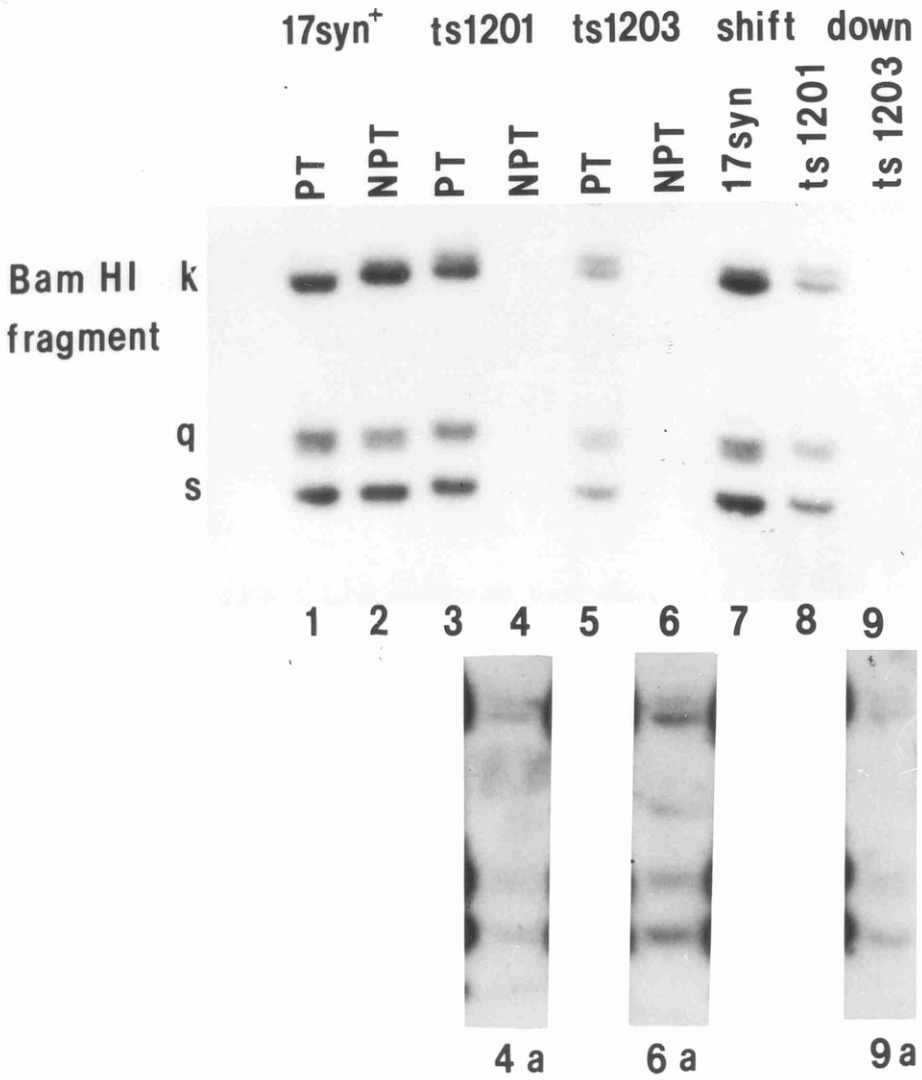

17syn+
ts1201
ts1203
shift down
PT
NPT
PT
NPT
PT
NPT
17syn
ts 1201
ts 1203
Bam HI k
fragment
q
s
1 2 3 4 5 6 7 8 9
4 a
6 a
9 a

FIGURE 31

Autoradiogram of the analysis of encapsidated (DNase-resistant) DNA. Unlabelled DNase-resistant DNA was prepared from HFL cells infected with wild-type HSV-1, ts1201 or ts1203 (moi 5 pfu per cell) as described in Section 2.3.3). Virus-infected cells were incubated at the PT (31^{o}) or NPT (38.5^{o}) or were shifted down from the NPT to the PT in the presence of cycloheximide at 10h pi prior to harvesting at 18h pi. One-twentieth of the sample from a 50mm petri dish was cleaved with BamHI, and the resulting fragments were separated by electrophoresis through a 0.8% agarose gel. The fragments were transferred to a nitrocellulose sheet and hybridised to *in vitro* labelled [^{32}P]-pGX2 DNA (contains HSV-1 fragment BamHI k). Tracks 4a, 6a and 9a are longer exposures of tracks 4, 6 and 9 respectively.

TABLE 9

Densitometric analysis of encapsidated DNA

(a) Permissive temperature

Virus	wt HSV-1	ts1201	ts1203
Amount of encapsidated DNA	100.00* 100.00	84.89* 115.91	17.34* 34.37

(b) Non-permissive temperature

Virus	wt HSV-1	ts1201	ts1203
Amount of encapsidated DNA	100.00* 100.00	<1.00* <1.00	<1.00* <1.00

(c) Temperature downshift

Virus	wt HSV-1	ts1201	ts1203
Amount of encapsidated DNA	100.00 100.00 (100)	49.24 54.44 (51.84))	<1.00 <1.00 (<1.00)

TABLE 9

Densitometric analysis of Southern blots of encapsidated (DNase-resistant) DNA. The amount of DNA encapsidated by each mutant (represented by the amount of [^{32}P]-labelled probe hybridised to fragment BamHI k) was expressed as a percentage of the DNA encapsidated by wt HSV-1. The results of two experiments are given in each case. All samples were harvested at 18h pi.

* These samples were not treated with cycloheximide from 10-18h pi.

(a) Amount of DNA encapsidated at the PT.

(b) Amount of DNA encapsidated at the NPT.

(c) Amount of DNA encapsidated after temperature downshift from the NPT to the PT at 10h pi.

At the PT, a similar amount of virus DNA was encapsidated in wild-type virus and ts1201-infected cells, whereas the amount of DNA packaged in ts1203-infected cells was only 17% of that seen in wild-type virus-infected cells.

At the NPT, the amount of DNA encapsidated by both ts1201 and ts1203 was <1% of the DNA packaged by wild-type virus (Fig. 31, Table 9). On longer exposure of the blots to film, background levels of encapsidation were detected for both mutants (Fig. 31, tracks 49, 6a and 9a). On temperature downshift of virus-infected cells from 38.5° to 31° at 10h pi in the presence of cycloheximide, the amount of virus DNA encapsidated in ts1201-infected cells after an 8h incubation at the PT was approximately 52% of that seen in wild-type virus-infected cells. However, no increase in the amount of DNA packaged in ts1203-infected cells was seen, confirming that the defect of this mutant is irreversible in the absence of protein synthesis.

A faint band which migrated slightly faster than fragment BamHI s was often noted during these experiments. Since the BamHI k probe has a high (G+C) content, this band may have arisen as a result of spurious hybridisation between the probe and a DNA fragment which also has a high (G+C) content.

4.1.6 Processing of p40 in ts1203-infected cells

Previously, Preston et al. (1983) showed that ts1201 was unable to process p40 correctly to its lower MW forms at the NPT, and suggested that this defect might be linked to the failure of ts1201 to encapsidate DNA. Since ts1203 also fails to package DNA at the NPT, it was of interest to investigate processing of p40 in cells infected with this mutant.

Immunoprecipitation experiments were performed using a monoclonal antibody MA5010B, which was specific for p40. Virus-infected cells,

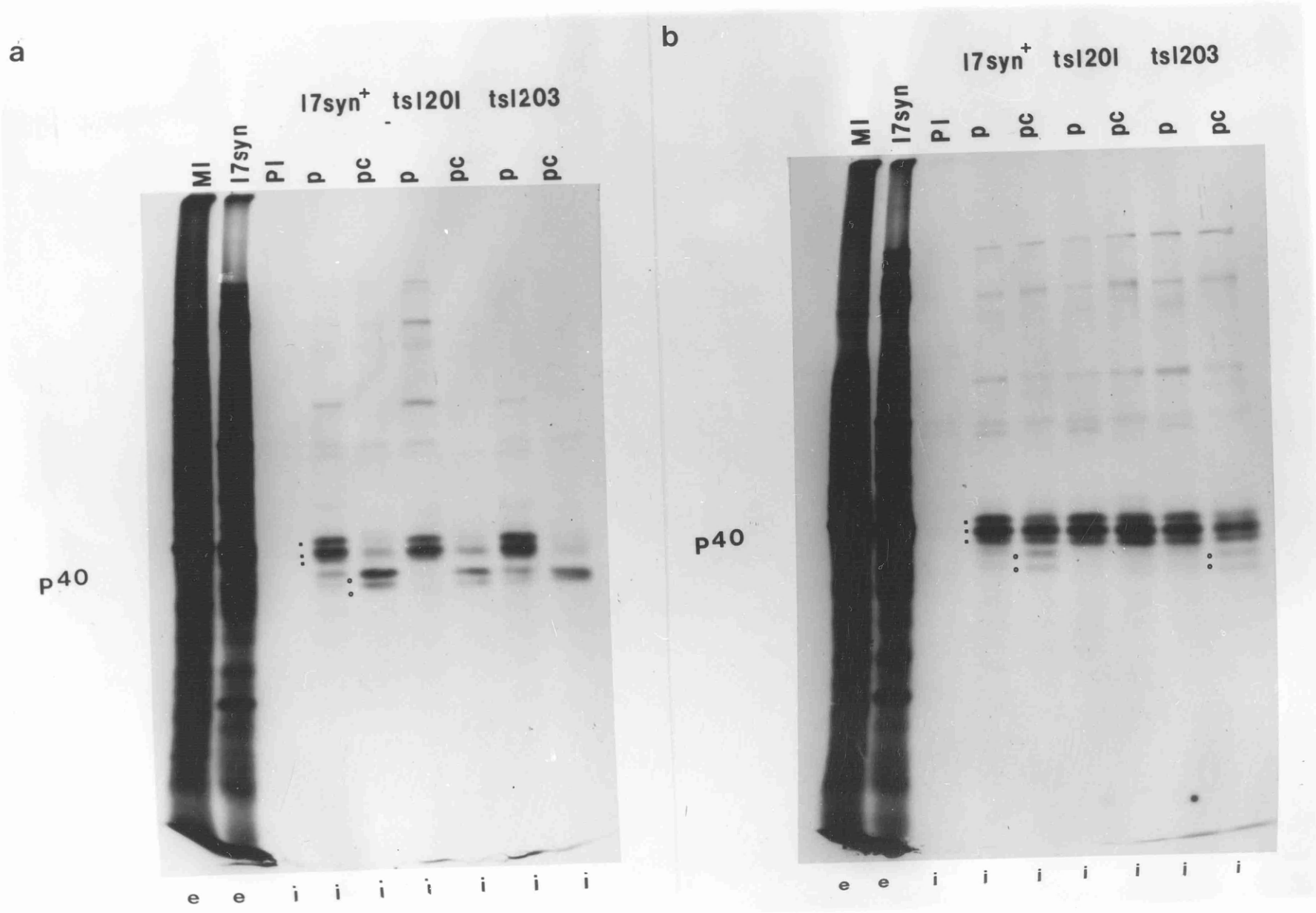

a
MI
17syn
PI
17syn+
ts1201
ts1203
p
pc
p40
e e i i i i i i i
b
MI
17syn
PI
17syn+
ts1201
ts1203
p
pc
p40
e e i i i i i i i

FIGURE 32

Autoradiograms of immune precipitates of p40 from HFL cells infected at (A) 31° and (B) 38.5° with wild-type HSV-1, ts1201 or ts1203. Virus-infected cell polypeptides were pulse-labelled with [^{35}S]-methionine at 5h pi for 30min. Cells were either harvested immediately, or after incubation for a further 5h in EF10 containing cycloheximide. Immunoprecipitation was performed as described in Section 2.7.7, using MA5010B which was specific for p40 of HSV-1. Pre-immune samples were wild-type virus-infected cell extracts which were incubated with control non-immune rabbit serum in place of MA5010B. The immunoprecipitated polypeptides were analysed on an 8% SDS polyacrylamide gel.

p =	pulse-labelled
pc =	pulse-labelled followed by chase period
PI =	pre-immune sample
e =	mock-infected or wild-type virus-infected cell extract
i =	immunoprecipitate
filled squares =	high MW forms of p40
open circles =	lower MW forms of p40

incubated at the PT or NPT, were pulse-labelled with [^{35}S]-methionine for 30min at 5h pi as described in Section 2.7.3. The cells were either harvested immediately or after a 5h 'chase' period in the absence of [^{35}]-methionine in medium containing cycloheximide to prevent further protein synthesis. The results of the immunoprecipitation experiment are shown in Fig. 32. This data demonstrates that both ts1203 and ts1201 process p40 correctly to its lower MW forms at the PT (Fig. 32a). At least five virus-specific polpeptides were resolved, ranging in MW from approximately 35,000 to 45,000. At the NPT, ts1203 behaved as wild-type virus, producing all forms of p40 (Fig. 32b). This is in contrast to ts1201, which failed to process p40 correctly to its lower MW forms at the restrictive temperature (Fig. 32b). One explanation for this finding is that ts1203 is defective in a later stage of DNA encapsidation than ts1201. In addition, this result indicates that processing of p40 must represent only one stage in packaging of virus DNA.

4.1.7 The following two experiments were performed to investigate why p40 was not processed to its lower MW forms in ts1201-infected cells at the NPT:

4.1.7a Phosphorylation of p40

Previous reports had demonstrated that p40 synthesized in cells infected with HSV-1 strains MAL (Heilman, 1982) and F (Braun et al., 1984b) was phosphorylated. However, it is noteworthy that the extent of p40 phosphorylation detected by Braun et al. (1984b) was variable. Since it was possible that phosphorylation of p40 is a pre-requisite to cleavage, an experiment was performed to determine whether or not the high MW forms of p40 synthesized in ts1201-infected cells at the NPT were phosphorylated. Wild-type virus- and ts1201-infected cell polypeptides were

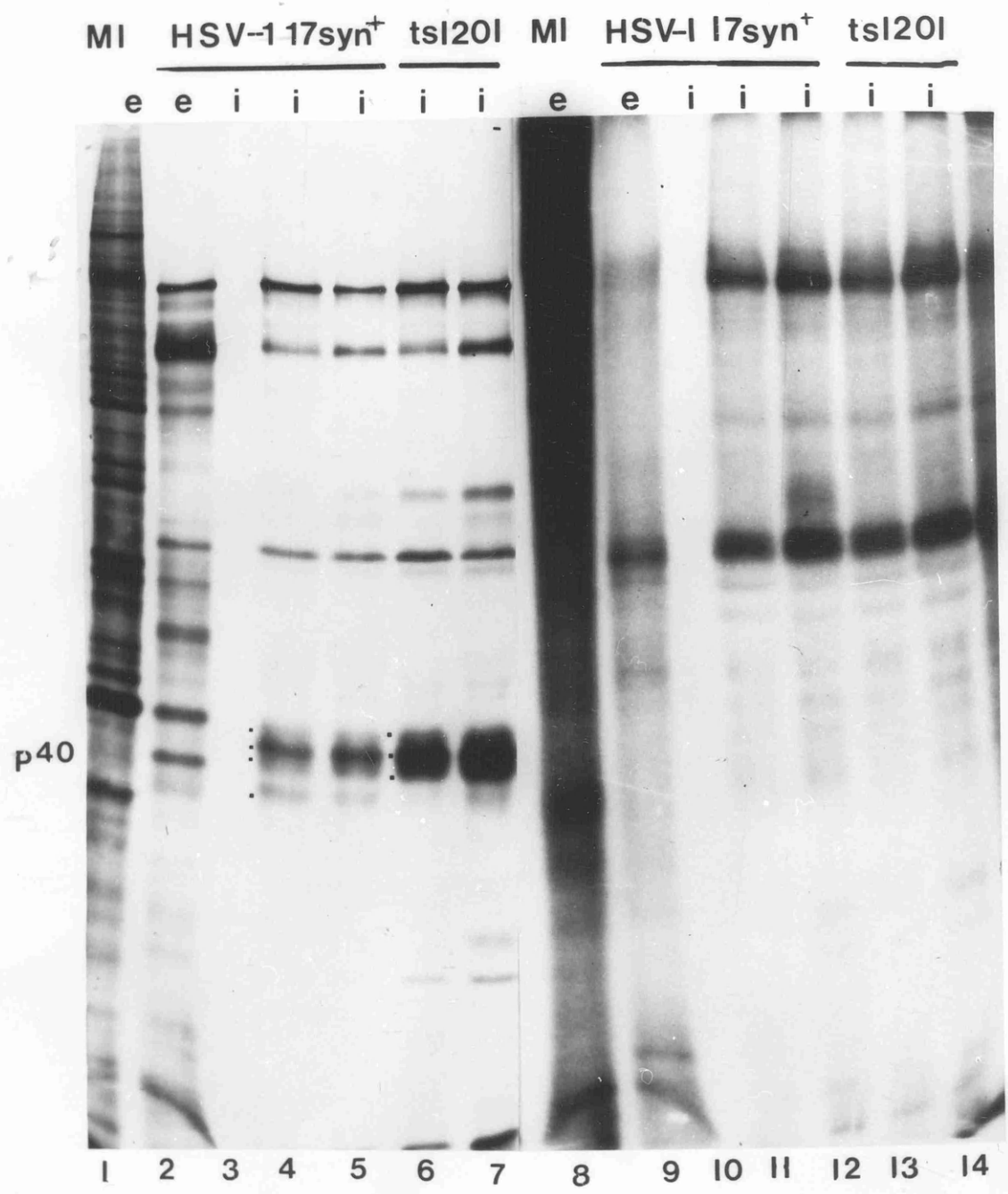

MI
HSV-1 17syn+
tsI201
MI
HSV-I I7syn+
tsI201
e e i i i i i e e i i i i i
p40
1 2 3 4 5 6 7 8 9 10 11 12 13 14

FIGURE 33

Autoradiogram of immune precipitates of p40 from HFL cells infected at 38.5° with wild-type HSV-1 or ts1201. Virus-infected cell polypeptides were labelled with [^{35}S]-methionine or [^{32}P]-orthophosphate from either 6-12h pi or 12-18h pi. Cells were harvested in RIPA buffer and immunoprecipitation was performed as described in Section 2.7.7, using MA5010B which was specific for p40 of HSV-1. Pre-immune samples were wild-type virus-infected cell extracts which were incubated with control non-immune rabbit serum in place of MA5010B. The immunoprecipitated polypeptides were analysed on an 8% SDS polyacrylamide gel.

e = mock-infected or wild-type virus-infected cell extract.

i = immunoprecipitate.

Tracks 1 to 7 = virus-infected cell polypeptides labelled with [^{35}S]-methionine.

Tracks 8 to 14 = Virus-infected cell polypeptides labelled with [^{32}P]-orthophosphate.

Tracks 1 and 8 = mock-infected cell extract.

Tracks 2 and 9 = wild-type virus-infected cell extract.

Track 3 and 10 = pre-immune sample.

Track 4 and 11 = wild-type virus-infected cell polypeptides labelled from 6-12h pi.

Tracks 5 and 12 = wild-type virus-infected cell polypeptides labelled from 12-18h pi.

Tracks 6 and 13 = ts1201-infected cell polypeptides labelled from 6-12h pi.

Tracks 7 and 14 = ts1201-infected cell polypeptides labelled from 12-18h pi.

radiolabelled with either [^{35}S]-methionine or [^{32}P]-orthophosphate from 6-12h pi or 12-18h pi at the NPT. [^{35}]-methionine-labelled and phosphorylated forms of p40 were detected by immunoprecipitation of virus-infected cell extracts with anti-p40 antibody MA5010B, and electrophoresis of immunoprecipitates on an SDS polyacrylamide gel. Figure 33 shows that although p40 was synthesized in both wild-type virus and ts1201-infected cells (Fig. 33, tracks 4, 5, 6 and 7), neither the processed nor unprocessed forms of p40 contained detectable amounts of phosphate (Fig. 33, tracks 11, 12, 13 and 14). Since this experiment was performed only in HFL cells, the possibility that phosphorylation of p40 is dependent on the host cell type cannot be ruled out. Alternatively, it is conceivable that monoclonal antibody MA5010B fails to recognise the phosphorylated forms of p40. However, this preliminary result indicates that p40 synthesized in wt HSV-1 strain 17-infected HFL cells does not contain detectable amounts of phosphate. Thus, phosphorylation of p40 is probably not a prerequisite for cleavage of the polypeptide in this virus strain, and therefore the failure to phosphorylate p40 is unlikely to account for the processing defect of ts1201.

4.1.8 High MW forms of p40 in ts1201-infected cells are translocated to the nucleus

Braun et al. (1984b) reported on the basis of cell fractionation experiments that the higher MW forms of p40 were the cytoplasmic precursors to nuclear products. Assuming that the processing site was within the nucleus, it was possible that p40 synthesized in ts1201-infected cells at the NPT was not processed to its lower MW forms because the polypeptide was not transported from cytoplasm to nucleus.

Immunofluroescence assays were performed to determine the intracellular location of p40 synthesized by wild-type virus and ts1201.

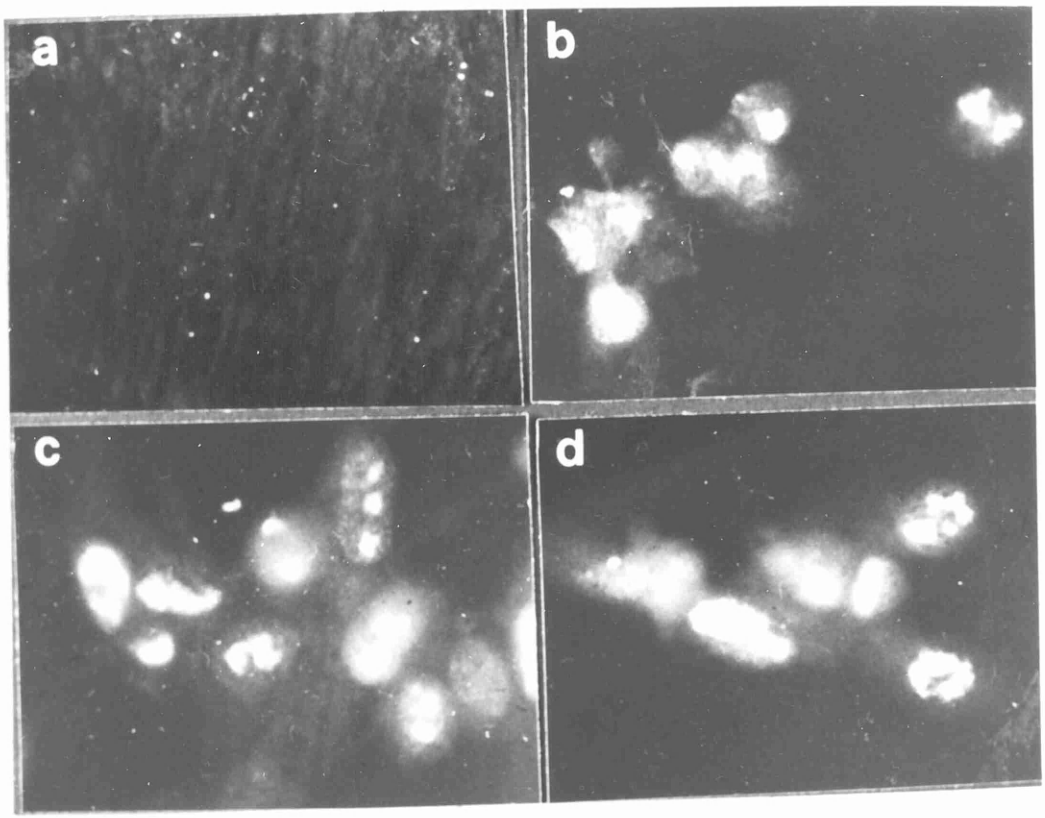

a
b
c
d

FIGURE 34

Localisation of p40 in ts1201 and wt HSV-1-infected cells. Monolayers of HFL cells were infected with 5 pfu of wild-type HSV-1, ts1201 per cell or mock-infected. Cells were harvested at 10h pi and prepared for indirect immunofluorescence assay.

(a) Mock-infected cells.

(b) Cells infected with wild-type HSV-1 at 38.5°.

(c) Cells infected with ts1201 at 31°.

(d) Cells infected with ts1201 at 38.5°.

The monoclonal antibody used in all cases was MA5010B, which was specific for p40 of HSV-1.

Virus-infected HFL cells on coverslips were incubated for 10h at the NPT, at which point the cells were washed and fixed in preparation for immunofluorescence. The intracellular distribution of p40 was detected with monoclonal antibody MA5010B. Figures 34b and 34d show that p40 was localised in the nucleus in both wild-type virus- and ts1201-infected cells at the NPT, indicating that aberrant processing of p40 does not result from a failure to transport the higher MW polypeptides from the cytoplasm to the cell nucleus. It is interesting that the fluorescence observed with MA5010B was granular in appearance, and was occasionally localised in distinct areas of the nucleus. Although this effect may result from the fixation procedure, it is tempting to speculate that this pattern of fluorescence reflects the association of p40 with groups of nucleocapsids.

4.1.9 p40 is associated with ts1203 and ts1201 capsids at the NPT

On the basis of experiments in which HSV nucleocapsids were isolated using detergent treatment of virus-infected cell nuclei, it was suggested that the processed forms of p40 were present in wild-type virus capsids which contained DNA, but not in empty capsids (Gibson and Roizman, 1974; Braun et al., 1984b). In view of this data, it was of interest to determine whether p40 was associated with empty capsids in ts1203-infected cells at the NPT, and if so whether the high MW forms of p40 were associated with ts1201 capsids.

To this end, an attempt was made to isolate and purify capsids from wild-type virus-, ts1201- and ts1203-infected cells at the NPT as described in Section 2.2.6. Capsids, obtained by this procedure and visualised under the electron microscope after negative staining, appeared relatively free of contaminating cell debris. However, analysis of capsid proteins by SDS-PAGE revealed that the samples were heavily contaminated with other virus-infected cell polypeptides. Attempts to

FIGURE 35

Localisation of p40 on wild-type virus, ts1201 and ts1203 capsids by immune electron microscopy. Monolayers of HFL cells were infected at 38.5° with 5 pfu of wt HSV-1, ts1201 or ts1203 per cell. Virus-infected cells were harvested at 10h pi when half of each sample was embedded in epon resin (B, D and F) (Section 2.9.1) and half in Lowicryl resin (A, C and E). Immune electron microscopy was performed on thin-section preparations of the Lowicryl-embedded samples by Dr. F.J. Rixon. These thin-sections were treated with a monoclonal antibody specific for p40 (MA5010B), and then with a protein A-colloidal gold conjugate, before viewing in the electron microscope.

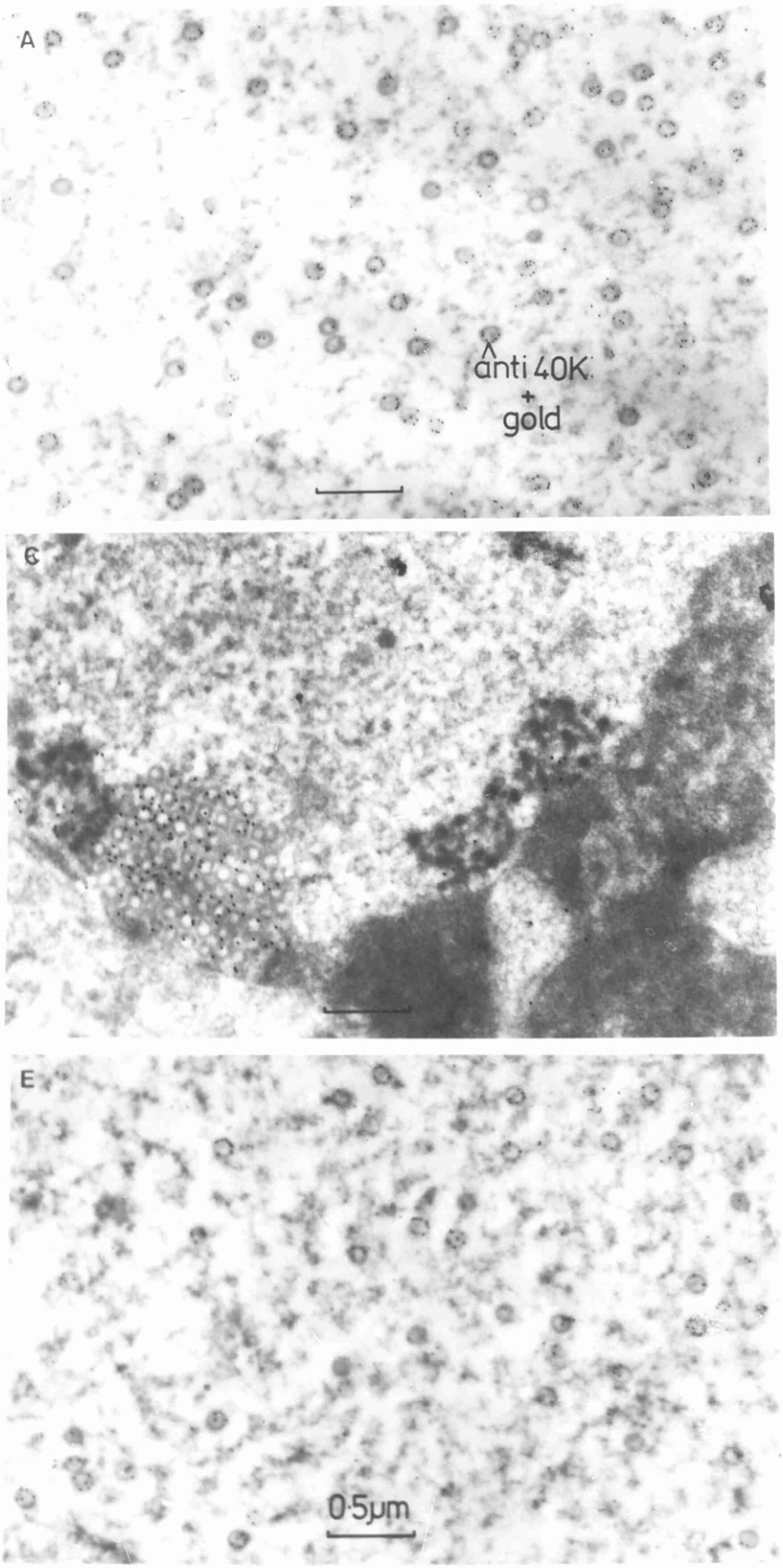
A and B, nuclei of cells infected with wt HSV-1.

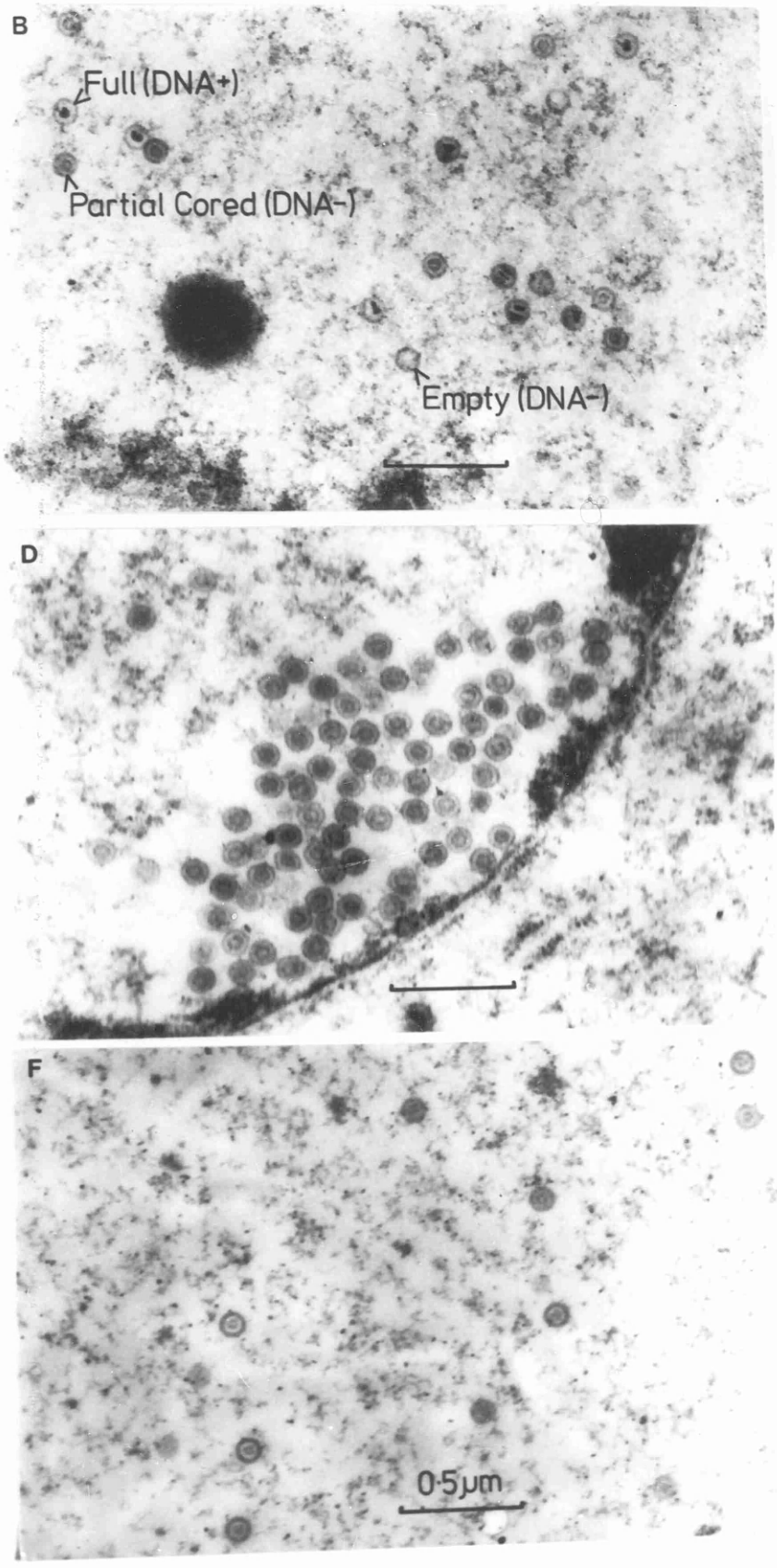
C and D, nuclei of cells infected with ts1201.

E and F, nuclei of cells infected with ts1203.

Full, partially cored and empty capsids are indicated.

The bars represent 0.5um.


A
anti 40K
+
gold
B
E
0·5µm


B
Full (DNA+)
Partial Cored (DNA-)
Empty (DNA-)
D
F
0·5μm

isolate nucleocapsids from HFL cells, or the use of sucrose velocity gradients to band capsids, failed to improve purification.

As an alternative approach, the technique of immune electron microscopy was used to investigate whether p40 was associated with ts1203 capsids at the NPT. These experiments, performed in collaboration with Dr. F.J. Rixon, involved the treatment of thin-section preparations of virus-infected cells with anti-p40 monoclonal antibody, followed by treatment with a protein A-colloidal gold particle conjugate. When viewed under the electron microscope, the colloidal gold particles were easily visualised and the location of p40 in the cell could be deduced. From Fig. 35 (a, c and e) it is clear that p40 was associated with capsid structures in wild-type virus-, ts1201- and ts1203-infected cells. It was difficult to distinguish between different types of capsids in these samples, which had been embedded in a hydrophilic, acrylic resin (Lowicryl). However, duplicate samples, which were embedded in epon resin (see Section 2.9.1), confirmed that DNA encapsidation did not occur in either ts1203- or ts1201-infected cells (Fig. 35b, d and f).

4.1.10 Cloning of DNA sequences containing the ts1203 mutation

As the first step in determining the alteration in the DNA sequence responsible for the ts1203 phenotype, the SalI fragment containing the ts1203 mutation, and the corresponding fragment from ts1203-rev 1 (a spontaneous revertant of ts1203), were cloned into plasmid vector pUC9. The restriction endonuclease digestion patterns of these two recombinant plasmids (designated pCA1 and pCA2 respectively) were compared with that of plasmid pGX31, which contains the corresponding SalI fragment from wt HSV-1 (Fig. 36, tracks 2, 3 and 4). Digestion of all three plasmids with SalI plus BstEII gave three corresponding bands as expected (compare Figs. 27 and 36, tracks 5, 6 and 7), and digestion with KpnI plus BstEII

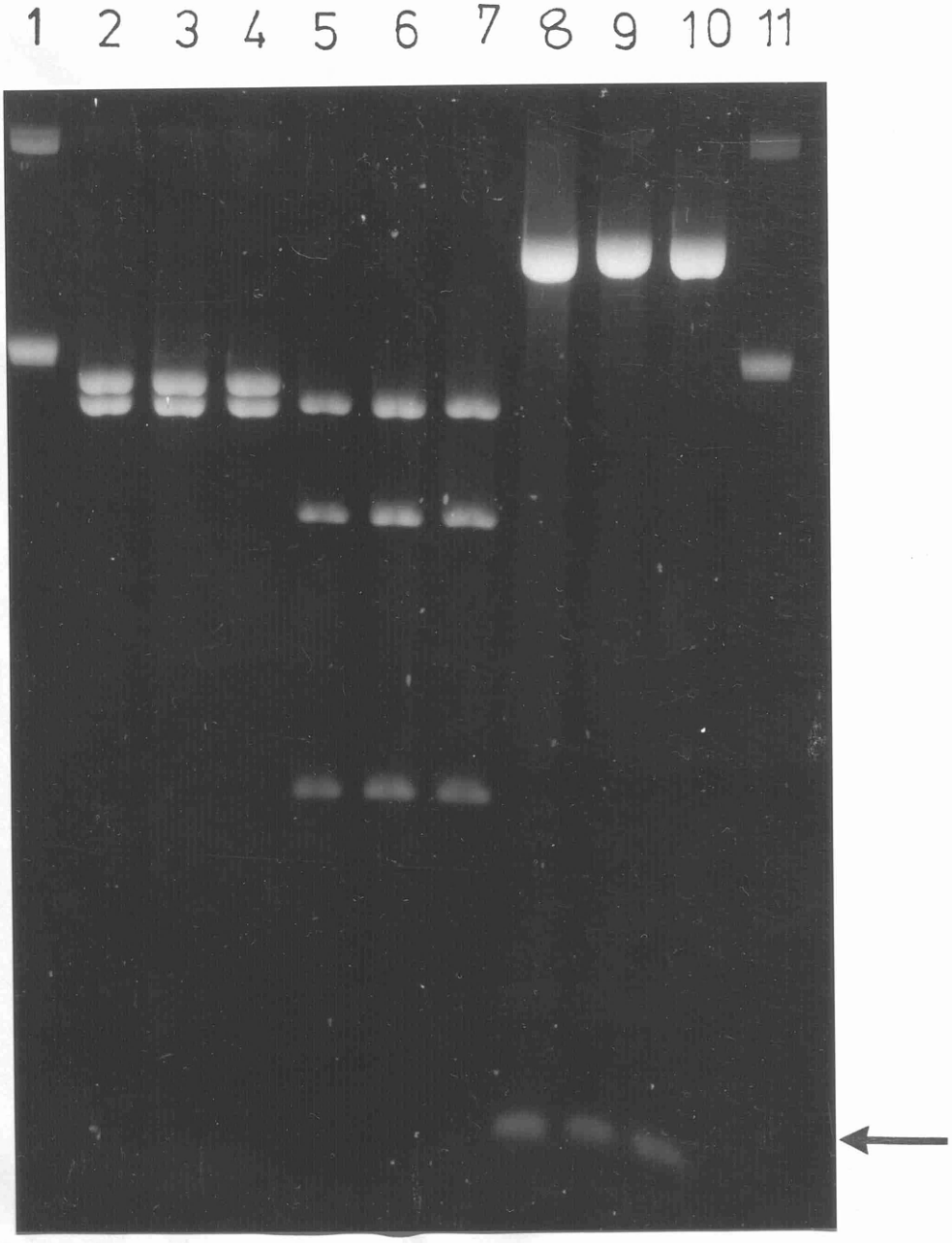

1 2 3 4 5 6 7 8 9 10 11

FIGURE 36

Restriction analysis of pGX31, pCA1 and pCA2. Plasmid DNAs (1ug) were digested with the appropriate restriction endonucleases under standard conditions. Digestion products were analysed by electrophoresis on a 0.8% agarose gel.

Tracks 1 and 11 = Uncut pGX31.

Tracks 2, 3 and 4 = pGX31, pCA1 and pCA2 respectively, cleaved with SalI.

Tracks 5, 6 and 7 = pGX31, pCA1 and pCA2 respectively, cleaved with SalI plus BstEII.

Tracks 8, 9 and 10 = pGX31, pCA1 and pCA2 respectively, cleaved with KpnI plus BstEII.

The arrow indicates the 450bp KpnI-BstEII DNA fragment in which the ts1203 mutation maps.

showed that the plasmids contained the 450bp fragment in which the ts1203 mutation had been mapped (Fig. 36, tracks 8, 9 and 10). The plasmids were tested in marker rescue experiments with ts1203 DNA (Table 10). The failure of cloned ts1203 SalI d to give ts^+ progeny indicated that the mutant DNA sequence had been cloned. The SalI d sequence cloned from ts1203-rev-1 successfully rescued the ts1203 mutation. These results indicated that the correct SalI fragment had been cloned in each case.

4.1.11 Mapping restriction endonuclease sites by partial digestion

Restriction enzymes, which could be used to cleave the cloned DNAs into small fragments suitable for subcloning into the single-stranded phage vector M13 prior to sequencing by the dideoxy chain termination method, were identified (Sanger et al., 1980). Restriction endonuclease sites within the 450bp KpnI/BstEII fragment were mapped by 3' end-labelling of the fragment with partial digestion with [^{32}P] and polyacrylamide gel electrophoresis of the digestion products. An autoradiogram of [^{32}P]-labelled partial digestion products of cleavage of the 450bp fragment with restriction enzymes AluI, HaeIII, HpaII, SmaI and ThaI is shown in Fig. 37. Three sites were recognised by AluI, two sites by HaeIII, two sites by HpaII and at least eight sites by ThaI. No sites for SmaI cleavage were contained in the 450bp fragment. Thus, overlapping DNA fragments suitable for sequencing could be obtained by cleavage of the 450bp KpnI/BstEII fragment singly with AluI and HaeIII.

4.2 DISCUSSION

The previous section describes the characterisation of ts1203 which, like ts1201, fails to package DNA into capsids at the restrictive temperature. Biochemical and genetic analyses of the two mutants

TABLE 10

Marker rescue of ts1203 using pGX31, pCA1 and pCA2

Plasmid DNA	eop NPT/PT ($\times 10^{-3}$)
None	<0.001
pGX31	0.69
pCA1	<0.001
pCA2	0.22

TABLE 10

Marker rescue of ts1203 using pGX31, pCA1 and pCA2. Plasmid DNAs pGX31 (contains HSV-1 DNA fragment SalI d), pCA1 (contains ts1203 DNA fragment SalI d) and pCA2 (contains ts1203 rev-1 DNA fragment SalI d) were cleaved with SalI and used in marker rescue experiments.

The relative efficiency of plating (eop NPT/PT) was calculated from the yield of progeny virus at the PT and NPT from the transfected cells.

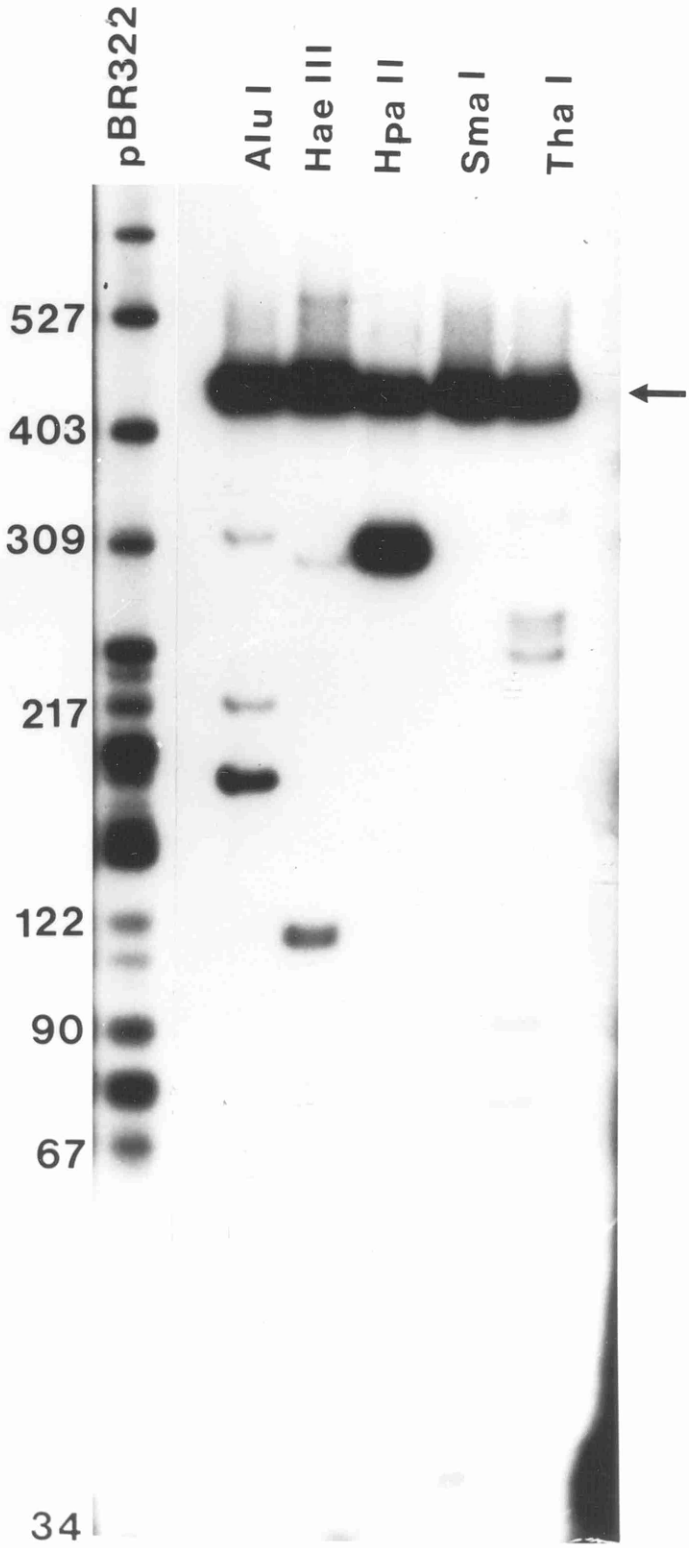

pBR322
Alu I
Hae III
Hpa II
Sma I
Tha I
527
403
309
217
122
90
67
34

FIGURE 37

Mapping of restriction endonuclease sites within the 450bp KpnI/BstEII fragment by partial digestion. An amount of 3'-terminally [^{32}P]-labelled Kpn/BstEII fragment, equivalent to $1x10^4$ cpm, was partially digested with restriction enzymes AluI, HaeIII, HpaII, SmaI or ThaI. Partial digestion products were separated on a 10% polyacrylamide gel. 3'-terminally [^{32}P]-labelled HpaII fragments of pBR322 were used as size markers. The arrow marks the undigested 450bp KpnI/BstEII fragment.

indicated that ts1203 and ts1201 were blocked at different steps in the pathway of herpesvirus DNA encapsidation.

Genetic analysis of ts1203

The lesion in ts1203 maps within a 450bp fragment located between mu 0.377-0.380 on the HSV genome. This mutation is to the right of the ts1201 lesion (mu 0.33) within HSV DNA fragment EcoRI f.

Several other ts mutants of HSV with structural defects map in this region of the genome (Fig. 28). Ts1204 and ts1208 both have a defect in assembly of functional nucleocapsids at the NPT, and map within HSV DNA fragment EcoRI f (Addison et al., 1984; see Section 3.2). TsN20 (mu 0.445-0.448) assembles many empty and partial cored capsids at the restrictive temperature, as well as a small number of 'aberrant capsid' structures and full capsids (Schaffer et al., 1974; Coen et al., 1984). Another mutant, tsZ47 (mu 0.312-0.415), maps within HSV DNA fragment EcoRI f, and has been characterised as an immune cytolysis-resistant mutant. Although tsZ47 synthesizes wild-type amounts of DNA at the NPT, it is not yet known whether this mutant also has a capsid assembly defect. Further analyses would be required to determine whether these mutants, together with ts1203 and ts1201, represent a cluster of essential structural genes on the HSV genome.

Several mutants of PRV with defects in DNA encapsidation have been isolated (Ben-Porat et al., 1982; Ladin et al., 1982). The lesions in these mutants map in the right hand end of the U_L region of the PRV genome, and include tsN, tsIE13 and ts101 (mu 0.19-0.27), tsUH3 and ts106 (mu 0.09-0.19) (Ihara et al., 1982). DNA homology studies have indicated that the HSV and PRV genomes are essentially colinear, except for the region between mu 0.1-0.4 which appears to be inverted (Davison and Wilkie, 1983). It would be of interest to determine, for example, by

cross-hybridisation studies, whether any of the genes represented by these PRV mutants are analogous to the HSV gene in which the ts1203 mutation lies.

Processing of virus DNA

Analysis of virus-infected cell DNA by Southern blotting revealed that although 36% of DNA synthesized by wild-type virus at the NPT was cleaved to unit length genomes, greater than 99% of the DNA synthesized by both ts1203 and ts1201 was 'endless' at this temperature. This result supports data obtained by Ladin et al. (1980), in a study of PRV ts mutants which failed to package virus DNA into capsids at the NPT. Using sedimentation analysis of virus-infected cell DNA on sucrose gradients, these workers demonstrated that mutant DNA synthesized at the NPT was in a rapidly sedimenting form, quite distinct from that of mature virion DNA. In addition, restriction enzyme digestion profiles of these mutant DNAs revealed the under-representation of terminal fragments and the appearance of a new joint fragment. On the basis of these results, the authors reported that at the NPT, the mutant DNAs were in concatemeric structures consisting of tandem arrays of the virus genome. The use of the Southern blotting technique in this study to detect HSV joint-spanning and terminal fragments provides additional evidence that the DNA synthesized by mutants which fail to encapsidate DNA is in an endless form. Although no sedimentation analysis of ts1201 or ts1203 mutant virus DNA was performed, it is reasonable to assume that the unpackaged mutant virus DNA is in the form of concatemers.

The ts1201 defect was reversible since, upon shift-down of infected cells from the NPT to the PT in the absence of further protein synthesis, ts1201 DNA was cleaved and packaged into capsids. These results suggest that DNA cleavage occurs concurrently with DNA packaging. However,

less DNA was cleaved in ts1201-infected cells than in wild-type virus-infected cells under these conditions. It is possible that this difference could be accounted for by the amount of wild-type virus DNA already cleaved at the NPT before temperature downshift. During this period, cleavage of ts1201 DNA would not occur. In contrast to the result obtained with ts1201, an increase in the amount of cleavage of ts1203 DNA was not seen upon temperature downshift in the presence of cycloheximide. This finding indicates that the ts1203 defect is irreversible in the absence of de novo protein synthesis. It is also notable that DNA encapsidation was less efficient in ts1203-infected cells at the PT than in cells infected with wild-type virus or ts1201. This defect is probably reflected in the small plaque size of ts1203 (data not shown).

Although the amount of DNA synthesized by wild-type virus at 38.5° was comparable to the amount synthesized at 31°, DNA maturation in wild-type virus-infected cells at the high temperature was less efficient than at the lower temperature. This may be a consequence of the thermolability of a virus polypeptide(s) involved in the DNA encapsidation process.

It is not known whether the initiation of a rolling circle method of DNA replication occurs at a specific sequence on the HSV genome, or is the result of random nicks along the DNA strand. Therefore, it follows that it is unknown whether concatemeric DNA molecules contain random or specific terminal sequences, for example, an a sequence or an ori-containing sequence. However, it is interesting to note that plasmids containing ori sequences, but lacking an a sequence, are capable of forming concatemers (Stow, 1982), a finding which supports the idea that initiation of a rolling circle mode of DNA replication occurs at one of the ori sequences. This result suggests that concatemeric HSV DNA molecules are unlikely to end specifically in an a sequence. For this reason, it was

assumed that low levels of terminal fragments detected in mutant virus-infected cell DNA samples at the NPT arise as a result of specific cleavage of DNA at the a sequence during encapsidation. Quantitation of the relative proportions of 'endless' and cleaved DNA in virus-infected cells has therefore been possible. As expected, the quantitation of cleavage of 'endless' DNA correlated well with estimates of encapsidated DNA. Clearly, some experimental error in such a multi-step process is unavoidable, however, the quantitations are valid in so far as the results obtained were reproducible in several experiments. These analyses also provided confirmation of electron microscopic observations on thin-sections of mutant virus-infected cells.

Irreversibility of the ts1203 defect

The ts defect of ts1203 was irreversible upon temperature downshift to the PT in the presence of cycloheximide. There are three possible explanations for this finding. Firstly, the ts mutation in ts1203 may have resulted in the synthesis of a polypeptide which is unstable, for example, the protein may have an altered configuration at the NPT which renders it susceptible to degradation. Alternatively, the mutated polypeptide may be stable at the NPT, but may be unable to regain a functional configuration upon temperature downshift to the PT. In both these cases, de novo synthesis of the mutant polypeptide would be required at the PT before the encapsidation process could proceed. Thirdly, the capsids assembled at the NPT may be aberrant and incapable of packaging virus DNA even if the mutant polypeptide regains a functional conformation upon temperature downshift to the PT. This possibility will be discussed in more detail later. It is feasible that the mutant polypeptide might have impaired function at the PT, as a consequence of a less compatible amino acid substitution, compared to the wild-type protein.

Virus-induced protein profiles failed to reveal any remarkable differences between ts1203 and wild-type virus at the NPT (data not shown). Alternative approaches to identification of the affected gene product will be indicated later.

Association of p40 with empty capsids

To determine whether or not the lower MW forms of p40 in ts1203-infected cells grown at the NPT were associated with the intermediate capsids assembled by this mutant, attempts were made to purify the mutant virus capsids. These experiments, however, were unsuccessful. There were a variety of reasons for this failure. First, the yield of capsids was generally low and, in some instances, no capsid band could be detected in the Percoll gradient. This problem could be partially overcome by using low-passage cells to propagate the virus. Second, capsid preparations were often heavily contaminated with non-structural virus polypeptides, and with cell debris. Further treatment of these capsid preparations with detergents followed by rebanding of capsids on Percoll or sucrose gradients did not resolve this problem. It is possible that the contaminating material could be reduced by using a different cell type to propagate the virus. Third, problems were encountered in isolating the capsid band from the gradient. On several occasions, a capsid band which was clearly visible on a Percoll gradient was not detected as a radioactive peak, following fractionation of the gradient. Again, this problem could, in part, be due to a low yield of capsids from the mutant virus-infected cells. However, it was felt that improvement could be made to the fractionation system.

Immune electron microscopy, performed in collaboration with Dr. F.J. Rixon, provided a successful alternative method to determine whether or not p40 was associated with HSV capsids which did not contain

DNA. In the light of previous data, the results obtained during these experiments are of great interest. It had been shown that p40 was absent from capsids lacking a DNA core which had been isolated by detergent treatment of infected cell nuclei (Gibson and Roizman, 1972; Braun et al., 1984b). However, using ts mutants which fail to encapsidate DNA, immune electron microscopy data has shown unequivocally that p40 is associated with intermediate capsids. Recently, Braun et al. (1984b) reported that only the lower MW forms of p40 were associated with detergent-extracted, full capsids. Our results did not agree with their findings. Immune electron microscopic observations on ts1201-infected cells at the NPT clearly demonstrated that the high MW forms of p40 become associated with capsids prior to DNA encapsidation. One explanation for these conflicting results is that p40 may be initially loosely associated with the capsid surface, and that a strong attachment is only made once DNA encapsidation has occurred. Thus, during detergent treatment, p40 is removed from empty capsids, whereas with the technique of immune microscopy, p40 remains loosely associated with the capsids. These results have been corroborated by the findings of Irmiere and Gibson (1983, 1985) in a study of structural polypeptides in HCMV 'A' capsids which lack DNA and any internal structure, B capsids which lack DNA but contain some internal structure, and non-infectious enveloped particles (NIEPS) which appear to be enveloped B capsids. These workers detected an HCMV 36,000 MW 'assembly protein' in B capsids and in NIEPS, but not in A capsids. It is notable that these B capsids were isolated by freeze-thaw treatment of infected cell nuclei, and not by the use of detergents. Assuming that the HCMV 'assembly protein' and the HSV p40 are analogous, these results demonstrate that (i) p40/36K becomes a capsid component before DNA packaging, and before processing of the polypeptide to lower MW species occurs, (ii) processing of p40 occurs before DNA

encapsidation, and (iii) removal of p40 is not required for envelopment of the capsid. With regard to the final point, it should be noted that NIEPS which lack DNA, have not yet been reported in HSV-infected cells.

General models for DNA encapsidation

The exact functions of the polypeptides which are ts in ts1203- and ts1201-infected cells are unknown. However, a brief review of DNA packaging models proposed for other virus systems may provide some basis for speculation.

The pathways of DNA encapsidation for the bacteriophage λ , T4 and P22, have been studied in detail, and share several features in common with the encapsidation of herpesvirus DNA. These bacteriophage have an icosahedral-shaped head or capsid, into which ds DNA, taken from a pool of concatemeric molecules, is packaged. The availability of in vitro DNA packaging systems for these bacteriophage has facilitated the elucidation of the DNA encapsidation pathway, which is summarised below.

The correct assembly of immature capsid shells is probably directed by 'scaffolding' or 'assembly core' proteins which are absent from the mature particle (Casjens and King, 1975; Murialdo and Becker, 1978; Earnshaw and Casjens, 1980). The DNA is replicated in the form of concatemers, and a specific recognition step is required to ensure that only virus DNA is packaged into the empty capsids. In the phage system, this involves the interaction of a phage-specified enzyme called 'terminase' with a specific phage DNA sequence designated cos (Emmons, 1984; Feiss and Becker, 1983). The HSV a sequence is probably involved in an analogous process. This protein:DNA complex makes specific contact with a cylindrical 'connector' structure which, in the phage system, is situated at the capsid vertex where the tail attaches (Serwer, 1976; Coombs and Eiserling, 1977). This protein structure forms the entrance through which

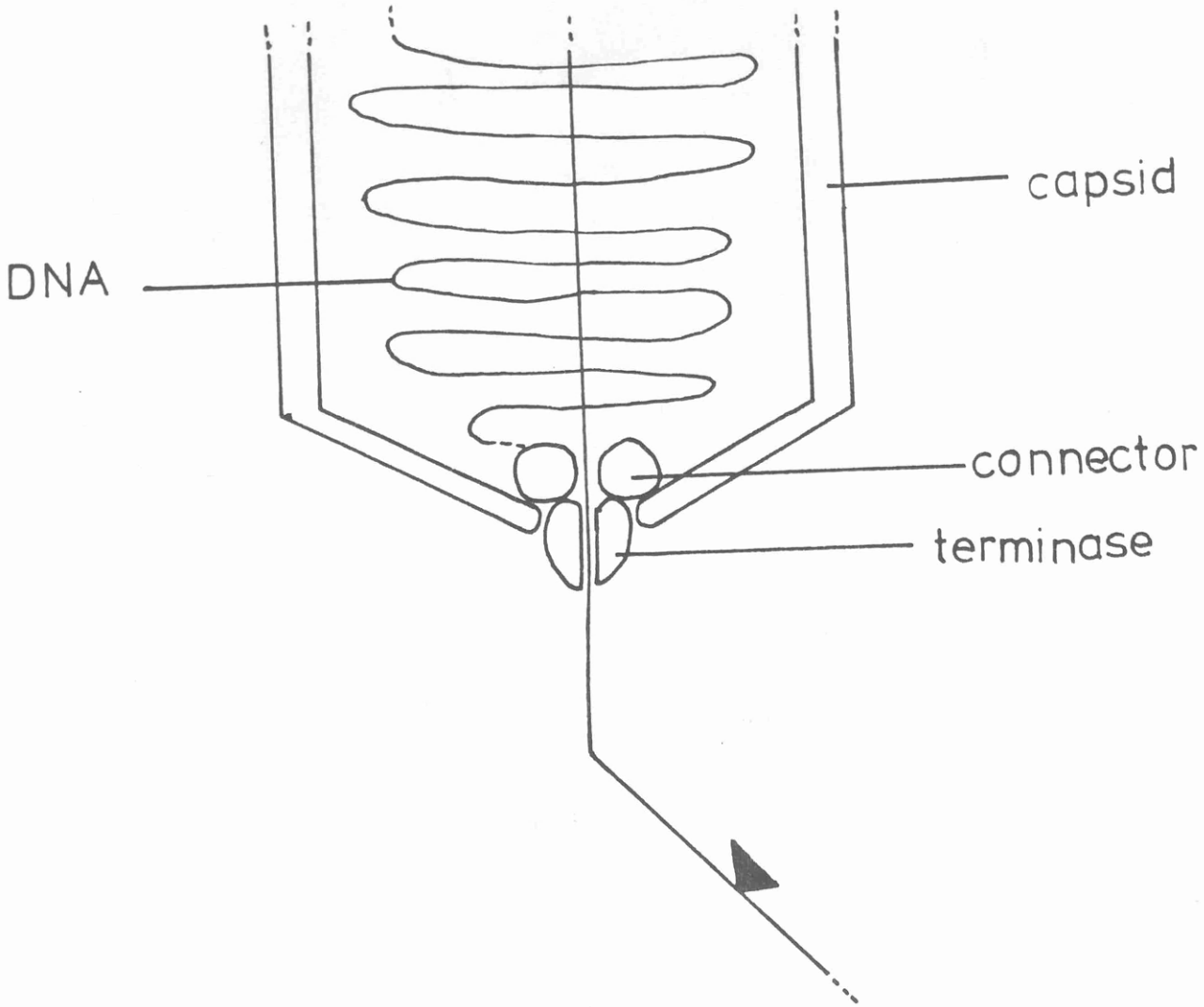

capsid
DNA
connector
terminase

FIGURE 38

Scanning for the terminal cos site of phage λ DNA, showing part of a nearly filled head. The 'terminase' protein is shown positioned at the connector structure of the capsid vertex. The DNA strand is being 'scanned' by terminase until the cos site (filled triangle) is encountered, and cut. This model is taken from Feiss and Becker (1983). Details are given in the text.

DNA is packaged into the capsid shell (Fig. 39). The structure and position of the entrance into herpesvirus capsids is not known. Analysis of in vitro λ packaging systems has demonstrated that energy (in the form of ATP) is required to drive DNA encapsidation. On the basis of X-ray crystallography observations, Earnshaw et al. (1976) reported that the DNA in phage P22 heads was packed as an ordered toroidal superhelix. It is postulated that because of its stiffness and charged nature, the DNA seeks a radius of maximum curvature on entering the head. Thus, the newly packaged DNA makes contact with the inner wall of the capsid, and coiling proceeds inwards. Polyamines are thought to stabilize the DNA within the phage capsid shell (Earnshaw and Casjens, 1980) and have also been found associated with packaged HSV DNA (Gibson and Roizman, 1971). Termination of packaging is achieved in the λ system by scanning of the DNA strand until the next cos site is recognised by terminase (Fig. 38). At this point, the DNA is cleaved by the introduction of staggered, single-strand nicks (Feiss and Widner, 1982). A similar model whereby the HSV DNA strand is scanned for a sequences, is easily envisaged. Indeed, a staggered nick-repair mechanism for cleavage and packaging of HSV DNA was recently proposed by Varmuza and Smiley (1985) (Fig. 8). In this model, the L and S termini of HSV DNA arise by staggered single-stranded nicks, the positions of which are directed by signals within the a sequence. Repair synthesis produces the two termini, each bearing an a sequence.

Poffenberger and Roizman (1985) proposed an addition to the "scanning" model of DNA packaging, based on the analysis of mutant HSV-1(F) 1358, which has a non-inverting genome due to a 15kbp deletion spanning the L-S junction. These workers showed that a small percentage (5% or less) of packaged 1358 DNA was in the form of covalently closed circles. In addition, a large fraction (30-40%) of 1358 DNA formed

covalent head-to-tail linkages soon after infection, and in the absence of de novo viral protein synthesis. The authors proposed that the cleaved termini of full length HSV DNA molecules are held together during packaging, probably by proteins bound to the a sequence. A small fraction of the termini would be covalently linked during or after packaging. Whether this model is generally predictive for standard HSV DNA is open to question and difficult to authenticate, since all four possible head-to-tail junctions are produced in abundance during isomerisation of the genome, and are represented in standard HSV virion DNA. Furthermore, this model requires a mechanism to achieve the juxtaposition of two termini following cleavage of the unit-length molecule from the DNA concatemer, and before packaging is complete. Perhaps an alternative interpretation of these results would be that cleavage and packaging of the HSV genome occurs by a mechanism similar to that proposed for phage , and that the terminal proteins are bound to the DNA either prior to or during encapsidation. The small percentage of circular molecules seen by Poffenberger and Roizman (1985) in packaged 1358 DNA could be formed fortuitously once the DNA was encapsidated.

The insertion of virus DNA into a preformed capsid shell has also been proposed for adenovirus. Adenovirus DNA is replicated by strand-displacement of circular unit-length molecules (Lechner and Kelly, 1977; Sussenbach and Kuijk, 1978). Again, a specific recognition step, similar to that involving the phage DNA cos sequence and the HSV DNA a sequence, has been proposed as one of the initial steps in adenovirus DNA encapsidation (Hammarskjold and Winberg, 1980). However, a detailed pathway of adenovirus DNA packaging is not known.

An alternative model of nucleocapsid assembly has been proposed for both herpesviruses and adenovirus. In this model, capsids are formed around a nucleoprotein core in a stepwise assembly process, which results

in the accumulation of structures of increasing sedimentation value. Weber et al. (1985) demonstrated that concurrent adenovirus DNA synthesis was essential for virus assembly. Sedimentation velocity analysis in sucrose gradients of nucleoprotein complexes (NPCs) isolated from infected cell nuclei, revealed that virus structural proteins and S1-sensitive virus DNA were present in a broad peak throughout the gradient. On the basis of these results, the authors suggested that capsid assembly and DNA synthesis were physically linked, and that stepwise assembly of capsids around the genome was occurring. A similar proposal was made by Pignatti and Cassai (1980) who isolated NCPs from HSV-infected cells using non-ionic detergent treatment. Electron microscopic examination of sucrose gradient-purified NPCs revealed that they consisted of hexagonal capsules (110-120nm diameter) containing electron dense cores which resembled extended nucleocapsid structures. The authors postulated that the NPCs were capsid assembly intermediates, and that addition of other structural polypeptides resulted in the production of mature nucleocapsids. However, the possibility that the NPCs were degraded nucleocapsid structures was not addressed in these experiments, and unequivocal evidence that any structural polypeptides were absent from these complexes has not been presented.

Function of the gene product which is ts in ts1201-infected cells

At the NPT, ts1201 fails both to process p40 to its lower MW forms and to encapsidate virus DNA. The reason p40 remains unprocessed in ts1201-infected cells in unclear. Braun et al. (1984b), on the basis of cell fractionation experiments, reported that the higher MW forms of p40 were cytoplasmic precursors to the lower MW nuclear products. Therefore, the possibility existed that the processing defect in ts1201 resulted from a failure to transport the primary p40 translation products to the nucleus.

Immunofluorescence experiments, however, revealed that the high MW forms of p40 synthesized in ts1201-infected cells at the NPT accumulated in the nucleus, a finding which contradicts the results of Braun et al. (1984b). One explanation for these conflicting results might be that the high MW forms of p40 are loosely associated with the nucleus, and 'leak out' into the cytoplasmic extract during cell fractionation. Clearly, immunofluorescence assay of fixed infected cells is a better method of determining the intracellular location of virus polypeptides.

An alternative reason for the ts1201 processing defect was suggested by previous reports that p40 synthesized in cells infected with HSV-1 strains MAL (Heilman, 1982) and F (Braun et al., 1984b), was phosphorylated. Thus, it was possible that phosphorylation of p40 was a prerequisite for cleavage, and that p40 synthesized in ts1201-infected cells at the NPT was unphosphorylated. However, immunoprecipitation experiments failed to detect any phosphorylation of p40 synthesized either by wt HSV-1 strain 17, or ts1201. Since processing of p40 to its lower MW forms occurs normally in HSV-1 strain 17-infected cells, it is unlikely that phosphorylation of the polypeptide is required for conversion to the lower MW forms in this virus strain. The remaining possibility, that at the NPT the mutant p40 adopts a configuration which is unable to be processed to lower MW forms, has not been investigated.

Clearly, the characterisation of ts1201 shows that processing of p40 is a prerequisite for HSV DNA encapsidation. However, analysis of ts1203 suggests that events additional to p40 modification are required before DNA packaging can occur. Perhaps modification of p40 on the capsid surface acts as a signal for the attachment of other tegument polypeptides, one or several of which might be involved in the virus DNA attachment/cleavage/packaging process. It is tempting to speculate that acquisition of tegument polypeptides on the capsid surface results in the

disaggregation of the crystalline arrays of capsids seen in ts1201-infected cells at the NPT to the random distribution of capsids seen in ts1203-infected cells at this temperature. Alternatively, modification of p40 itself might be responsible for this effect.

Function of the gene product which is ts in ts1203-infected cells

Since the gene product which is ts in ts1203-infected cells has not yet been identified, any predictions of its function must be purely speculative. On one hand, the polypeptide might be involved in capsid shell assembly, i.e. a scaffolding protein. In this case, aberrant capsids would be assembled such that DNA encapsidation was impossible, both at the NPT, and upon temperature downshift to the PT. Alternatively, the polypeptide might be controlling a step in the DNA encapsidation pathway per se. In this case the polypeptide could be involved in a sequence recognition in a manner analogous to the phage terminase enzyme, or perhaps have a role in linkage of DNA to the capsid, or be required for insertion of the DNA into the capsid. Functional analysis must obviously await identification of the gene product.

4.3 FUTURE PROSPECTS

The ultimate aim of the characterisation of HSV ts mutants is the determination of the function of virus gene products. As with ts1204, the early steps in this process have been completed for the gene product which is ts in ts1203-infected cells. Marker rescue experiments have localised the ts1203 lesion to a 450bp region in DNA fragment BamHI g. Additionally, this fragment, and the corresponding fragment from ts1203 rev-1 DNA have been cloned, and are available for DNA sequence analysis to determine the precise bp change which is responsible for the ts1203 phenotype. As detailed for ts1204, the use of synthetic oligopeptide

technology to produce antisera against the affected gene product should allow identification of the polypeptide in wt virus-infected cell extracts. Such gene-specific antisera would be useful for determining the DNA-binding properties of the protein. In addition, it would be important to investigate whether the protein was structural or non-structural. Such experiments might eventually lead to further definition of steps in the pathway of herpesvirus DNA encapsidation.

It would be of interest to investigate the possibility that p40 is processed by a specific cleavage event. Thus, the ability of protease inhibitors such as TPCK or PMSF to prevent processing of p40 would be indicative that a specific cleavage step was involved. Further analysis might involve production of antisera against oligopeptides from the N- or C-terminus of the predicted polypeptide. Use of these antisera to immunoprecipitate p40 would indicate which forms, if any, had arisen from a cleavage event. Thus, cleavage products lacking either the N- or C-terminus would not be immunoprecipitated by the corresponding antiserum.

The function of p40 might be further elucidated by determining whether or not the protein has DNA-binding properties. Bayliss et al. (1975) showed that several proteins of approximately 40,000 MW bound to DNA:cellulose columns. Braun et al. (1984a), however, using a filter-binding assay to analyse the DNA-binding properties of capsid polypeptides, reported that only capsid polypeptide VP19C (Vmw50) could bind DNA. It might be of value to determine whether the eluate of DNA:cellulose columns contains p40, for example, by the technique of Western blotting. These experiments might then indicate whether p40 was involved in protein:protein interactions in the capsid tegument rather than protein:DNA interactions, in the DNA encapsidation process.

This study has clearly shown the advantages of comparative detailed analyses of ts mutants which, on preliminary examination, have

similar phenotypic defects. The isolation and characterisation of other ts mutants defective in DNA encapsidation is required both to define additional parameters by which mutants can be analysed, and to further identify steps in the herpesvirus DNA encapsidation process.

REFERENCES

REFERENCES

ABODEELY, R.A., LAWSON, L.A. and RANDALL, C.C. (1970). Morphology and entry of enveloped and de-enveloped equine abortion (herpes) virus. J. Virol., 5, 513-523.

ADDISON, C., RIXON, F.J., PALFREYMAN, J.W., O'HARA, M. and PRESTON, V.G. (1984). Characterisation of a herpes simplex virus type 1 mutant which has a temperature-sensitive defect in penetration of cells and assembly of capsids. Virology, 138, 246-259.

ALWINE, J.C., STEINHART, W.L. and HILL, C.W. (1974). Transcription of herpes simplex type 1 DNA in nuclei isolated from infected HEp-2 and KB cells. Virology, 60, 302-307.

ANDERSON, K.P., COSTA, R.H., HOLLAND, L.E. and WATSON, E.K. (1980). Characterisation of herpes simplex virus type 1 RNA present in the absence of de novo protein synthesis. J. Virol., 34, 9-27.

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 virus type 2. J. Gen. Virol., 40, 103-119.

AURELIAN, L. and ROIZMAN, B. (1964). The host range of herpes simplex virus: Interferon, viral DNA and antigen synthesis in abortive infection of dog kidney cells. Virology, 22, 452-461.

AVERETT, D.R., LUBBERS, C., ELION, G.B. and SPECTOR, T. (1983). Ribonucleotide reductase induced by herpes simplex virus type 1: Characterisation of a distinct enzyme. J. Biol. Chem., 258, 9,831-9,838.

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.

BALACHANDRAN, N. and HUTT-FLETCHER, L.M. (1985). Synthesis and processing of glycoprotein gG of herpes simplex virus type 2. J. Virol., 54, 825-832.

BARINGER, J.R. and SWOVELAND, P. (1973). Recovery of herpes simplex virus from human trigeminal ganglions. New England J. of Med., 288, 648-650.

BATTERSON, W., FURLONG, D. and ROIZMAN, B. (1983). Molecular genetics of herpes simplex virus. VIII. Further characterisation of a temperature-sensitive mutant defective in release of viral DNA and in other stages of the viral reproductive cycle. J. Virol., 45, 397-407.

BATTERSON, W. and ROIZMAN, B. (1983). Characterisation of the herpes simplex virion-associated factor responsible for the induction of alpha genes. J. Virol., 46, 371-377.

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., ASCHER, Y., FRIEDMANN, A. and KESSLER, E. (1978). Circular, linear and branched herpes simplex virus molecules from arginine deprived cells. J. Gen. Virol., 41, 629-633.

BECKER, Y., DYM, H. and SAROV, J. (1968). Herpes simplex virus DNA. Virology, 36, 184-192.

BEN-PORAT, T., BROWN, L. and VEACH, R.A. (1982). Recombination occurs mainly between parental genomes and precedes DNA replication in pseudorabies virus-infected cells. J. Virol., 44, 134-143.

BEN-PORAT, T., HOFFMANN, P., BROWN, L., FELDMAN, L. and BLANKENSHIP, M.L. (1982a). Partial characterisation of temperature-sensitive mutants of pseudorabies virus. Virology, 122, 251-267.

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. and KAPLAN, A.S. (1971). Phospholipid metabolism of herpesvirus-infected and uninfected rabbit kidney cells. Virology, 45, 252-264.

BEN-PORAT, T., KAPLAN, A.S., STEHN, B. and RUBENSTEIN, A.S. (1976).

Concatemeric forms of intracellular herpesvirus DNA. Virology, 69, 547-560.

BEN-PORAT, T. and RIXON, F.J. (1979). Replication of herpesvirus DNA. IV. Analysis of concatemers. Virology, 94, 61-70.

BEN-PORAT, T., STEHN, B. and KAPLAN, A.S. (1976). Fate of parental herpesvirus DNA. Virology, 71, 412-422.

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.

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., 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.

BEREZNEY, R. and COFFEY, D.S. (1974). Identification of a nuclear protein matrix. Biochem. Biophys. Res. Commun., 60, 1,410-1,417.

BEREZNEY, R. and COFFEY, D.S. (1977). Nuclear matrix. Isolation and characterisation of a framework structure from rat liver nuclei. J. Cell. Biol., 73, 616-637.

BIBOR-HARDY, V., BERNARD, M. and SIMARD, R. (1985). Nuclear

matrix modifications at different stages of infection by herpes simplex virus type 1. J. Gen. Virol., 66, 1,095-1,103.

BIBOR-HARDY, V., POUCHELET, M., ST.-PIERRE, E., HERZBERG, M. and SIMARD, R. (1982a). The nuclear matrix is involved in herpes simplex virogenesis. Virology, 121, 296-306.

BIBOR-HARDY, V., SUH, M., POUCHELET, M. and SIMARD, R. (1982b). Modifications of the nuclear envelope of BHK cells after infection with herpes simplex virus type 1. J. Gen. Virol., 63, 81-94.

BLACK, L.W. and SHOWE, M.K. (1983). Morphogenesis of the T4 head. In: "Bacteriophage T4". Ed. C.K. Matthews, E.M. Kutter, G. Mosig and P.B. Berget. American Soc. Micro.

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.

BOND, V.C. and PERSON, S. (1984). Fine structure physical map locations of alterations that affect cell fusion in herpes simplex virus type 1. Virology, 132, 368-376.

BOND, V.C., PERSON, S. and WARNER, S.C. (1982). The isolation and characterisation of mutants of herpes simplex virus type 1 that induce cell fusion. J. Gen. Virol., 61, 245-254.

BRAUN, D.K., BATTERSON, W. and ROIZMAN, B. (1984a). Identification and genetic mapping of a herpes simplex virus capsid protein that binds DNA. J. Virol., 50, 645-648.

BRAUN, D.K., PEREIRA, L., NORRILD, B. and ROIZMAN, B. (1983). Application of denatured, electrophoretically separated and immobilized lysates of herpes simplex virus-infected cells for the detection of monoclonal antibodies and for studies of the viral proteins. J. Virol., 46, 103-112.

BRAUN, D.K., ROIZMAN, B. and PEREIRA, L. (1984b). Characterisation of post-translational products of herpes simplex virus gene 35 proteins binding to the surfaces of full capsids but not empty capsids. J. Virol., 49, 142-153.

BROWN, S.M., HARLAND, J. and SUBAK-SHARPE, J.H. (1984). Isolation of restriction endonuclease site deletion mutants of herpes simplex virus. J. Gen. Virol., 65, 1,053-1,068.

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, 24, pp. 33-39. Ed. G. deThe, W. Henle and F. Rapp.

BROWN, S.M., RITCHIE, D.A. and SUBAK-SHARPE, J.H. (1973). Genetic studies with herpes simplex virus type 1. The isolation of temperature-sensitive mutants, their arrangement into complementation groups and recombination analysis leading to a linkage map. J. Gen. Virol., 18, 329-346.

BUSBY, D.W.G., HOUSE, W. and MACDONALD, J.R. (1964). In: "Virological Techniques", published by 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 microscopic studies of temperature-sensitive mutants of herpes simplex virus type 2. J. Virol., 18, 727-737.

CABRERA, C.V., WOHLENBERG, C., OPENSHAW, H., REY-MENDEZ, M., PUGA, A. and NOTKINS, A.L. (1980). Herpes simplex virus DNA sequences in the CNS of latently infected mice. Nature (London), 288, 288-290.

CAMACHO, A. and SPEAR, P.G. (1978). Transformation of hamster embryo fibroblasts by a specific fragment of the herpes simplex virus genome. Cell, 15, 993-1,002.

CAMPBELL, M.E.M., PALFREYMAN, J.W. and PRESTON, C.M. (1984). Identification of herpes simplex virus DNA sequences which encode a trans-acting polypeptide responsible for stimulation of immediate-early transcription. J. Mol. Biol., 180, 1-19.

CARADONNA, S.J. and CHENG, Y.-C. (1981). Induction of uracil-DNA glycosylase and dUTP nucleotidohydrolase activity in herpes simplex virus-infected human cells. J. Biol. Chem., 256, 9,834-9,837.

CASAREALE, D., SAIRENJI, T. and HUMPHREYS, R.E. (1984). Isolation and characterisation of an EBV superinfection-enhanced, host cell-coded, 53,000 dalton protein. Intervirology, 22, 24-31.

CASJENS, S. and KING, J. (1975). Virus assembly. Ann. Rev. Biochem., 44, 555-611.

CHANTLER, J.K. and STEVELY, W.S. (1973). Virus-induced proteins in pseudorabies-infected cells. I. Acid-extractable proteins of the nucleus. J. Virol., 11, 815-822.

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.

CHEBATH, J., MERLIN, G., METZ, R., BENECH, P. and REVEL, M. (1983). Interferon-induced 56,000 Mr protein and its mRNA in human cells: molecular cloning and partial sequence of cDNA. Nucleic Acids Res., 11, 1,213-1,226.

CHOU, J. and ROIZMAN, B. (1985). Isomerization of herpes simplex virus 1 genome: Identification of the cis-acting and recombination sites within the domain of the a sequence. Cell, 41, 803-811.

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.

CHU, C.T. and SCHAFFER, P.A. (1975). Qualitative complementation test for temperature-sensitive mutants of herpes simplex virus. J. Virol., 16, 1,131-1,136.

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., MCLAUCHLAN, J. and MCGEOCH, D.J. (1979). Orientation of herpes simplex virus type 1 immediate-early mRNAs. Nucleic Acids Res., 7, 77-91.

CLEMENTS, J.B., WATSON, R.J. and WILKIE, N.M. (1977). Temporal regulation of herpes simplex virus transcription: Location of transcripts on the viral genome. Cell, 12, 275-285.

COATES, J.A.V. (1982). Genetic and biochemical studies with temperature-sensitive mutants of herpes simplex virus type 1. Ph.D. Thesis. University of Glasgow.

COEN, D.M., GELEP, P.T., ASCHMAN, S.K., WELLER, S.K. and SCHAFFER, P.A. (1984). Fine mapping and molecular cloning of mutations at the herpes simplex virus DNA polymerase locus. J. Virol., 49, 236-247.

COHEN, G.H. (1972). Ribonucleotide reductase activity of synchronised KB cells infected with herpes simplex virus. J. Virol., 9, 408-418.

COHEN, G.H., DIETZSCHOLD, D., PONCE DE LEON, M., LONG, D., GOLUB, E., VARRICHIO, A., PEREIRA, L. and EISENBERG, R.J. (1984). Localisation and synthesis of an antigenic determinant of herpes simplex virus glycoprotein D that stimulates the production of neutralising antibody. J. Virol., 49, 102-108.

COHEN, G.H., PONCE DE LEON, M., DIGGLEMAN, H., LAWRENCE, W.C., VERNON, S.K. and EISENBERG, R.J. (1980). Structural analysis of the capsid polypeptides of herpes simplex virus types 1 and 2. J. Virol., 34, 521-531.

COHEN, S.N., CHUNG, A.C.Y. and HSU, L. (1972). Non-chromosomal antibiotic resistance in bacteria: Genetic transformation by R factor DNA. Proc. Natl. Acad. Sci., USA, 69, 2,110-2,114.

COLE, C.N. and SANTANGELO, G.M. (1983). Analysis in Cos-1 cells of processing and polyadenylation signals by using derivatives of the herpes simplex virus type 1 thymidine kinase gene. Mol. Cell. Biol., 3, 267-279.

COLE, C.N. and STACY, T.P. (1985). Identification of sequences in the herpes simplex virus thymidine kinase gene required for efficient processing and polyadenylation. Mol. Cell. Biol., 5, 2,104-2,113.

CONLEY, A.J., KNIPE, D.M., JONES, P.C. and ROIZMAN, B. (1981). Molecular genetics of herpes simplex virus. VII. Characterisation of a temperature-sensitive mutant produced by in vitro mutagenesis and defective in DNA synthesis and accumulation of alpha polypeptides. J. Virol., 37, 191-206.

COOK, M.L., BASTONE, V.B. and STEVENS, J.G. (1974). Evidence that neurons harbour latent herpes simplex virus. Infection and Immunity, 9, 946-951.

COOMBS, P.H. and EISERLING, F.A. (1977). Studies on the structure, protein composition and assembly of the neck of bacteriophage T4. J. Mol. Biol. 116, 375-405.

COOPER, G.M. (1973). Phosphorylation of 5-bromo-deoxy cytidine in cells infected with herpes simplex virus. Proc. Natl. Acad. Sci., USA, 12, 3,788-3,792.

CORDINGLEY, M.G., CAMPBELL, M.E.M. and PRESTON, C.M. (1983). Functional analysis of a herpes simplex type 1 promoter: Identification of far-upstream regulatory sequences. Nucleic Acids Res., 11, 2,347-2,365.

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, 996-1,001.

CROMBIE, I.K. (1975). Genetic and biochemical studies with herpes simplex virus type 1. Ph.D. 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.

DALZIEL, R.G. and MARSDEN, H.S. (1984). Identification of two herpes simplex virus type-1-induced proteins (21K and 22K) which interact specifically with the a sequence of herpes simplex virus DNA. J. Gen. Virol., 65, 1,467-1,475.

DARBY, G., FIELD, H.J. and SALISBURY, S.A. (1981). Altered substrate specificity of herpes simplex thymidine kinase confers acyclovir resistance. Nature (London), 289, 81-83.

DARGAN, D.J. and SUBAK-SHARPE, J.H. (1983). Ultrastructural characterisation of herpes simplex virus type 1 (strain 17) temperature-

sensitive mutants. J. Gen. Virol., 64, 1,311-1,326.

DARLINGTON, R.W. and MOSS, L.H. (1968). Herpesvirus envelopment. J. Virol., 2, 48-55.

DAVIDSON, I. and STOW, N.D. (1985). Expression of an immediate-early polypeptide and activation of a viral origin of DNA replication in cells containing a fragment of herpes simplex virus DNA. Virology, 141, 77-78.

DAVISON, A.J. (1981). Detailed structural aspects of the HSV genome. Ph.D. thesis, University of Glasgow.

DAVISON, A.J. and WILKIE, N.M. (1981). Nucleotide sequences of the joint between the L and S segments of herpes simplex types 1 and 2. J. Gen. Virol., 55, 315-331.

DAVISON, A.J. and WILKIE, N.M. (1983a). Location and orientation of homologous sequences in the genomes of five herpesviruses. J. Gen. Virol., 64, 1,927-1,942.

DAVISON, A.J. and WILKIE, N.M. (1983b). Inversion of the two segments of the herpes simplex virus genome in intertypic recombinants. J. Gen. Virol., 64, 1-18.

DAVISON, M.-J., PRESTON, V.G. and MCGEOCH, D.J. (1984). Determination of the sequence alteration in the DNA of the herpes simplex type 1 temperature sensitive mutant tsK. J. Gen. Virol., 65, 859-863.

DEBROY, C., PEDERSON, N. and PERSON, S. (1985). Nucleotide sequence of a herpes simplex virus type 1 gene that causes cell fusion. Virology,

145, 36-48.

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.

DELUCA, N., BZIK, D.J., BOND, V.C., PERSON, S. and SNIPES, W. (1982). Nucleotide sequences of herpes simplex virus type 1 (HSV-1) affecting virus entry, cell fusion and production of glycoprotein gB (VP7). Virology, 122, 411-423.

DELUCA, N., BZIK, D.J., PERSON, S. and SNIPES, W. (1981). Early events in herpes simplex virus type-1 infection: Photosensitivity of fluorescein isothiocyanate-treated virions. Proc. Natl. Acad. Sci., USA, 78, 912-916.

DELUCA, N.A., COURTNEY, M.A. and SCHAFFER, P.A. (1984). Temperature-sensitive mutants in herpes simplex virus type 1 ICP4 permissive for early gene expression. J. Virol., 52, 767-776.

DENHARDT, D.T. (1966). A membrane-filter technique for the detection of complementary DNA. Biochem. Biophys. Res. Commun., 23, 641-646.

DENNIS, D. and SMILEY, J.R. (1984). Transactivation of a late herpes simplex virus promoter. Molec. Cell. Biol., 4, 544-551.

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.

DOMS, R.W., HELENIUS, A. and WHITE, J. (1985). Membrane fusion activity of the influenza virus haemagglutinin: the low pH-induced conformational change. J. Biol. Chem., 260, 2,973-2,981.

DOWDLE, W.R., NAHMIAS, A.J., HARWELL, R.W. and PAULS, F.P. (1967). Association of antigenic type of herpes virus hominis with site of viral recovery. J. Imm., 99, 974-980.

DUBBS, D.R. and KIT, S. (1964). Mutant strains of herpes simplex virus deficient in thymidine kinase-inducing ability. Virology, 22, 493-502.

DULBECCO, R. and VOGT, M. (1954). Plaque formation and isolation of pure lines with poliomyelitis. J. Exp. Medicine, 99, 167-182.

DUTIA, B.M. (1983). Ribonucleotide reductase induced by herpes simplex virus has a virus-specified component. J. Gen. Virol., 64, 513-521.

DUYCKINK-SMITH, J. and de HARVEN, E. (1974). Herpes simplex virus and human cytomegalovirus replication in WI-38 cells. II. An ultrastructural study of viral penetration. J. Virol., 14, 945-956.

EARNSHAW, W., CASJENS, S. and HARRISON, S.C. (1976). Assembly of the head of bacteriophage P22: X-ray diffraction from heads, proheads and related structures. J. Mol. Biol., 104, 387-397.

EARNSHAW, W.C. and CASJENS, S.R. (1980). DNA packaging by the double-stranded DNA bacteriophages. Cell, 21, 319-331.

EBERLE, R. and COURTNEY, R.J. (1980). Preparation and

characterisation of specific antisera to individual glycoprotein antigens comprising the major glycoprotein region of herpes simplex virus type 1. J. Virol., 35, 902-917.

ECKER, J.R., KUDLER, L. and HYMAN, R.W. (1984). Variation in the structure of varicella-zoster virus DNA. Intervirology, 21, 25-37.

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, 41, 3,597-3,604.

EISENBERG, R.J., LONG, D., HOGUE-ANGELETTI, R. and COHEN, G.H. (1984). Amino-terminal sequence of glycoprotein D of herpes simplex virus types 1 and 2. J. Virol., 49, 265-268.

EISENBERG, S.P., COEN, D.M. and MCKNIGHT, S.L. (1985). Promoter domains required for expression of plasmid-borne copies of the herpes simplex virus thymidine kinase gene in virus-infected mouse fibroblasts and microinjected frog oocytes. Mol. Cell. Biol., 5, 1,940-1,947.

ELION, G.B., FURMAN, P.A., FYFE, J.A., MIRANDA, P. de, BEAUCHAMP, L. and SCHAEFFER, H.J. (1977). Selectivity of action of an antiherpetic agent, 9-(2-hydroxyethoxymethyl) guanine. Proc. Natl. Acad. Sci., USA, 74, 5,716-5,720.

ELKAREH, A., MURPHEY, A.J.M., FICHTER, T., EFSTRATIADIS, A. and SILVERSTEIN, S. (1985). "Transactivation" control signals in the promoter of the herpesvirus thymidine kinase gene. Proc. Natl. Acad. Sci., USA, 82, 1,002-1,006.

EMMONS, S.W. (1974). Bacteriophage lambda derivatives carrying two copies of the cohesive end site. J. Mol. Biol., 83, 511-525.

ESPARZA, J., PURIFOY, D.J.M., SCHAFFER, P.A. and BENYESH-MELNICK, M. (1974). Isolation, complementation and preliminary phenotypic characterisation of temperature-sensitive mutants of herpes simplex virus type 2. Virology, 57, 554-565.

EVERETT, R.D. (1983). DNA sequence elements required for regulated expression of the HSV-1 glycoprotein D gene lie within 83bp of the RNA cap site. Nucleic Acids Res., 11, 6,647-6,666.

EVERETT, R.D. (1984a). Trans-activation of transcription by herpes virus products: Requirement for two HSV-1 immediate-early polypeptides for maximum activity. EMBO J., 3, 3,135-3,141.

EVERETT, R.D. (1984b). A detailed analysis of an HSV-1 early promoter: Sequences involved in trans-activation by viral immediate-early gene products are not early specific. Nucleic Acids Res., 12, 3,037-3,056.

FALTYNEK, C.R., MCCANDLESS, S., CHEBATH, J. and BAGLIONI, C. (1985). Different mechanisms for activation of gene transcription by interferons alpha and gamma. Virology, 144, 173-180.

FARNAM, A.E. and NEWTON, A.A. (1959). The effect of some environmental factors on herpes virus grown in HeLa cells. Virology, 1, 449-461.

FEISS, M. and BECKER, A. (1983). DNA cutting and packaging. In:

"Lambda II". pp. 305-330. Ed. R. Hendrix, J. Roberts, F. Stahl and R. Weisberg. Cold Spring Harbor Laboratory Monograph Series.

FEISS, M. and WIDNER, N. (1982). Bacteriophage lambda DNA packaging: Scanning for the terminal cohesive end site during packaging. Proc. Natl. Acad. Sci., USA, 79, 3,498-3,502.

FELDMAN, L., BLANKENSHIP, M.L. and BEN-PORAT, T. (1981). Isolation and characterisation of a temperature-sensitive uncoating mutant of pseudorabies virus. J. Gen. Virol., 54, 333-342.

FENWICK, M., MORSE, L.S. and ROIZMAN, B. (1979). Anatomy of herpes simplex virus DNA. XI. Apparent clustering of functions affecting rapid inhibition of host DNA and protein synthesis. J. Virol., 29, 825-827.

FENWICK, M.L. and CLARK, J. (1982). Early and delayed shut-off of host protein synthesis in cells infected with herpes simplex virus. J. Gen. Virol., 61, 121-125.

FENWICK, M.L. and MCMENAMIN, M.M. (1984). Early virion-associated suppression of cellular protein synthesis by herpes simplex virus is accompanied by inactivation of mRNA. J. Gen. Virol., 65, 1,225-1,228.

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.

FIELD, H.J., DARBY, G. and WILDY, P. (1980). Isolation and characterisation of acyclovir-resistant mutants of herpes simplex virus. J. Gen. Virol., 49, 115-124.

FIELD, H.J. and WILDY, P. (1978). The pathogenicity of thymidine kinase deficient mutants of herpes simplex virus in mice. J. Hyg., 81, 267-277.

FINGEROTH, J.D., WEIS, J.J., TEDDER, T.F., STROMINGER, J.K., BIRO, P.A. and FEARON, D.T. (1984). Epstein-Barr virus receptor of human B lymphocytes is the C3d receptor CR2. Proc. Natl. Acad. Sci., USA, 81, 4,510-4,514.

FISHER, F.B. and PRESTON, V.G. (1986). Isolation and characterisation of herpes simplex virus type 1 mutants which fail to induce dUTPase activity. Virology. In Press.

FRAME, M.C., MCGEOCH, D.J., RIXON, F.J. and ORR, A.C. (1985a). The 10K virion phosphoprotein encoded by US gene 9 from herpes simplex virus type 1. Submitted for publication. (Virology).

FRAME, M.C., MARSDEN, H.S. and DUTIA, B.M. (1985). The ribonucleotide reductase induced by herpes simplex virus type 1 involves minimally a complex of two polypeptides (136K and 38K). J. Gen. Virol., 66, 1,581-1,587.

FRAME, M.C., MARSDEN, H.S. and MCGEOCH, D.J. (1985b). Novel herpes simplex virus type 1 glycoproteins identified by antiserum against a synthetic oligopeptide from the predicted gene US4 product. J. Gen. Virol. Submitted for publication.

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.

FREEMAN, M.J. and POWELL, K.L. (1982). DNA-binding properties of a herpes simplex virus immediate-early protein. J. Virol., 44, 1,084-1,087.

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. and ROIZMAN, B. (1971). Herpes simplex virus: Genome size and redundancy studies by renaturation kinetics. J. Virol., 8, 591-593.

FRIEDMAN, R.L., MANLY, S.P., MCMAHON, M., KERR, I.M. and STARK, G.R. (1984). Transcriptional and post-translational regulation of interferon induced gene expression in human cells. Cell, 38 745-755.

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.

FRINK, R.J., ANDERSON, K.P. and WAGNER, E.K. (1981). Herpes simplex virus type 1 HindIII fragment L encodes spliced and complementary mRNA species. J. Virol., 39, 559-572.

FRINK, R.J., DRAPER, K.G. and WAGNER, E.K. (1981). Uninfected cell polymerase efficiently transcribes early but not late herpes simplex virus type 1 mRNA. Proc. Natl. Acad. Sci., USA, 78, 6,139-6,143.

FRINK, R.J., EISENBERG, R., COHEN, G. and WAGNER, E.K. (1983). Detailed analysis of the portion of the herpes simplex virus type 1 genome

encoding glycoprotein C. J. Virol., 45, 634-647.

FULLER, A.O. and SPEAR, P.G. (1985). Specificities of monoclonal and polyclonal antibodies that inhibit adsorption of herpes simplex virus to cells and lack of inhibition by potent neutralising antibodies. J. Virol., 55, 475-482.

FURLONG, D. (1978). Direct evidence for six-fold symmetry of the herpesvirus hexon capsomere. Proc. Natl. Acad. Sci., USA, 75, 2,764-2,766.

FURLONG, D., SWIFT, H. and ROIZMAN, B. (1972). Arrangement of herpesvirus deoxyribonucleic acid in the core. J. Virol., 10, 1,071-1,074.

GALL, W.E. and EDELMAN, G.M. (1981). Lateral diffusion of surface molecules in animal cells and tissues. Science, 213, 903-905.

GALLOWAY, D.A., COPPLE, C.D. and MCDOUGALL, J.K. (1980). Analysis of viral DNA sequences in hamster cells transformed by herpes simplex virus type 2. Proc. Natl. Acad. Sci., USA, 77, 880-884.

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.

GALLOWAY, D.A. and MCDOUGALL, J.K. (1983). The oncogenic potential of herpes simplex viruses: Evidence for a 'hit and run' mechanism. Nature (London), 302, 21-24.

GALLOWAY, D.A., NELSON, J.A. and MCDOUGALL, J.K. (1984). Small fragments of herpesvirus DNA with transforming activity contain insertion

sequence-like structures. Proc. Natl. Acad. Sci., USA, 81, 4,736-4,740.

GIBSON, W. (1981). Structural and non-structural proteins of strain Colburn cytomegalovirus. Virology, 111, 516-537.

GIBSON, W. and ROIZMAN, B. (1971). Compartmentalisation of spermine and spermidine in herpes simplex virions. Proc. Natl. Acad. Sci., USA, 68, 2,818-2,821.

GIBSON, W. and ROIZMAN, B. (1972). Proteins specified by herpes simplex virus. VIII. Characterisation and composition of multiple capsid forms of subtypes 1 and 2. J. Virol., 10, 1,044-1,052.

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.

GLORIOSO, J.C., LEVINE, M., HOLLAND, T.C. and SZCZESIUL, M.S. (1980). Mutant analysis of herpes simplex virus-induced cell surface antigens: resistance to complement-mediated immune cytolysis. J. Virol., 35, 672-681.

GODSON, G.N. and VAPNEK, D. (1973). A simple method of preparing large amounts of OX174 RF supercoiled DNA. Biochim. Biophys. Acta., 299, 516-520.

GODOWSKI, P.J. and KNIPE, D.M. (1983). Mutations in the major DNA-binding protein gene of herpes simplex virus type 1 result in increased levels of viral gene expression. J. Virol., 47, 478-486.

GODOWSKI, P.J. and KNIPE, D.M. (1985). Identification of a herpes simplex virus function that represses late gene expression from parental viral genomes. J. Virol., 55, 357-365.

GOODPASTURE, E.W. (1929). Herpetic infection with especial reference to involvement of the nervous system. Medicine, 8, 223-243.

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, 679-681.

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., VELDHEISEN, G. and WILKIE, N.M. (1973). Infectious herpesvirus DNA. Nature New Biology, 245, 265-266.

GRAY, C.P. and KAERNER, H.C. (1984). Sequence of the putative origin of replication in the U_L region of herpes simplex virus type 1 ANG DNA. J. Gen. Virol., 65, 2,109-2,119.

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.

HAGUENAU, F. and MICHELSON-FISKE, S. (1975). Cytomegalovirus: Nucleocapsid assembly and core structure. Intervirology, 5, 293-299.

HALLIBURTON, I.W., RANDALL, R.E., KILLINGTON, R.A. and WATSON,

D.H. (1977). Some properties of recombinants between type 1 and type 2 herpes simplex viruses. J. Gen. Virol., 36, 471-484.

HALLIBURTON, I.W. and TIMBURY, M.C. (1973). Characterisation of temperature sensitive mutants of herpes simplex virus type 2. Growth and DNA synthesis. Virology, 54, 60-68.

HAMMARSKJOLD, M.L. and WINBERG, G. (1980). Encapsidation of adenovirus 16 DNA is directed by a small DNA sequence at the left end of the genome. Cell, 20, 787-795.

HANAHAN, D. (1983). Studies on transformation of Escherichia coli with plasmids. J. Mol. Biol., 166, 557-580.

HARRIS-HAMILTON, E. and BACHENHEIMER, S.L. (1985). Accumulation of herpes simplex virus type 1 RNAs of different kinetic classes in the cytoplasm of infected cells. J. Virol., 53, 144-151.

HAY, J., MOSS, H. and HALLIBURTON, I.W. (1971). Induction of deoxyribonucleic acid polymerase and deoxyribonuclease activities in cells infected with herpes simplex virus type 2. Biochem. J., 124, 64-71.

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., JACOB, R.J., WADSWORTH, S. and ROIZMAN, B. (1975). Anatomy of herpes simplex virus DNA. IV. Evidence for four populations of molecules differing in the relative orientation of their long and short components. Proc. Natl. Acad. Sci., USA, 72, 4,243-4,247.

HEILMAN, C.J., Jr. (1982). Antigenic and structural analysis of herpes simplex virus proteins p40 and p80. Ph.D. Thesis. John Hopkins University, Baltimore, USA.

HEILMAN, C.J., Jr., ZWEIG, M., STEPHENSON, J.R. and HAMPAR, B. (1979). Isolation of a nucleocapsid polypeptide of herpes simplex virus types 1 and 2 possessing immunologically type-specific and cross-reactive determinants. J. Virol., 29, 34-42.

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, J.W., SPEAR, P.G. and ROIZMAN, B. (1972). Proteins specified by herpes simplex virus. VII. Viral proteins in the plasma membrane. J. Virol., 9, 431-439.

HILL, T.M., SINDEN, R.R. and SADLER, J.R. (1983). Herpes simplex virus types 1 and 2 induce shut off of host protein synthesis by different mechanisms in friend erythroleukaemia cells. J. Virol., 45, 241-250.

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.

HOCHBERG, E. and BECKER, Y. (1968). Adsorption, penetration and uncoating of herpes simplex virus. J. Gen. Virol., 2, 231-241.

HOFFMAN, P.J. and CHENG, Y.C. (1978). The deoxyribonuclease induced after infection of KB cells by herpes simplex virus type 1 or type 2. I. Purification and characterisation of the enzyme. J. Biol. Chem., 253, 3,557-3,562.

HOFFMAN, P.J. and CHENG, Y.C. (1979). DNase induced after infection of KB cells by herpes simplex virus type 1 or type 2. II. Characterisation of an associated endonuclease activity. J. Virol., 32, 449-457.

HOLLAND, L.E., ANDERSON, K.P., SHIPMAN, C. 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.

HOLLAND, L.E., SANDRI-GOLDIN, R.M., GOLDIN, A.L., GLORIOSO, J.C. and LEVINE, M. (1984). Transcriptional and genetic analysis of the herpes simplex virus type 1 genome: co-ordinates 0.29-0.45. J. Virol., 49, 947-959.

HOLLAND, T.C., HOMA, F.L., MARLIN, S.D., LEVINE, M. and GLORIOSO, J. (1984). Herpes simplex virus type 1 glycoprotein C-negative mutants exhibit multiple phenotypes, including secretion of truncated glycoproteins. J. Virol., 52, 566-574.

HOLLAND, T.C., MARLIN, S.D., LEVINE, M. and GLORIOSO, J. (1983). Antigenic variants of herpes simplex virus selected with glycoprotein-specific monoclonal antibodies. J. Virol., 45, 672-682.

HOLMES, D.S. and QUIGLEY, M. (1981). A rapid boiling method for the preparation of bacterial plasmids. Anal. Biochem., 114, 193-197.

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; study of the functional organisation of the genome. J. Virol., 34, 716-742.

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, 1,347-1,365.

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 ROIZMAN, B. (1975). Regulation of herpesvirus macromolecular synthesis; sequential transition of polypeptide synthesis requires functional viral polypeptides. Proc. Natl. Acad. Sci., USA, 72, 1,276-1,280.

HONESS, R.W. and ROIZMAN, B. (1975). Proteins specified by herpes simplex virus. XIII. Glycosylation of viral polypeptides. J. Virol., 16, 1,308-1,326.

HONESS, R.W. and WATSON, D.H. (1977). Herpes simplex virus resistance and sensitivity to phosphonacetic acid. J. Virol., 21, 584-600.

HOPE, R.G. and MARSDEN, H.S. (1983). Processing of glycoproteins

induced by herpes simplex virus type 1: Sulphation and nature of oligosaccharide linkage. J. Gen. Virol., 64, 1,943-1,953.

HOPE, R.G., PALFREYMAN, J.W., SUH, M. and MARSDEN, H.S. (1982). Sulphated glycoproteins induced by herpes simplex virus. J. Gen. Virol., 58, 399-415.

HSU, M.-C., SCHEID, A. and CHOPPIN, P.W. (1981). Activation of the sendai virus protein (F) involves a conformational change with exposure of a new hydrophobic region. J. Biol. Chem., 256, 3,557-3,563.

HUGHES, R.G., Jr. and MUNYON, W.H. (1975). Temperature-sensitive mutants of herpes simplex virus type 1 defective in lysis but not in transformation. J. Virol., 16, 275-283.

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 characterisation of the ribonucleotide reductase induced by herpes simplex virus infection of mammalian cells. J. Virol., 37, 580-588.

HYMAN, R.W. (1980). Identification of proteins tightly bound to herpes simplex virus DNA. Virology, 105, 254-255.

HYMAN, R.W., OAKES, J.E. and KUDLER, L. (1977). In vitro repair of pre-existing nicks and gaps in herpes simplex virus DNA. Virology, 76, 286-294.

IHARA, S., LADIN, B.F. and BEN-PORAT, T. (1982). Comparison of the physical and genetic maps of pseudorabies virus shows that the genetic map is circular. Virology, 122, 268-278.

IRMIERE, A. and GIBSON, W. (1985). Isolation of human cytomegalovirus intranuclear capsids, characterisation of their protein constituents, and demonstration that the B-capsid assembly protein is also abundant in non-infectious enveloped particles. J. Virol., 56, 277-283.

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.

JACOB, R.J. and ROIZMAN, B. (1977). Anatomy of herpes simplex virus DNA. VIII. Properties of the replicating DNA. J. Virol., 23, 394-411.

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. (1976). Herpesvirus proteins: induction of nucleoside phosphotransferase activity after herpes simplex virus infection. J. Virol., 17, 1,056-1,059.

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.P. (1983).

Immortalisation and neoplastic transformation of normal diploid cells by defined cloned DNA fragments of herpes simplex virus type 2. Proc. Natl. Acad. Sci., USA, 80, 5,902-5,906.

JEAN, J.H. and BEN-PORAT, T. (1976). Appearance in vivo of single-stranded complementary ends on parental herpesvirus DNA. Proc. Natl. Acad. Sci., USA, 73, 2,674-2,678.

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.

JOHNSON, D.C. and SPEAR, P.G. (1982). Monensin inhibits the processing of herpes simplex virus glycoproteins, their transport to the cell surface, and the egress of virions from infected cells. J. Virol., 43, 1,102-1,112.

JOHNSON, D.C., WITTELS, M. and SPEAR, P.G. (1984). Binding to cells of virosomes containing herpes simplex virus type 1 glycoproteins and evidence for fusion. J. Virol., 52, 238-247.

JONES, K.A. and TIJAN, R. (1985). Sp1 binds to promoter sequences and activates herpes simplex virus 'immediate-early' gene transcription in vitro. Nature (London), 317, 179-182.

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.

JONES, R.J. and HYMAN, R.W. (1983). Specious hybridisation between

herpes simplex virus DNA and human cellular DNA. Virology, 131, 555-560.

JONGENEEL, C.V. and BACHENHEIMER, S.L. (1981). Structure of replicating herpes simplex virus DNA. J. Virol., 39, 656-660.

JONSSON, V., WELLS, A. and KLEIN, G. (1982). Receptors for the complement C3d component and the Epstein-Barr virus are quantitatively co-expressed on a series of B cell lines and their derived somatic cell hybrids. Cellular Immunology, 72, 263-273.

KAERNER, H.C., MAICHLE, I.B., OTT, A. and SCHRODER, C.H. (1979). Origin of two different classes of defective HSV-1 Angellotti DNA. Nucleic Acids Res., 6, 1,467-1,478.

KAERNER, H.C., OTT-HARTMAN, A., SCHATTER, R., SCHRODER,C. and GRAY, C. (1981). Amplification of a short nucleotide sequence in the repeat units of defective herpes simplex virus type 1 Angelotti DNA. J. Virol., 39, 75-81.

KAPLAN, A.S. and BEN-PORAT, T. (1964). Mode of replication of pseudorabies virus DNA. Virology, 23, 90-95.

KATSUMOTO, T., HIRANO, A., KURIMURA, T. and TAKAGI, A. (1981). In situ electron microscopical observation of cells infected with herpes simplex virus. J. Gen. Virol., 52, 267-278.

KEIR, H.M. and GOLD, E. (1963). Deoxyribonucleic acid nucleotidyl-transferase and deoxyribonuclease from cultured cells infected with herpes simplex virus. Biochem. Biophys. Acta., 72, 263-276.

KEIR, H.M., SUBAK-SHARPE, J.H., SHEDDEN, W.I.H., WATSON, D.H. and WILDY, P. (1966). Immunological evidence for a specific DNA polymerase produced after infection by herpes simplex virus. Virology, 30, 154-157.

KESSLER, S.W. (1975). Rapid isolation of antigens from cells with a staphylococcal protein A-antibody adsorbent: parameters of the interaction of antibody-antigen complexes with protein A. J. Immunol., 115, 1,617-1,624.

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.

KINCHINGTON, P.R., REINHOLD, W.C., CASEY, T.A., STRAUS, S.E., HAY, J. and RUYECHAN, W.T. (1985). Inversion and circularisation of the varicella-zoster virus genome. J. Virol., 56, 194-200.

KING, J., LENK, E.V. and BOTSTEIN, D. (1973). Mechanism of head assembly and DNA encapsidation in salmonella phage P22. II. Morphogenetic pathway. J. Mol. Biol., 80, 697-731.

KIT, S. and DUBBS, D.R. (1963). Acquisition of thymidine kinase activity by herpes simplex infected mouse fibroblast cells. Biochem. Biophys. Res. Comm., 11, 55-59.

KIT, S., DUBBS, D.R. and SCHAFFER, P.A. (1978). Thymidine kinase activity in biochemically transformed mouse cells after superinfection by thymidine-kinase-negative temperature-sensitive herpes simplex virus mutants. Virology, 85, 456-463.

KLEIN, R.J. (1976). Pathogenic mechanisms of recurrent herpes simplex virus infections. Archives of Virology, 51, 1-13.

KNIPE, D.M., BATTERSON, W., NOSAL, C., ROIZMAN, B. and BUCHAN, A. (1981). Molecular genetics of herpes simplex virus. VI. Characterisation of a temperature-sensitive mutant defective in the expression of all early viral gene products. J. Virol., 38, 539-547.

KNOPF, K.-W. (1979). Properties of herpes simplex virus DNA polymerase and characterisation of its associated exonuclease activity. Eur. J. Biochem., 98, 231-244.

KOMENT, R.W. and RAPP, F. (1975). Temperature-sensitive host range mutants of herpes simplex virus type 2. J. Virol., 15, 812-819.

KOMENT, R.W. and RAPP, F. (1975a). Variation in susceptibility of different cell types to temperature-sensitive host range mutants of herpes simplex virus type 2. Virology, 64, 164-169.

KOMENT, R.W. and RAPP, F. (1975b). In vivo characteristics of temperature-sensitive host range mutants of herpes simplex virus type 2. Intervirology, 5, 10-20.

KRISTIE, T.M. and ROIZMAN, B. (1984). Separation of sequences defining basal expression from those conferring alpha gene recognition within the regulatory domains of herpes simplex virus 1 alpha genes. Proc. Natl. Acad. Sci., USA, 81, 4,065-4,069.

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, 1,151-1,164.

LADIN, B.F., IHARA, S., HAMPL, H. and BEN-PORAT, T. (1982). Pathway of assembly of herpesvirus capsids: an analysis using DNA$^+$ temperature-sensitive mutants of pseudorabies virus. Virology, 116, 554-561.

LAEMMLI, O.K. and FAVRE, M. (1973). Maturation of the head of bacteriophage T4. I. DNA packaging events. J. Mol. Biol., 80, 575-599.

LANG, J.C., SPANDIDOS, D.A. and WILKIE, N.M. (1984). Transcriptional regulation of a herpes simplex virus immediate-early gene is mediated through an enhancer-type sequence. EMBO J., 3, 389-395.

LANGELIER, Y. and BUTTIN, G. (1981). Characterisation of ribonucleotide reductase induction in BHK-21/C13 syrian hamster cell line upon infection by herpes simplex virus (HSV). J. Gen. Virol., 57, 21-31.

LARDER, B.A. and DARBY, G. (1985). Selection and characterisation of acyclovir-resistant herpes simplex virus type 1 mutants inducing altered DNA polymerase activities. Virology, 146, 262-271.

LATHANGUE, N.B., SHRIVER, K., DAWSON, C. and CHAN, W.L. (1984). Herpes simplex virus infection causes the accumulation of a heat shock protein. EMBO J., 3, 267-277.

LECHNER, R.L. and KELLY, T.J. (1977). The structure of replicating adenovirus 2 DNA molecules. Cell, 12, 1,007-1,020.

LEE, G.T.-Y. and SPEAR, P.G. (1980). Viral and cellular factors that influence cell fusion by herpes simplex virus. Virology, 107, 402-414.

LEE, L.-F., NAZERIAN, K., WITTER, R.L., LEINBACH, S.S. and BOEZI, J.A. (1978). A phosphonoacetate-resistant mutant of herpesvirus of turkeys. J. Natl. Cancer Inst., 60, 1,141-1,146.

LEE, P.M., CHERRY, R.J. and BACHI, T. (1983). Correlation of rotational mobility and flexibility of Sendai virus spike glycoproteins with fusion activity. Virology, 128, 65-76.

LEINBACH, S.S., RENO, J.M., LEE, L.-F., ISBELL, A.F. and BOEZI, J. (1976). Mechanism of phosphonoacetate inhibition of herpes virus-induced DNA polymerase. Biochemistry, 15, 426-430.

LEMASTER, S. and ROIZMAN, B. (1980). Herpes simplex virus phosphoproteins. II. Characterisation of the virion protein kinase and of the polypeptides phosphorylated in the virion. J. Virol., 35, 798-811.

LITTLE, S.P., JOFRE, J.T., COURTNEY, R.J. and SCHAFFER, P.A. (1981). A virion-associated glycoprotein essential for infectivity of herpes simplex virus type 1. Virology, 115, 149-160.

LITTLE, S.P. and SCHAFFER, P.A. (1981). Expression of the syncytial (syn) phenotype in HSV-1 strain KOS: Genetic and phenotypic studies of mutants in two syn loci. Virology, 112, 686-702.

LOCKER, H. and FRENKEL, N. (1979). Structure and origin of defective genomes contained in serially passaged HSV type 1 (Justin). J. Virol., 29,

1,065-1,077.

LONBERG-HOLM, K., CROWELL, R.L. and PHILIPSON, L. (1976). Unrelated animal viruses share receptors. Nature (London) 259, 679-681.

MCCOMBS, R.M., BRUNSCHWIG, J.P., MIRKOVIC, R. and BENYESH-MELNICK, M. (1971). Electron microscopic characterisation of a herpes-like virus isolated from tree-shrews. Virology, 45, 816-820.

MCCREADY, S.J., GODWIN, J., MASON, D.W., BRAZELL, I.A. and COOK, P.R. (1980). DNA is replicated at the nuclear cage. J. Cell. Science, 46, 365-386.

MACDONALD, D. (1980). Herpes simplex virus-induced early proteins. Ph.D. Thesis. University of Glasgow.

MCDOUGALL, J.K., CRUM, C.P., FENOGLIO, C.M., GOLDSTEIN, L.C. and GALLOWAY, D.A. (1982). Herpesvirus-specific RNA and protein in carcinoma of the uterine cervix. Proc. Natl. Acad. Sci., USA, 79, 3,853-3,857.

MCGEOCH, D.J., DOLAN, A., DONALD, S. and RIXON, F.J. (1985). Sequence determination and genetic content of the short unique region in the genome of herpes simplex virus type 1. J. Mol. Biol., 181, 1-13.

MACHTIGER, N.A., PANCAKE, B.A., EBERLE, R., COURTNEY, R.J., TEVITHIA, S.S. and SCHAFFER, P.A. (1980). Herpes simplex virus glycoproteins; isolation of mutants resistant to immune cytolysis. J. Virol., 34, 336-346.

MACKEM, S. and ROIZMAN, B. (1980). Regulation of herpes-virus macromolecular synthesis: transcription-initiation sites and domains of alpha genes. Proc. Natl. Acad. Sci., USA, 77, 7,122-7,126.

MACKEM, S. and ROIZMAN, B. (1982a). Regulation of alpha genes of herpes simplex virus: the alpha 27 gene promoter-thymidine kinase chimera is positively regulated in converted L cells. J. Virol., 43, 1,015-1,023.

MACKEM, S. and ROIZMAN, B. (1982b). Structural features of the herpes simplex virus alpha gene 4,0 and 27 prmoter-regulatory sequences which confer alpha regulation on chimeric thymidine kinase genes. J. Virol., 44, 939-949.

MACKEM, S. and ROIZMAN, B. (1982c). Differentiation between alpha promoter and regulator regions of herpes simplex virus 1: the functional domains and sequence of a moveable alpha regulator. Proc. Natl. Acad. Sci., USA, 79, 4,917-4,921.

MCKNIGHT, S.L. (1980). The nucleotide sequence and transcript map of the herpes simplex virus thymidine kinase gene. Nucleic Acids Res., 8, 5,949-5,964.

MCKNIGHT, S.L., GAVIS, E.R., KINGSBURY, R. and AXEL, R. (1981). Analysis of transcriptional regulatory signals of the HSV thymidine kinase gene: identification of an upstream control region. Cell, 25, 385-395.

MCKNIGHT, S.L. and KINGSBURY, R. (1982). Transcriptional control signals of a eukaryotic protein-coding gene. Science, 217, 316-324.

MCKNIGHT, S.L., KINGSBURY, R., SPENCE, A. and SMITH, M. (1984).

The distal transcriptional signals of the herpes virus TK gene share a common hexonucleotide control sequence. Cell, 37, 253-262.

MCLAUCHLAN, J. and CLEMENTS, J.B. (1983). DNA sequence homology between two co-linear loci on the HSV genome which have different transforming abilities. EMBO J., 2, 1,953-1,961.

MCLAUCHLAN, J., GAFFNEY, D., WHITTON, J.L. and CLEMENTS, J.B. (1985). The consensus sequence YGTGTTYY located downstream from the AATAAA signal is required for efficient formation of mRNA 3' termini. Nucleic Acids Res., 13, 1,347-1,368.

MCLENNAN, J.L. and DARBY, G. (1980). Herpes simplex virus latency: the cellular location of virus in dorsal root ganglia and the fate of the infected cell following virus activation. J. Gen. Virol., 51, 233-243.

MACNAB, J.C.M., ORR, A. and LATHANGUE, N.B. (1985). Cellular proteins expressed in herpes simplex virus transformed cells also accumulate on herpes simplex virus infection. EMBO J., 4, 3,223-3,228.

MACNAB, J.C.M., VISSER, L., JAMIESON, A.J. and HAY, J. (1980). Specific viral antigens in rat cells transformed by herpes simplex virus type 2 and in rat tumours induced by inoculation of transformed cells. Cancer Res., 40, 2,074-2,079.

MACPHERSON, I. and STOKER, M. (1962). Polyoma transformation of hamster cell clones - an investigation of genetic factors affecting cell competence. Virology, 16, 147-151.

MAEDA, T., KAWASAKI, K. and OHNISHI, S. (1981). Interaction of

influenza virus haemaglutinin with target membrane lipids is a key step in virus-induced haemolysis and fusion at pH5.2. Proc. Natl. Acad. Sci., USA, 78, 4,133-4,137.

MAITLAND, N.J., KINROSS, J.H., BUSUTTIL, A., LUDGATE, S.M., SMART, G.E. and JONES, K.W. (1981). The detection of DNA tumour virus-specific RNA sequences in abnormal human cervical biopsies by in situ hybridisation. J. gen. Virol., 55, 123-127.

MANSERVIGI, R. (1974). Method for isolation and selection of temperature sensitive mutants of herpes simplex virus. Applied Microbiology, 27, 1,034-1,040.

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, 3,913-3,917.

MAO, J.C.H., ROBISHAW, E.E. and OVERBY, L.R. (1975). Inhibition of DNA polymerase activity from herpes simplex virus-infected W-38 cells by phosphonoacetic acid. J. Virol., 15, 1,281-1,283.

MARCIANO-CABRAL, F., 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.

MARK, G.E. and KAPLAN, A.S. (1971). Synthesis of proteins in cells infected with herpesvirus. VII. Lack of migration of structural viral proteins to the nucleus of arginine-deprived cells. Virology, 45, 53-60.

MARSDEN, H.S., BUCKMASTER, A., PALFREYMAN, J.W., HOPE, R.G. and

MINSON, A.C. (1984). Characterisation of the 92,000 dalton glycoprotein induced by herpes simplex virus type 2. J. Virol., 50, 547-550.

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., LANG, J., DAVISON, A.J., HOPE, R.G. and MACDONALD, D.M. (1982). Genomic location and lack of phosphorylation of the HSV immediate-early polypeptide IE12. J. Gen. Virol., 62, 17-27.

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.

MATTHEWS, R.E.F. (1982). Classification and nomenclature of viruses. Intervirology, 17, 4-181).

MATZ, B., SUBAK-SHARPE, J.H. and PRESTON, V.G. (1983). Physical mapping of temperature-sensitive mutations of herpes simplex virus type 1 using cloned restriction endonuclease fragments. J. Gen. Virol., 64, 2,261-2,270.

MESSER, L.I. (1978). A genetic and biochemical study of complementation between ts mutants of HSV-1 and HSV-2. Ph.D. thesis, University of Glasgow.

MIYAMOTO, K. and MORGAN, C. (1971). Structure and development of viruses as observed in the electron microscope. XI. Entry and uncoating of

herpes simplex virus. J. Virol., 8, 910-918.

MOCARSKI, E.S., DEISS, L.P. and FRENKEL, N. (1985). Nucleotide sequence and structural features of a novel U_S - a junction present in a defective herpes simplex virus genome. J. Virol., 55, 140-146.

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 genome inversions. Cell, 24, 555-565.

MOCARSKI, E.S. and ROIZMAN, B. (1981). Site-specific inversion sequence of the herpes simplex virus genome: Domain and structural features. Proc. Natl. Acad. Sci., USA, 78, 7,047-7,051.

MOCARSKI, E.S. and ROIZMAN, B. (1982). Structure and role of the herpes simplex virus DNA termini in inversion, circularisation and generation of virion DNA. Cell, 31, 89-97.

MOCARSKI, E.S. and ROIZMAN, B. (1982b). Herpes virus dependent amplification and inversion of cell-associated viral thymidine kinase gene flanked by a sequences and linked to an origin of DNA replication. Proc. Natl. Acad. Sci., USA, 79, 5,626-5,630.

MORGAN, C., ELLISON, S.A., ROSE, H.M. and MOORE, D.H. (1954). Structure and development of viruses as observed in the electron microscope. J. Exp. Med., 100, 195-202.

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-exonuclease 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.

MOSS, B., GERSHOWITZ, 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 virus 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.

MULLER, M.T., BOLLES, C.S. and PARRIS, D.S. (1985). Association of type 1 DNA topoisomerase with herpes simplex virus. J. Gen. Virol., 66, 1,565-1,574.

MURCHIE, M.-J. and MCGEOCH, D.J. (1982). DNA sequence analysis of immediate-early gene region of the herpes simplex virus type 1 genome (map co-ordinates 0.950-0.978). J. Gen. Virol., 62, 1-15.

MURIALDO, H. and BECKER, A. (1978). Head morphogenesis of complex double-stranded DNA bacteriophages. Microbiol. Rev., 42, 529-576.

NACHTIGAL, M., NACHTIGAL, S., LUNGEANEU, A. and MACNAB, J.C.M. (1982). Chromosome changes in rat embryo cell lines transformed by temperature-sensitive mutants and sheared DNA of herpes simplex virus. Cancer Genetics and Cytogenetics, 7, 313-326.

NAZERIAN, K. (1974). DNA configuration in the core of Marek's disease virus. J. Virol., 13, 1,148-1,150.

NII, S. (1971). Electron microscope observations on FL cells infected with herpes simplex virus. II. Envelopment. Biken J., 14, 325-348.

NII, S., MORGAN, C. and ROSE, H.M. (1968). Electron microscopy of herpes simplex virus. II. Sequence of development. J. Virol., 2, 517-536.

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, 2,370-2,374.

NISHIOKA, Y. and SILVERSTEIN, S. (1978). Requirement of protein synthesis for the degradation of host mRNA in Friend erythroleukaemia cells infected with HSV-1. J. Virol., 27, 619-627.

NOBLE, A.G., LEE, G.T.-Y., SPRAGUE, R., PARISH, M.L. and SPEAR, P.G. (1983). Anti-gD monoclonal antibodies inhibit cell fusion induced by herpes simplex virus type 1. Virology, 129, 218-224.

NOTARIANNI, E.L. and PRESTON, C.M. (1982). Activation of cellular

stress protein genes by herpes simplex virus temperature-sensitive mutants which overproduce immediate-early polypeptides. Virology, 123, 113-122.

O'HARE, P. and HAWYARD, G.S. (1984). Expression of recombinant genes containing herpes simplex virus delayed-early and immediate-early regulatory regions and trans-activation by herpesvirus infection. J. Virol., 52, 522-531.

O'HARE, P. and HAYWARD, G.S. (1985). Evidence for a direct role for both the 175,000- and 110,000- molecular weight immediate-early proteins of herpes simplex virus in the transactivation of delayed-early promoters. J. Virol., 53, 751-760.

OLOFFSON, S., LUNDSTROM, M., MARSDEN, H.S., JEANSSON, S. and VAHLNE, A. Characterisation of a herpes simplex virus type 2-specificed glycoprotein with affinity for N-acetyl galactosamine specific lectin and its identification as g92K or gG. J. Virol. Submitted for publication.

ONARATO, L., STIRMER, B. and SHOWE, M. (1978). Isolation and characterisation of bacteriophage T4 mutant preheads. J. Virol., 27, 409-426.

O'NEILL, F.J. and RAPP, F. (1971). Early events required for induction of chromosomal abnormalities in human cells by herpes simplex virus. Virology, 44, 544-553.

PALFREYMAN, J.W., HAARR, L., CROSS, A., HOPE, R.G. and MARSDEN, H.S. (1983). Processing of herpes simplex virus type-1 glycoproteins: Two-dimensional gel analysis using monoclonal antibodies. J. Gen. Virol., 64, 873-881.

PALMER, E.L., MARTIN, M.L. and GARY, G.W., Jr. (1975). The ultrastructure of disrupted herpesvirus nucleocapsids. Virology, 65, 260-265.

PANCAKE, B.A., ASCHMAN, D.P. and SCHAFFER, P.A. (1983). Genetic and phenotype analysis of herpes simplex virus type 1 mutants conditionally resistant to immune cytolysis. J. Virol., 47, 568-585.

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.

PARA, M.F., BAUCKE, R.B. and SPEAR, P.G. (1982). Glycoprotein gE of herpes simplex virus type 1: Effects of anti-gE on virion infectivity and on virus-induced Fc-binding receptors. J. Virol., 41, 129-136.

PARDOLL, D.M., VOGELSTEIN, B. and COFFEY, D.S. (1980). A fixed state of DNA replication in eukaryotic cells. Cell, 19, 527-536.

PARK, M., KITCHENER, H.C. and MACNAB, J.C.M. (1983). Detection of herpes simplex virus type-2 DNA sequences in human cervical carcinoma tissue. EMBO J, 2, 1,029-1,034.

PARRIS, D.S., COURTNEY, R.J. and SCHAFFER, P.A. (1978). Temperature-sensitive mutants of herpes simplex virus type 1 defective in transcriptional and post-transcriptional functions required for viral DNA synthesis. Virology, 90, 177-186.

PARSEGIAN, V.A., FULLER, N.L. and RAND, R.P. (1979). Measured work of deformation and repulsion of lipid bilayers. Proc. Natl. Acad. Sci., USA, 76, 2,750-2,754.

PEDEN, K., MOUNTS, P. and HAYWARD, G. (1982). Homology between mammalian cell DNA sequences and human herpesvirus genomes detected by a hybridization procedure with high complexity probe. Cell, 31, 71-80.

PERDUE, M.L., COHEN, J.C., KEMP, M.C., RANDALL, C.C. and O'CALLAGHAN, D.J. (1975). Characterisation of three species of nucleocapsids of equine herpesvirus type 1 (EHV-1). Virology, 64, 187-205.

PERDUE, M.L., COHEN, J.C., RANDALL, C.C. and O'CALLAGHAN, D.J. (1976). Biochemical studies of the maturation of herpesvirus nucleocapsid species. Virology, 74, 194-208.

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.

PERSSON, R., BACCHETTI, S. and SMILEY, J.R. (1985). Cells that constitutively express the herpes simplex virus immediate-early protein ICP4 allow efficient activation of viral delayed-early genes in trans. J. Virol., 54, 414-421.

PERTOFT, D.H. (1980). Herpes simplex virus - a simple purification procedure. Separation News, 3, 2-7.

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 tumicamycin on herpes simplex virus glycoproteins and infectious virus production. J. Virol., 34, 142-153.

POFFENBERGER, K.L. and ROIZMAN, B. (1985). Studies on non-inverting genome of a viable herpes simplex virus. I. Presence of head-to-tail linkages in packaged genomes and requirements for circularisation after infection. J. Virol., 53, 587-595.

POFFENBERGER, K.L., TABARES, E. and ROIZMAN, B. (1983). Characterisation of a viable non-inverting herpes simplex virus 1 genome derived by insertion and deletion of sequences at the junction of components L and S. Proc. Natl. Acad. Sci., USA, 80, 2,690-2,694.

POGUE-GEILE, K.L., LEE, G.T.-Y., SHAPIRA, S.K. and SPEAR, P.G. (1984). Fine mapping of mutations in the fusion-inducing MP strain of herpes simplex virus type 1. Virology, 136, 100-109.

POST, L.E., MACKEM, S. and ROIZMAN, B. (1981). Regulation of genes of herpes simplex virus: Expression of chimeric genes produced by fusion of thymidine kinase with alpha gene promoters. Cell, 24, 555-565.

POWELL, K.L. and COURTNEY, R.J. (1975). Polypeptides synthesized in herpes simplex virus type 2 infected Hep-2 cells. Virology, 66, 217-228.

POWELL, K.L., LITTLER, E. and PURIFOY, D.J.M. (1981). Non-structural proteins of herpes simplex virus. II. Major virus-specific DNA-binding protein. J. Virol., 39, 894-902.

POWELL, K.L. and PURIFOY, D.J.M (1977). Non-structural proteins of

herpes simplex virus. I. Purification of the induced DNA polymerase. J. Virol., 24, 618-626.

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., PURIFOY, D.J.M. and COURTNEY, R.J. (1975). The synthesis of herpes simplex virus proteins in the absence of virus DNA synthesis. Biochem. Biophys. Res. Commun., 66, 262-271.

PRESTON, C.M. (1977). The cell-free synthesis of herpesvirus-induced polypeptides. Virology, 78, 349-353.

PRESTON, C.M. (1979a). 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. (1979b). Abnormal properties of an immediate early polypeptide in cells infected with the herpes simplex virus type 1 mutant tsK. J. Virol., 32, 357-369.

PRESTON, C.M., CORDINGLEY, M.G. and STOW, N.D. (1984). Analysis of DNA sequences which regulate the transcription of a herpes simplex virus immediate-early gene. J. Virol., 50, 708-716.

PRESTON, C.M. and MCGEOCH, D.J. (1981). Identification and mapping of two polypeptides encoded within the herpes simplex virus type 1 thymidine kinase gene sequences. J. Virol., 38, 593-605.

PRESTON, C.M. and TANNAHILL, D. (1984). Effects of orientation and position on the activity of a herpes simplex virus immediate-early gene far-upstream region. Virology, 137, 439-444.

PRESTON, V.G. (1981). Fine-structure mapping of herpes simplex virus type 1 temperature-sensitive mutations within the short repeat region of the genome. J. Virol., 39, 150-161.

PRESTON, V.G., COATES, J.A.V. and RIXON, F.J. (1983). Identification and characterisation of a herpes simplex virus gene product required for encapsidation of virus DNA. J. Virol., 45, 1,056-1,064.

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.

PRESTON, V.G. and FISHER, F.B. (1984). Identification of the herpes simplex virus type 1 gene encoding the dUTPase. Virology, 138, 58-68.

PRESTON, V.G., PALFREYMAN, J.W. and DUTIA, B.M. (1984). Identification of a herpes simplex virus type 1 polypeptide which is a component of the virus-induced ribonucleotide reductase. J. Gen. Virol., 65, 1,457-1,466.

PUGA, A., CANTIN, E. and NOTKINS, A.L. (1982). Homology between murine and human cellular DNA sequences and the terminal repetition of the S component of herpes simplex virus type 1 DNA. Cell, 31, 81-87.

PUGA, A., ROSENTHAL, J.D., OPENSHAW, H. and NOTKINS, A.L. (1978).

Herpes simplex virus DNA and mRNA sequences in acutely and chronically infected trigeminal ganglia of mice. Virology, 89, 102-111.

PURIFOY, D.J.M., LEWIS, R.B. and POWELL, K.L. (1977). Identification of the herpes simplex virus DNA polymerase gene. Nature (London), 269, 621-623.

PURIFOY, D.J.M. and POWELL, K.L. (1976). DNA binding proteins induced by herpes simplex virus type 2 in HEp-2 cells. J. Virol., 19, 717-731.

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.

QUINLAN, M.P. and KNIPE, D.M. (1985). Stimulation of expression of a herpes simplex virus DNA-binding protein by two viral proteins. Mol. Cell. Biol., 5, 957-963.

QUINN, J.P. and MCGEOCH, D.J. (1985). DNA sequence of the region in the genome of herpes simplex virus type 1 containing the genes for DNA polymerase and the major DNA binding protein. Nucleic Acids Res., 13, 8,143-8,159.

RANDALL, R.E., HONESS, R.W. and O'HARE, P. (1983). Proteins specified by herpesvirus saimiri: Identification and properties of virus-specific polypeptides in productively infected cells. J. Gen. Virol., 64, 19-35.

READ, G.S. and FRENKEL, N. (1983). Herpes simplex virus mutants defective in the virion-associated shut-off of host polypeptide synthesis and exhibiting abnormal synthesis of alpha (immediate-early) viral polypeptides.

J. Virol., 46, 498-512.

READ, G.S., PERSON, S. and KELLER, P.M. (1980). Genetic studies of cell fusion induced by herpes simplex virus type 1. J. Virol., 35, 105-113.

REYES, G.R., LAFEMINA, R., HAYWARD, S.D. and HAYWARD, G.S. (1979). Morphological transformation by DNA fragments of human herpesviruses: Evidence for two transforming regions and lack of correlation with biochemical transfer of the thymidine kinase gene. Cold Spring Harbor Symp. Quant. Biol., 44, 629-641.

RITCHIE, D.A., BROWN, S.M., SUBAK-SHARPE, J.H. and JAMIESON, A.T. (1977). Heterozygosis and genetic recombination in herpes simplex virus type 1. Virology, 82, 323-333.

RIXON, F.J. (1977). Studies on herpes simplex virus DNA synthesis. Ph.D. Thesis. University of Glasgow.

RIXON, F.J. and BEN-PORAT, T. (1979). Structural evolution of the DNA of pseudorabies-defective viral particles. Virology, 97, 151-163.

RIXON, F.J. and CLEMENTS, J.B. (1982). Detailed structural analysis of two spliced immediate-early mRNAs. Nucleic Acids Res., 10, 2,241-2,256.

RIXON, F.J. and MCGEOCH, D.J. (1984). A 3' co-terminal family of mRNAs from the herpes simplex virus type 1 short region: two overlapping reading frames encode unrelated polypeptides, one of which has a highly reiterated amino acid sequence. Nucleic Acids Res., 12, 2,473-2,487.

ROBINSON, A.J., YOUNGHUSBAND, H.B. and BELLETT, A.J.D. (1973). A

circular DNA-protein complex from adenovirus. Virology, 56, 54-69.

ROCK, D.L. and FRASER, N.W. (1983). Detection of HSV-genome in central nervous system of latently infected mice. Nature (London), 302, 523-525.

ROCK, D.L. and FRASER, N.W. (1985). Latent herpes simplex virus type 1 DNA contains two copies of the virion DNA joint region. J. Virol., 55, 849-852.

ROIZMAN, B. (1962). Polykaryocytosis induced by viruses. Proc. Natl. Acad. Sci., USA, 48, 228-233.

ROIZMAN, B. (1962a). Polykaryocytosis. Cold Spring Harbor Symp. Quant. Biol., 27, 327-342.

ROIZMAN, B. (1969). The herpesviruses - a biochemical definition of the group. In: "Current Topics in Microbiology and Immunology", 49, pp. 3-79.

ROIZMAN, B. (1979). The structure and isomerisation of herpes simplex virus genomes. Cell, 16, 481-494.

ROIZMAN, B. and AURELIAN, L. (1965). Abortive infection in canine cells by herpes simplex virus. I. Characterisation of viral progeny from co-operative infection with mutants differing in capacity to multiply in canine cells. J. Mol. Biol., 11, 528-538.

ROIZMAN, B., BORMAN, G.S. and KAMALI-ROUSTA, M. (1965). Macromolecular synthesis in cells infected with herpes simplex virus. Nature (London), 206, 1,374-1,375.

ROIZMAN, B. and FURLONG, D. (1974). The replication of herpesviruses. In: "Comprehensive Virology", Vol. 3, Eds. H. Frenkel-Conrat and R.R. Wagner. pp. 229-403. Plenum Press, New York and London.

ROIZMAN, B., NORRILD, B., CHAN, C. and PEREIRA, L. (1984). Identification and preliminary mapping with monoclonal antibodies of a herpes simplex virus 2 glycoprotein lacking a known type 1 counterpart. Virology, 133, 242-251.

ROIZMAN, B. and ROANE, P.R., Jr. (1964). The multiplication of herpes simplex virus. II. The relationship between protein synthesis and the duplication of viral DNA in infected HEp-2-cells. Virology, 22, 262-269.

ROSENTHAL, K.S., LEUTHER, M.D. and BARISAS, B.G. (1984). Herpes simplex virus binding and entry modulate cell surface protein mobility. J. Virol., 49, 980-983.

RUBENSTEIN, A.S., GRAVELL, M. and DARLINGTON, R. (1972). Protein kinase activity in enveloped herpes simplex virions. Virology, 50, 287-290.

RUSSELL, J. and PRESTON, C.M. (1986). An in vitro latency system for herpes simplex virus type 2. Virology. In press.

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 the genetic loci specifying the social behaviour of infected cells. J. Virol., 29, 677-697.

SACKS, W.R., GREENE, C.C., ASCHMAN, D.P. and SCHAFFER, P.A.

(1985). Herpes simplex virus type 1 ICP27 is an essential regulatory protein. J. Virol., 55, 796-805.

SANDRI-GOLDIN, R.M., GOLDIN, A.L., HOLLAND, L.E., GLORIOSO, J.C. and LEVINE, M. (1983). Expression of herpes simplex virus beta and gamma genes integrated in mammalian cells and their induction by an alpha gene product. Mol. Cell. Biol., 3, 2,028-2,044.

SANDRI-GOLDIN, R.M., LEVINE, M. and GLORIOSO, J. (1981). Method of induction of mutations in physically defined regions of the herpes simplex virus genome. J. Virol., 38, 41-49.

SANDERS, P.G., WILKIE, N.M. and DAVISON, A.J. (1982). Thymidine kinase deletion mutants of herpes simplex virus type 1. J. Gen. Virol., 63, 277-284.

SANGER, F., COULSON, A.R., BARRELL, B.G., SMITH, A.J.H. and ROE, B. (1980). Cloning in single-stranded bacteriophage as an aid to rapid DNA sequencing. J. Mol. Biol., 143, 161-178.

SARMIENTO, M., HAFFEY, M. and SPEAR, P.G. (1979). Membrane proteins specified by herpes simplex virus. III. Role of glycoprotein VP7 (B_2) in virion infectivity. J. Virol., 29, 1,149-1,158.

SCHAFFER, P.A. (1985). Temperature-sensitive and other mutants of HSV. Personal communication.

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 characterisation. Virology, 52, 57-71.

SCHAFFER, P.A., BRUNSCHWIG, J.P., MCCOMBS, R.M. and BENYESH-MELNICK, M. (1974). Electron microscopic studies of temperature-sensitive mutants of herpes simplex virus type 1. Virology, 62, 444-457.

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.

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., VONKA, V., LEWIS, R. and BENYESH-MELNICK, M. (1970). Temperature-sensitive mutants of herpes simplex virus. Virology, 42, 1,144-1,146.

SCHEK, N. and BACHENHEIMER, S.L. (1985). Degradation of cellular mRNAs induced by a virion-associated factor during herpes simplex virus infection of Vero cells. J. Virol., 55, 601-610.

SCHLEGEL, R., WILLINGHAM, M.C. and PASTAN, I.H. (1982). Saturable binding sites for vesicular stomatitis virus on the surface of Vero cells. J. Virol., 43, 871-875.

SCHLOMAI, J., FRIEDMANN, A. and BECKER, Y. (1976). Replicative intermediates of herpes simplex virus DNA. Virology, 69, 647-659.

SCHRODER, C.H., STEGMANN, B., LAUPPE, H.F. and KAERNER, H.C. (1975). An unusual defective genotype derived from herpes simplex virus

strain ANG. Intervirology, 6, 270-284.

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.

SERWER, P. (1976). Internal proteins of bacteriophage T7. J. Mol. Biol., 107, 271-291.

SHELDRICK, P. and BERTHELOT, N. (1974). Inverted repetitions in the chromosome of herpes simplex virus. Cold Spring Harbor Symp. Quant. Biol., 39, 667-678.

SHOWE, M. and ONARATO, L. (1978). A kinetic model for form determination of the head of bacteriophage T4. Proc. Natl. Acad. Sci., USA, 75, 4,165-4,169.

SHULMAN, M., WILDE, C.D. and KOHLER, G. (1978). A better cell line for making hybridomas secreting specific antibodies. Nature (London), 276, 269-270.

SILVER, S. and ROIZMAN, B. (1985). γ_2-thymidine kinase chimeras are identically transcribed but regulated as γ_2 genes in herpes simplex virus genomes and as beta genes in cell genomes. Mol. Cell. Biol., 5, 518-528.

SILVERSTEIN, S. and ENGLEHARD, T. (1979). Alterations in the protein synthetic apparatus of cells infected with herpes simplex virus. Virology, 95, 334-342.

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., FONG, B. and LEUNG, W.C. (1981). Construction of a double-jointed herpes simplex virus DNA molecule: inverted repeats are required for segment inversion and direct repeats promote deletions. Virology, 111, 345-362.

SOUTHERN, E.M. (1975). Detection of specific sequences among DNA fragments separated by electrophoresis. J. Mol. Biol., 98, 503-518.

SPAETE, R.R. and FRENKEL, N. (1982). The herpes simplex virus amplicon: A new eukaryotic defective-virus cloning amplification vector. Cell, 30, 295-304.

SPAETE, R.R. and FRENKEL, N. (1985). The herpes simplex virus amplicon: Analyses of cis-acting replication functions. Proc. Natl. Acad. Sci., USA, 82, 694-698.

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, 991-1,008.

SPEAR, P.G. and ROIZMAN, B. (1972). Proteins specified by herpes simplex virus. V. Purification and structural proteins of the herpes virion. J. Virol., 9, 143-159.

STEINMAN, R.A., BRODIE, S.E. and COHN, Z.A. (1976). Membrane flow during pinocytosis. A stereological analysis. J. Cell. Biol., 68, 665-687.

STENBERG, R.M. and PIZER, L.I. (1982). Herpes simplex virus-induced changes in cellular and adenovirus RNA metabolism in an adenovirus 5-transformed cell line. J. Virol., 42, 474-487.

STEVELY, W.S., KATAN, M., STIRLING, V., SMITH, G. and LEADER, D.P. (1985). Protein kinase activities associated with the virions of pseudorabies and herpes simplex virus. J. Gen. Virol., 66, 661-673.

STEVENS, J.G. and COOK, M.L. (1971). Latent herpes simplex virus in spinal ganglia of mice. Science, 173, 843-845.

STEVENS, J.G. and COOK, M.L. (1973). Latent herpes simplex virus in sensory ganglia. In: Perspectives in Virology, Vol, VIII. pp.171-188, Ed. M. Pollard, Academic Press, NY.

STOW, N.D. (1978). Physical mapping of herpes simplex virus type 1 mutations. Ph.D. thesis, University of Glasgow.

STOW, N.D. (1982). Localization of an origin of DNA replication within the TR_S/IR_S repeated region of the herpes simplex virus type 1 genome. EMBO J., 1, 863-867.

STOW, N.D. and MCMONAGLE, E. (1983). Characterisation of the TR_S/IR_S origin of DNA replication of herpes simplex virus type 1. Virology, 130, 427-438.

STOW, N.D., MCMONAGLE, E. and DAVISON, A.J. (1983). Fragments from both termini of the herpes simplex virus type 1 genome contain signals required for the encapsidation of viral DNA. Nucleic Acids Res., 11, 8,205-8,220.

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.

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.

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, J.H. (1969). In: Proceedings of the First International Congress for Virology, Helsinki, 1968, p.252. Ed. J.L. Melnick, Karger, Basel.

SUBAK-SHARPE, J.H., BURK, 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, 737-747.

SUH, M., KESSOUS, A., POIRIER, N. and SIMARD, R. (1980). Immunoprecipitation of polypeptides from hamster embryo cells transformed by herpes simplex virus type 2. Virology, 104, 303-311.

SUSSENBACK, J.S. and KUIJK, M.G. (1978). Studies on the replication of adenovirus DNA. VI. Localisation of the origins of the displacement synthesis. Virology, 84, 509-517.

SVENNERHOLM, B., VAHLNE, A., JEANSSON, S., LUNDEN, R., OLOFSSON, S., SVANTESSON, G. and LYCKE, E. (1980). Separation of herpes simplex virus virions and nucleocapsids on Percoll gradients. J. Virological Methods, 1, 303-309.

SWANSTROM, R.I., PIVO, K. and WAGNER, E.K. (1975). Restricted transcription of the herpes simplex virus genome occurring early after infection and in the presence of metabolic inhibitors. Virology, 66, 140-150.

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.

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. (1967). The disaggregation of host polysomes in productive and abortive infection with herpes simplex virus. Virology, 32, 678-686.

TAKADA, K. and OSATO, T. (1981). Epstein-Barr virus susceptibility of B cells correlates to the presence of complement receptors but not surface immunoglobulins. Intervirology, 16, 118-122.

TAYA, Y., DEVOS, R., TAVERNIER, J., CHEROUTRE, H., ENGLER, G. and FIERS, W. (1982). Cloning and structure of the human immune interferon-chromosomal gene. EMBO J., 1, 953-958.

TERNI, M. and ROIZMAN, B. (1970). Variability of herpes simplex virus:

Isolation of two variants from simultaneous eruptions at different sites. J. Infect. Dis., 121, 212-220.

TIMBURY, M.C. (1971). Temperature-sensitive mutants of herpes simplex virus type 2. J. Gen. Virol., 13, 373-376.

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.

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., THERAULT, A. and ELTON, R.A. (1974). A stable syncytial mutant of herpes simplex type 2 virus. J. Gen. Virol., 23, 219-224.

TOGNON, M., FURLONG, D., CONLEY, A. and ROIZMAN, B. (1981). Molecular genetics of herpes simplex virus. V. Characterisation of a mutant defective in ability to form plaques at low temperatures and in a viral function which prevents accumulation of coreless capsids at nuclear pores late in infection. J. Virol., 40, 870-880.

VAHLNE, A., NYSTROM, B., SANDBERG, M., HAMBERGER, A. and LYCKE, E. (1978). Attachment of herpes simplex virus to neurons and glial cells. J. Gen. Virol., 40, 359-371.

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.

VARMUZA, S.L. and SMILEY, J.R. (1985). Signals for site-specific cleavage of herpes simplex virus DNA: Maturation involves two separate cleavage events at sites distal to the recognition sequences. Cell, 41, 793-802.

VAUGHAN, P.J., PURIFOY, D.J.M. and POWELL, K.L. (1985). DNA-binding protein associated with herpes simplex virus DNA polymerase. J. Virol., 53, 501-508.

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.

VERNON, S.K., LAWRENCE, W.C., COHEN, G.H., DURSO, M. and RUBIN, B.A. (1976). Morphological components of herpesviruses. II. Preservation of virus during negative staining procedures. J. Gen. Virol., 31, 183-191.

VERNON, S.K., LAWRENCE, W.C., LONG, C.A., RUBIN, B.A. and SHEFFIELD, J.B. (1982). Morphological components of herpesvirus. IV. Ultrastructural features of the envelope and tegument. J. Ultrastructure Research, 81, 163-171.

VERNON, S.K., PONCE DE LEON, M., COHEN, G.H., EISENBERG, R.J. and RUBIN, B.A. (1981). Morphological components of herpesvirus. III. Localization of herpes simplex virus type 1 nucleocapsid polypeptides by immune electron microscopy. J. Gen. Virol., 54, 39-46.

VLAZNY, D. and FRENKEL, N. (1981). Replication of herpes simplex virus

DNA: Localisation of replication recognition signals within defective genomes. Proc. Natl. Acad. Sci., USA, 78, 742-746.

VLAZNY, D., KWONG, A. and FRENKEL, N. (1982). Site-specific cleavage/packaging of herpes simplex virus DNA and the selective maturation of nucleocapsids containing full-length viral DNA. Proc. Natl. Acad. Sci., USA, 79, 1,423-1,427.

WADSWORTH, S., HAYWARD, G.S. and ROIZMAN, B. (1976). Anatomy of herpes simplex virus DNA. V. Terminal repetitive sequences. J. Virol., 17, 503-512.

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, 1,487-1,497.

WAGNER, E.K. and ROIZMAN, B. (1969). 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., TEWARI, K.K., KOLODNER, R. and WARNER, R.C. (1974). The molecular size of the herpes simplex virus type 1 genome. Virology, 57, 436-447.

WAGNER, M.J., SHARPE, J.A. and SUMMERS, W.C. (1981). Nucleotide sequence of the thymidine kinase gene of herpes simplex virus type 1. Proc. Natl. Acad. Sci., USA, 78, 1,441-1,445.

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.

WATSON, D.H. (1973). Morphology. In: Kaplan: The Herpesviruses, pp.27-43. Academic Press, New York.

WATSON, R.J. and CLEMENTS, J.B. (1978). Characterisation of transcription-deficient temperature-sensitive mutants of herpes simplex virus type 1. Virology, 91, 364-379.

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, 329-330.

WATSON, R.J., PRESTON, C.M. and CLEMENTS, J.B. (1979). Separation and characterisation of herpes simplex virus type 1 immediate-early mRNAs. J. Virol., 31, 42-52.

WATSON, R.J., SULLIVAN, M. and VANDE-WOUDE, G.F. (1981). Structures of two spliced HSV-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.

WATSON, R.J. and VANDE-WOUDE, G.F. (1982). DNA sequence of an immediate-early gene (IE mRNA 5) of herpes simplex virus type 1. Nucleic Acids Res., 10, 979-991.

WEBER, J.M., DERY, C.V., MIRZA, M.A. and HORVATH, J. (1985). Adenovirus DNA synthesis is coupled to virus assembly. Virology, 140, 351-359.

WEISSBACH, A., HONG, L.S.-C., AUKER, J. and MULLER, R. (1973). Characterisation of herpes simplex virus-induced deoxyribonucleic acid polymerase. J. Biol. Chem., 248, 6,270-6,277.

WELLER, S.K., SPADARO, A., SCHAFFER, J.E., MURRAY, A.W., MAXAM, A.M. and SCHAFFER, P.A. (1985). Cloning, sequencing and functional analysis of ori_L, a herpes simplex virus type 1 origin of DNA synthesis. Mol. Cell. Biol., 5, 930-942.

WELLS, A., KOIDE, N., EGGERTSEN, G., LUNDWALL, A., GODAL, T., STEEN, H.B. and KLEIN, G. (1984). Epstein-Barr virus binding to virus-carrying cell lines is enhanced in the presence of C3 and C3d. J. Gen. Virol., 65, 507-513.

WHITTON, J.L. and CLEMENTS, J.B. (1984). The junctions between the repetitive and short unique sequences of the herpes simplex virus genome are determined by the polypeptide-coding regions of two spliced immediate-early mRNAs. J. Gen. Virol., 65, 451-466.

WHITTON, J.L., RIXON, F.J., EASTON, A.J. and CLEMENTS, J.B. (1983). Immediate-early mRNA-2 of herpes simplex viruses 1 and 2 is unspliced: Conserved sequences around the 5' and 3' termini correspond to transcription regulatory signals. Nucleic Acids Res., 11, 6,271-6,287.

WIGDAHL, B.L., ISOM, H.C., DE CLERQ, E and RAPP, F. (1982). Activation of herpes simplex virus (HSV) type 1 genome by temperature sensitive mutants of HSV type 2. Virology, 116, 468-479.

WIGDAHL, B.L., SCHECK, A.C., DE CLERQ, E. and RAPP, F. (1982a). High efficiency latency and reactivation of HSV in human cells. Science,

214, 1,145-1,146.

WIGDAHL, B.L., SCHECK, A.C., ZIEGLER, R.J., DE CLERQ, E. and RAPP, F. (1984). Analysis of the herpes simplex virus genome during in vitro latency in human diploid fibroblasts and rat sensory neurons. J. Virol., 49, 205-213.

WILCOX, K.W., KOHN, A., SKLYNSKAYA, E. and ROIZMAN, B. (1980). Herpes simplex virus phosphoproteins. I. Phosphate cycles on and off some viral polypeptides can alter their affinity for DNA. J. Virol., 3, 167-182.

WILDY, P. (1955). Recombination with herpes simplex virus. J. Gen. Micro., 13, 346-360.

WILDY, P., RUSSELL, W.C. and HORNE, R.W. (1960). The morphology of herpes virus. Virology, 12, 204-222.

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. (1976). Physical maps for herpes simplex virus type 1 DNA for restriction endonucleases HindIII, HpaI and XbaI. J. Virol., 20, 222-233.

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., DAVISON, A.D., CHARTRAND, P., STOW, N.D., PRESTON, V.G. and TIMBURY, M.C. (1978). Recombination in herpes simplex virus:

Mapping of mutations and analysis of intertypic recombinants. Cold Spring Harbor Symp. Quant. Biol., 43, 827-840.

WILLIAMS, M.V., Jr. (1984). Demonstration of a herpes simplex virus type 2-induced deoxyuridine triphosphate nucleotidohydrolase in infected KB cells and in biochemically transformed HeLa cells. J. Gen. Virol., 65, 209-213.

WOHLRAB, F. and FRANCKE, B. (1980). Deoxyribopyrimidine triphosphatase activity specific for cells infected with herpes simplex virus type 1. Proc. Natl. Acad. Sci., USA, 80, 100-104.

WOHLRAB, F., GARRETT, B.K. and FRANCKE, B. (1982). Control of expression of the herpes simplex virus-induced deoxypyrimidine triphosphatase in cells infected with mutants of herpes simplex virus types 1 and 2 and intertypic recombinants. J. Virol., 43, 935-942.

WU, M., HYMAN, R.W. and DAVISON, N. (1979). Electron microscopic mapping of proteins bound to herpes simplex virus DNA. Nucleic Acids Res., 6, 3,427-3,441.

WUNNER, W.H., REAGAN, K.J. and KOPROWSKI, H. (1984). Characterisation of saturable binding sites for rabies virus. J. Virol., 50, 691-697.

YAMAMOTO, S. and KABUTA, H. (1976). Genetic analysis of polykaryocytosis by herpes simplex virus. II. Recombination between viruses with non-fusing and fusing ability. Kurume Medical Journal, 23, 209-217.

YAMAMOTO, S., KABUTA, H. and SVENAGA, Y. (1972). Mutants of

herpes simplex virus. II. IdU-resistant mutants and a preliminary experiment on genetic recombination. Kurume Medical Journal, 19, 237-243.

ZEZULAK, K.M. and SPEAR, P.G. (1983). Characterisation of a herpes simplex virus type 2 75,000 - molecular weight glycoprotein antigenically related to herpes simplex virus type 1 glycoprotein C. J. Virol., 47, 553-562.

ZIPSER, D., LIPSICH, L. and KWOH, J. (1981). Mapping functional domains in the promoter region of the herpes thymidine kinase gene. Proc. Natl. Acad. Sci., USA, 78, 6,276-6,280.

ZWEIG, M., HEILMAN, C.J., RABIN, H. and HAMPAR, B. (1980). Shared antigenic determinants between two distinct classes of proteins in cells infected with herpes simplex virus. J. Virol., 35, 644-652.

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Characterisation of a Herpes Simplex Virus Type 1 Mutant Which Has a Temperature-Sensitive Defect in Penetration of Cells and Assembly of Capsids

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A herpes simplex virus type 1 (HSV-1) mutant, *ts*1204, which has a temperature-sensitive (*ts*) mutation located within genome map coordinates 0.318 to 0.324, close to but outside the coding sequences of the glycoprotein gB gene, has been characterised. Although this mutant adsorbed to the cell surface at the nonpermissive temperature (NPT), it failed to penetrate the cell membrane. As a consequence of this defect, high multiplicities of infection of *ts*1204 blocked subsequent infection of cells by wild-type HSV-1. By contrast, at the NPT, superinfection of cells with HSV-2 was not inhibited by prior infection with *ts*1204. The penetration defect could be overcome either by brief incubation of mutant virus-infected cells at the permissive temperature, or by treatment of the cells with polyethylene glycol, a compound which promotes fusion of membranes. Upon continued incubation of *ts*1204-infected cells at the NPT, low numbers of capsids were assembled. Although these capsids all had some internal structure, they did not contain DNA. Another mutant, *ts*1208, which lies in the same complementation group as *ts*1204, penetrated cells normally at the NPT, but like *ts*1204, had a defect in the formation of functional capsids. Evidence presented in this paper suggests that the gene in which the *ts*1204 and *ts*1208 lesions map encodes a structural polypeptide.

INTRODUCTION

The virion of herpes simplex virus type 1 (HSV-1) has a complex structure consisting of an icosahedral-shaped capsid within an envelope. Inside the capsid is a cylindrical core around which DNA is wound (Furlong *et al.*, 1972). Between the envelope and the nucleocapsid is an ill-defined structure known as the tegument (Roizman and Furlong, 1974). Although over 30 structural polypeptides have been identified in the HSV virion (Heine *et al.*, 1974; Marsden *et al.*, 1978) the functions of very few are known. The best characterised of these are the envelope glycoproteins which have been implicated in virus attachment and penetration.

Evidence that glycoprotein gB is involved in virus penetration of host cells is based largely on the analysis of two HSV-1 mutants, *ts*B5 and *ts*J12, belonging to complementation group 1-9 (Schaffer *et al.*, 1978; Sarmiento *et al.*, 1979; Little *et al.*, 1981; DeLuca *et al.*, 1982). These mutants fail to process gB to its mature forms at the nonpermissive temperature (NPT) and, as a consequence, produce virions that adsorb to cells but are unable to penetrate. Treatment of these virus-infected cells with polyethylene glycol (PEG), an agent which promotes membrane fusion, enabled the aberrant virions to enter cells and replicate. These temperature-sensitive (*ts*) mutations, together with the syncytial lesion in *ts*B5 and a locus affecting the rate of entry of virus, map within the structural gene of gB

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(Little *et al.*, 1981; DeLuca *et al.*, 1982; Holland *et al.*, 1983; Bzik *et al.*, 1984). Recent work by Epstein *et al.* (1984) has suggested that glycoprotein gC may also have a role in cell fusion. These workers found that HSV-1 strains which synthesized low levels of gC relative to gB in a permissive cell line were able to penetrate the nonpermissive rat cell line XC whereas viruses which induced large amounts of gC remained on the cell surface. The above findings strongly support the view that HSV penetrates cells by fusion of the virus envelope with the cellular membrane (Morgan *et al.*, 1968) rather than by phagocytosis (Dales and Silverberg, 1969).

Once inside the cell, the nucleocapsids accumulate at the nuclear pores where the virus DNA is released (Becker *et al.*, 1969; Batterson *et al.*, 1983). New capsids are assembled in the nucleus and replicated concatemeric virus DNA is cleaved as it is packaged into capsids (Vlazny *et al.*, 1982; Stow *et al.*, 1983). Two structural proteins, p40 (ICP35, VP22a) and VP21, which are absent from empty capsids, are associated with capsids containing DNA (Gibson and Roizman, 1972; Heilman *et al.*, 1979; Braun *et al.*, 1984). The p40 is thought to be required for DNA encapsidation since a mutant, *ts*1201, is unable to package DNA at the NPT and fails to process p40 to its mature lower molecular weight forms (Preston *et al.*, 1983). It is not known, however, whether this polypeptide has a direct or indirect role in packaging of virus DNA.

In this paper we describe the characterisation of the mutant, *ts*1204, which lies in a newly identified complementation group of HSV-1 (Matz *et al.*, 1983). This mutant has two defects at the restrictive temperature, one in virus penetration of the host cell and the other in assembly of functional capsids. A second mutant *ts*1208, which fails to complement *ts*1204, has a defect in the latter process only.

MATERIALS AND METHODS

Cells. BHK-21 clone 13 cells were used for virus propagation and titration. In all other experiments, a cell line of human foetal lung (HFL) fibroblasts (Flow Laboratories 2002) was used unless otherwise stated. These cells were grown in Eagle's medium supplemented with 10% foetal calf serum and 1% nonessential amino acids.

Virus. The wild-type HSV-1 stock was strain 17. The isolation of wild-type HSV-1 strain 17 and *ts*A (Brown *et al.*, 1973), and *ts*K (Preston, 1979) has been described previously. A nonsyncytial (*syn*$^+$) form of *ts*K, obtained from the cross *ts*K*syn* $\times$ strain 17*syn*$^+$, was used in this paper. The mutants, 17*ts*VP1201, 17*ts*VP1204, and 17*ts*VP1208 were all derived from strain 17*syn*$^+$ and formed nonsyncytial plaques on BHK cells. For brevity, these mutants are referred to as *ts*1201, *ts*1204, and *ts*1208, respectively. The origin of *ts*1201 has been described previously by Preston *et al.* (1983). *ts*1204 and *ts*1208 were isolated from experiments in which *in vitro* mutagenised cloned fragments of HSV-1 DNA were recombined into the wild-type virus genome. Since the *ts* lesions of *ts*1204 and *ts*1208 did not map in the mutagenised fragments, they were considered to have arisen spontaneously. The revertant for growth at the NPT, *ts*1204 rev-1, was derived from a plaque-purified, low-passage stock of *ts*1204. The particle:PFU ratios of all virus stocks used in this study were approximately 20:1.

Electron microscopy. Cells (2×10^6 per dish) were infected with virus at a m.o.i. of 5 PFU per cell unless otherwise stated. At various times after infection, samples were harvested. The cells were washed with phosphate-buffered saline (PBS), scraped into 0.5 ml of PBS, pelleted by low-speed centrifugation, and resuspended in PBS containing 2.5% glutaraldehyde. The samples were subsequently treated as described by Atkinson *et al.* (1978), except that hydroxypropylmethacrylate was used instead of propylene oxide.

Indirect immunofluorescence. At various times postinfection with mutant virus, subconfluent HFL cells on coverslips were fixed for 10 min in a methanol-acetone mixture (3 parts to 1 part) at $-20°$, and air-dried. Cells were then preincubated with normal rabbit serum before addition

of a 1/40 dilution of ascites fluid containing the appropriate monoclonal antibody. After a 30-min incubation at room temperature (RT), the cells were extensively washed in PBS and treated with fluorescent isothiocyanate-conjugated rabbit anti-mouse immunoglobulin (Miles Laboratories) for 30 min at RT. Nonspecifically bound fluorescent label was removed by washing the cells extensively in PBS. The coverslips were rinsed once in deionised water and mounted in 50% glycerol on glass slides.

Marker rescue. Marker rescue was performed as described by Stow *et al.* (1978) with the modifications of Preston (1981). Cloned DNAs, pGX56 (contains HSV-1 *Bam*HI *u*), pGX134 (contains HSV-1 *Kpn*I *m*), pGX142 (contains HSV-1 *Kpn*I *t*) and pGX38 (contains HSV-1 *Eco*RI *f*), were cleaved with the appropriate restriction endonuclease before being used in the marker rescue experiments. The plasmid vector pAT153 was present in all clones except pGX38 which contained the vector pACYC184. The genome map coordinates for these HSV-1 DNA fragments are shown in Fig. 7.

Treatment of virus-infected cells with PEG. This treatment was performed essentially as described by Sarmiento *et al.* (1979). Briefly, sparsely seeded HFL cells on coverslips were infected with 5 PFU of virus per cell. After adsorption for 1 hr at 38.5°, the inocula were removed by washing monolayers once with PBS. The virus-infected cell monolayers were then treated with PBS containing PEG (50% w/v) for 1 min. Subsequent washes with PBS containing PEG were carried out as described by Sarmiento *et al.* (1979). Following this treatment, the cells were incubated at the NPT for 6 hr in medium containing 10% foetal calf serum before being fixed in methanol–acetone for immunofluorescence assay. Control virus-infected and mock-infected cell monolayers were processed in the same manner as above except that PEG was omitted from all solutions.

Virus-infected cell polypeptide synthesis. Cells (2×10^6 per dish) were infected at a m.o.i. of 20 PFU per cell, washed twice with cell culture medium at 1 hr postinfection, and overlaid with 2 ml of medium per dish. Virus-infected cell polypeptides were radiolabelled by incubation in PBS containing 100 μCi of [^{35}S]methionine/ml. After 30 min, samples were harvested and virus-infected cell polypeptide samples were analysed on 5 to 15% linear gradient sodium dodecyl sulphate (SDS) polyacrylamide gels with a 5% polyacrylamide stacking gel as described by Marsden *et al.* (1978).

Virus-induced immediate-early (IE) polypeptides. IE polypeptides were induced in wild-type and *ts*1204 virus-infected cells as described by Preston *et al.* (1978).

Monoclonal antibodies. These were prepared essentially as described by Palfreyman *et al.* (1983), except that the SP2/0-Ag14 cell line (Shulman *et al.*, 1978) was used as the parental myeloma cell in fusion. MA1147 was specific for the major DNA binding protein, Vmw136 (ICP8) of HSV-1 and the equivalent polypeptide of HSV-2. MA1098 was specific for the IE polypeptide Vmw175 (ICP4) of HSV-1.

RESULTS

ts1204 has a defect in a very early function. Preliminary electron microscopic observations of thin sections of cells infected with *ts*1204 at the NPT revealed that more than 60% of the cells showed no evidence of virus infection. Indirect immunofluorescence studies using the monoclonal antibody MA1147 specific for Vmw136 (ICP8), the major DNA binding protein of HSV-1, supported this finding. Greater than 95% of wild-type virus-infected cells showed typical bright nuclear fluorescence (Fig. 1b), whereas fewer than 40% of cells infected with *ts*1204 displayed this fluorescence. The remaining mutant virus-infected cells resembled mock-infected cells. To investigate the possibility that Vmw136 detected in cells infected with *ts*1204 at the NPT was produced by leak-through of virus which had a defect in a very early function, the *ts*1204 inoculum was warmed to 38.5° prior to addition to cells. This treatment did not result in any significant loss of virus infectivity at 31°

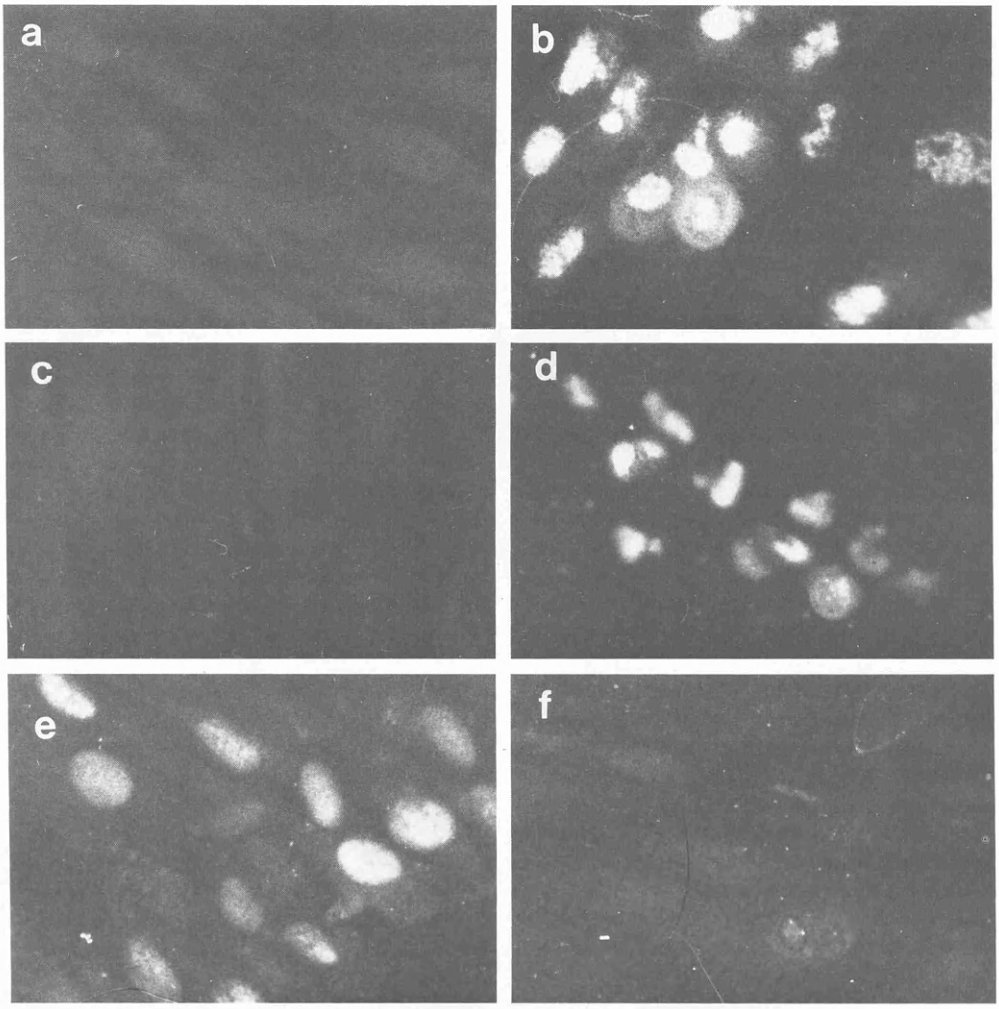

FIG. 1. Monolayers of HFL cells, infected with 5 PFU wild-type HSV-1 or *ts*1204, were harvested at 6 hr p.i. at the NPT and prepared for the indirect immunofluorescence assay. (a) Mock-infected cells. (b) Wild-type HSV-1 infected cells. (c) Cells infected with *ts*1204 which was warmed to the NPT prior to infection. (d) Cells infected with *ts*1204 at the NPT and shifted down to the PT at 2 hr p.i. (e) Wild-type HSV-1 infected cells treated with cycloheximide. (f) *ts*1204-infected cells treated with cycloheximide. The monoclonal antibody used in (a), (b), (c), and (d) was MA1147 which was specific for Vmw136, the major DNA binding protein of HSV-1. The monoclonal antibody used in (e) and (f) was MA1098 which was specific for Vmw175 of HSV-1.

(Matz *et al.*, 1983). The results show that fewer than 1% of cells infected with pre-warmed *ts*1204 at the NPT exhibited nuclear fluorescence (Fig. 1c). The effect of the mutation was reversible upon down-shift of mutant-infected cells from 38.5 to 31° at 1 hr p.i. After 6 hr incubation at the permissive temperature (PT), all cells showed nuclear immunofluorescence when tested with MA1147 (Fig. 1d). Although it was clear from these experiments that *ts*1204 did not synthesize detectable amounts of the major DNA binding protein, an early antigen, at the NPT, it was possible that virus-specified IE polypeptides were synthesized. In order to test

this possibility, IE polypeptides were induced in wild-type and *ts*1204 virus-infected cells at the NPT by cycloheximide treatment. Although IE mRNAs are normally made in small amounts very early in wild-type virus infection, these transcripts can be induced in large amounts by treatment of virus-infected cells with cycloheximide, a protein synthesis inhibitor, added from the time of infection. Upon removal of cycloheximide, these IE mRNAs can be translated in the presence of actinomycin D which prevents further mRNA synthesis. Wild-type virus-infected cells treated in this manner at the NPT, gave bright nuclear fluorescence in the indirect immunofluorescence test using MA1098, specific for Vmw175 (ICP4). By contrast, no fluorescence was detected in *ts*1204-infected cells, suggesting that no IE polypeptides were synthesized in mutant-infected cells (Figs. 1e and f).

ts1204 fails to induce virus-specific polypeptides at the NPT. Virus-infected cells, incubated at 38.5°, were pulse-labelled with [^{35}S]methionine for 30 min at 6 hr p.i., harvested, and analysed by SDS polyacrylamide gel electrophoresis (Fig. 2). When cold (4°) mutant inoculum was added to cells at the NPT, the polypeptide profile of *ts*1204-infected cells was similar to the pattern obtained for wild-type virus-infected cells except that there was reduced shutoff of host protein synthesis. If, on the other hand, the mutant was prewarmed to the NPT prior to addition to cells, the polypeptide profile resembled mock-infected cells, although there was an overall reduction in polypeptide synthesis. Some differences were seen in the intensity of several host polypeptides bands, but the significance of these observations is not yet understood.

ts1204 has a defect in penetration of cells. Immunofluorescence studies and polypeptides profiles suggested that *ts*1204-infected cells failed to synthesize virus-specific polypeptides at the NPT. These findings indicated that the mutant had a defect in adsorption, penetration, or uncoating. In order to distinguish between these possibilities, electron microscopic studies were performed using a high m.o.i.

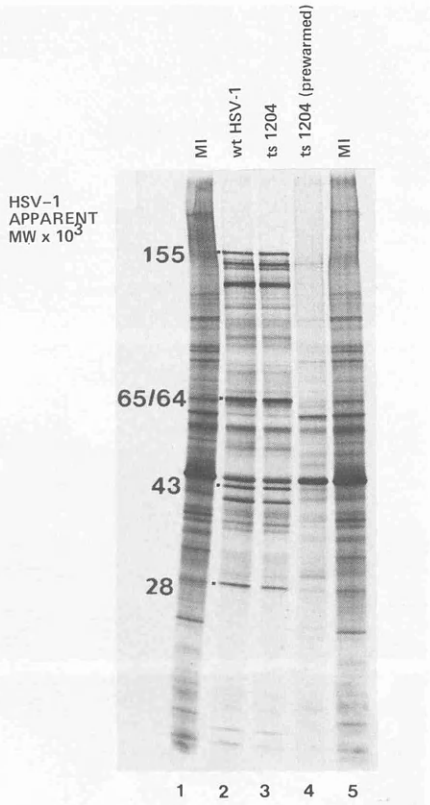

FIG. 2. Autoradiogram of polypeptides induced by wild-type HSV-1, *ts*1204, and mock-infected HFL cells at 38.5°. Virus-infected cells were labelled for 30 min at 6 hr p.i. with [^{35}S]methionine. Protein samples were analysed on a 5–15% gradient SDS polyacrylamide gel.

(200 PFU per cell) of *ts*1204 to infect cells at the NPT. Cell cultures were harvested at 6 hr p.i. and prepared for electron microscopy. No evidence of infection was detected in thin sections of the mutant virus-infected cells. Many enveloped virus particles containing DNA, however, were observed, closely associated with the outside surface of the cell membrane (Fig. 3). By contrast, no enveloped virions were seen on the surface of cells infected with 200 PFU per cell of wild-type virus at the NPT.

Enhancement of infection by ts1204 at the NPT using PEG. Electron microscopic studies suggested that *ts*1204 was unable to penetrate host cells at the NPT. There

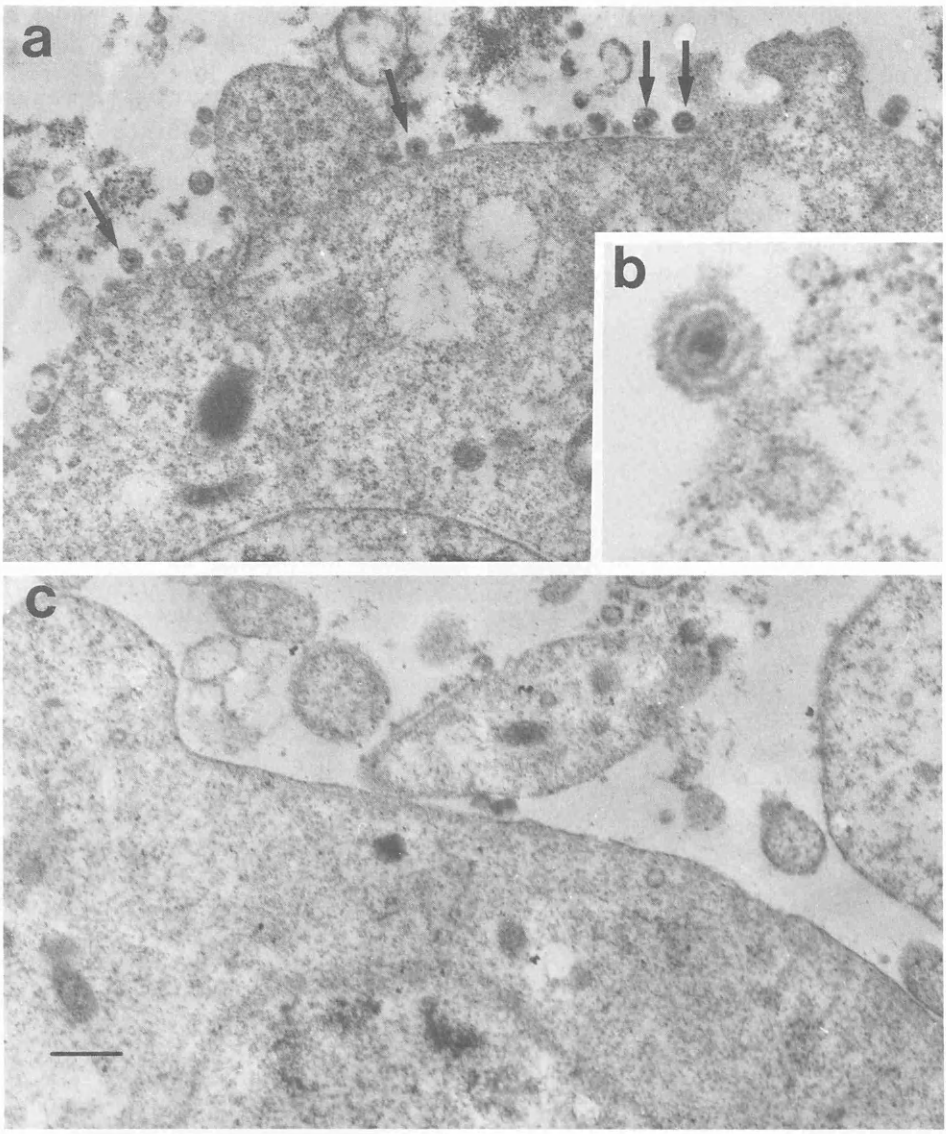

FIG. 3. Electron micrographs of thin sections of HFL cells infected with 200 PFU of *ts*1204 or wild-type HSV-1. Samples were harvested after incubation for 6 hr at the NPT. (a) Enveloped *ts*1204 virions on the surface of a cell. (b) Enveloped *ts*1204 virion in close association with the cell membrane. (c) Surface and interior of a cell infected with wild-type HSV-1. Arrows indicate enveloped virions containing DNA. The bar represents 0.5 μM.

are two alternative explanations for this finding. The virions either failed to attach to cellular receptors, or bound to the receptors but failed to penetrate the cells. Electron microscopic data supported the latter explanation since many *ts*1204 virus particles appeared to adhere to the cell surface (Fig. 3). To investigate these possibilities, cells infected with 5 PFU of *ts*1204 per cell at the NPT were treated with PEG. The proportion of cells infected with virus was determined by indirect immunofluorescence using MA1147. As a consequence of this treatment, 90% of

*ts*1204-infected cells showed nuclear fluorescence (Fig. 4). A similar proportion of wild-type virus-infected cells treated under the same conditions exhibited this pattern of fluorescence. In the absence of PEG, *ts*1204-infected cells resembled mock-infected cells. This result indicates that *ts*1204 attached to, but did not penetrate, host cells at the NPT.

Nature of ts1204 binding to cells. The previous findings showed that *ts*1204 remained on the cell surface at the NPT. Superinfection experiments using wild-type HSV-1 or HSV-2 were conducted in order to determine whether *ts*1204 bound to specific cellular receptors for HSV, or bound nonspecifically to cells at 38.5°. Cells were infected at the NPT with a high m.o.i. (200 PFU per cell) of *ts*K, which has a mutation in Vmw175 (Preston, 1979, Preston, 1981), or *ts*1204. After 1 hr incubation at 38.5°, unattached virus was removed by washing the cell monolayers extensively with PBS. The cells were then superinfected with 10 PFU per cell of HSV-1 wild-type strain 17 or HSV-2 wild-type strain HG52, and incubated for 1 hr at the NPT to allow for virus adsorption. After this time, cells were overlaid with tissue culture medium and incubation was continued for 6 hr at 38.5° before cells were fixed and prepared for indirect immunofluorescence. The presence of the nuclear virus antigen Vmw136, detected using MA1147, was taken as evidence that virus had entered the cell. *ts*K, which penetrates cells normally but induces a very limited number of virus-specific polypeptides at the NPT (Preston, 1979), did not synthesize Vmw136 at this temperature. Cells infected with this mutant resembled mock-infected cells in the immunofluorescence test. After superinfection with wild-type HSV-1, bright nuclear

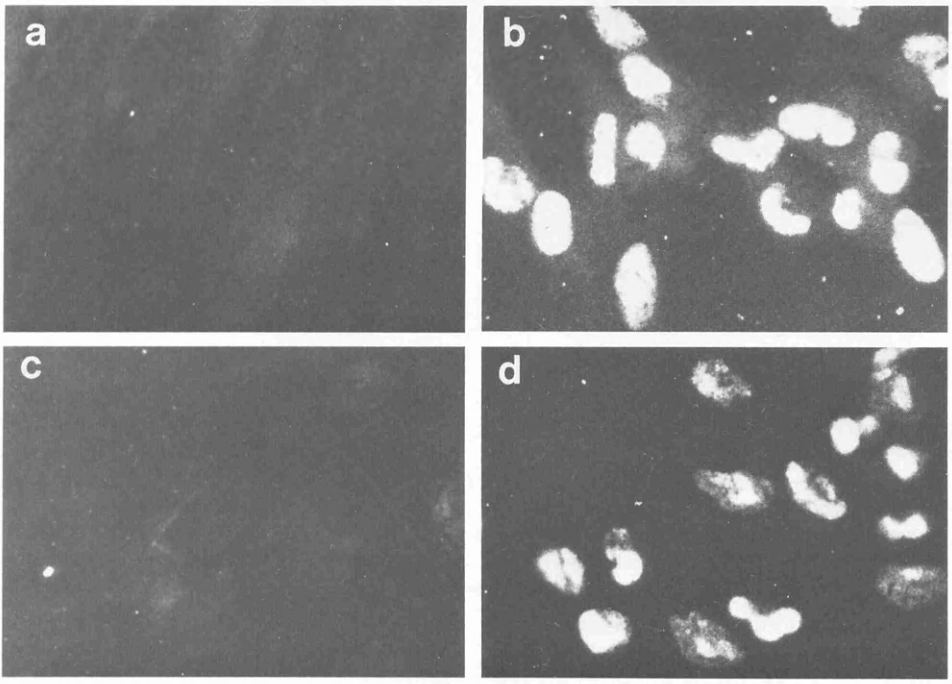

FIG. 4. Monolayers of HFL cells, infected with 5 PFU wild-type HSV-1 or *ts*1204 and harvested at 6 hr p.i. at the NPT and prepared for immunofluorescence assay. (a) Mock-infected cells. (b) Wild-type infected HSV-1 infected cells treated with PEG. (c) Cells infected with *ts*1204 which was prewarmed to the NPT prior to infection. (d) *ts*1204-infected cells treated with PEG. The monoclonal antibody used in all cases was MA1147 which was specific for Vmw136, the major DNA binding protein of HSV-1.

fluorescence was observed in *ts*K-infected cells, indicating that HSV-1 strain 17 was able to penetrate the cell surface (Figs. 5d and e). By contrast, no specific Vmw136 nuclear antigen was detected in *ts*1204-infected cells when the superinfecting virus was wild-type HSV-1 (Fig. 5b). If, however, the superinfecting virus was HSV-2 strain HG52, nuclear fluorescence was seen in greater than 90% of *ts*1204-infected cells (Fig. 5c). These results were confirmed by analysis of radiolabelled virus-infected cell polypeptides on SDS polyacrylamide gels. The pattern of polypeptides from *ts*1204-infected cells superinfected with wild-type HSV-1 resembled

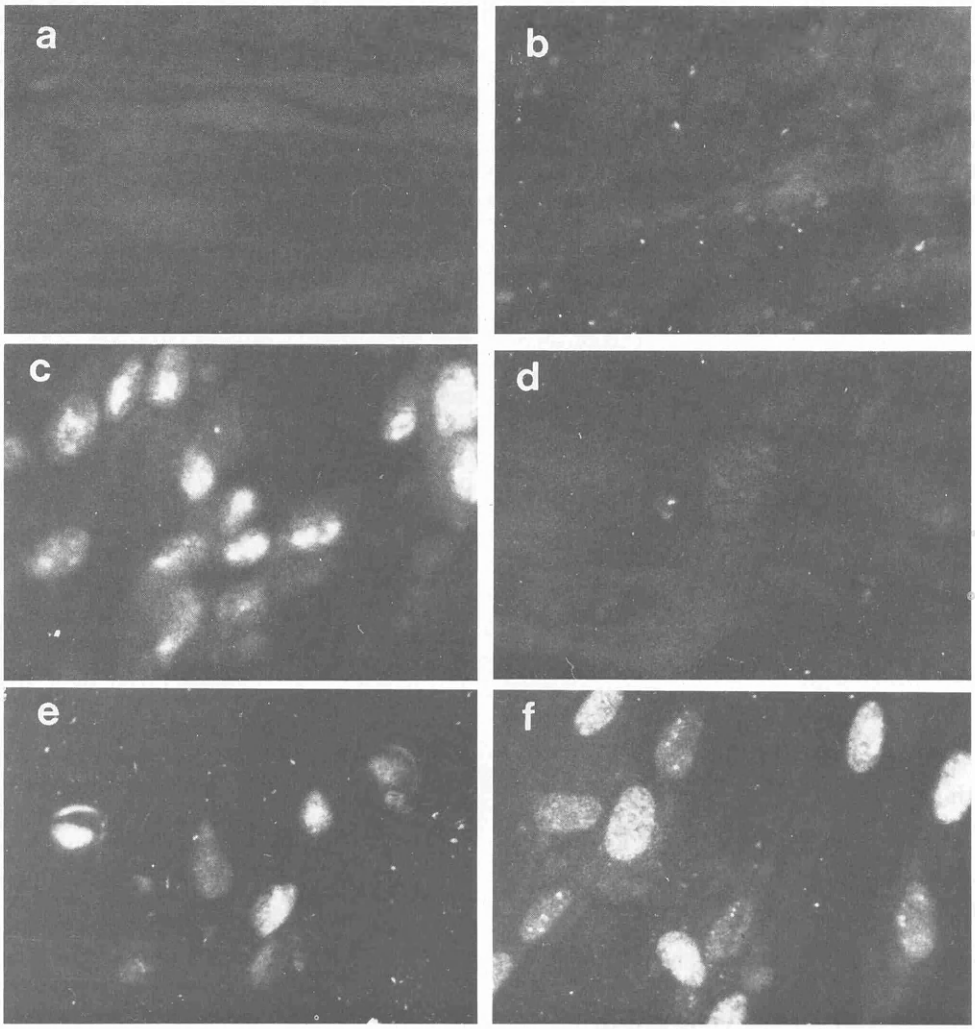

FIG. 5. Monolayers of HFL cells infected with 200 PFU *ts*1204 or *ts*K and superinfected with 5 PFU wild-type HSV-1 or wild-type HSV-2. (a) Cells infected with 200 PFU *ts*1204, prewarmed to the NPT, as a control. (b) Cells infected with *ts*1204 and superinfected with wild-type HSV-1. (c) Cells infected with *ts*1204 and superinfected with wild-type HSV-2. (d) Cells infected with 200 PFU *ts*K as a control. (e) Cells infected with *ts*K and superinfected with wild-type HSV-1. (f) Cells infected with *ts*K and superinfected with wild-type HSV-2. The monoclonal antibody used in all cases was MA1147 which was specific for Vmw136, the major DNA binding protein of HSV-1.

that of mock-infected cells, whereas the pattern of mutant virus-infected cells superinfected with HSV-2 was indistinguishable from that of HSV-2 (data not shown). These results indicated that *ts*1204 binds in a specific manner to cellular receptors at the NPT.

ts1204 has a second phenotypic defect. Electron microscopic studies on *ts*1204-infected cells grown at the NPT revealed that when the virus inoculum was not warmed before addition to cells, about 40% of cells showed signs of infection, such as margination of host chromatin and production of capsids in the nuclei (data not shown). A striking feature of these cells was that low numbers of capsids were seen, none of which contained dense cores. This observation suggested that *ts*1204 had a second phenotypic defect. To confirm this finding, virus was adsorbed to cells for 1 hr at the PT instead of 38.5° to enable the mutant to penetrate the cells. After this time, the cell cultures were transferred to 38.5° and incubation was continued for 9 hr. Electron microscopic examination of these cells showed that *ts*1204 appears to have a defect in capsid assembly, since very few capsids were produced at the NPT, and unlike wild-type HSV-1 (Fig. 6a), none of these contained DNA (Fig. 6b).

Characterisation of ts1208, a mutant which fails to complement ts1204. Previous work by Matz *et al.* (1983) showed that *ts*1204 would complement mutants in other cistrons, including those which had mutations in the gB gene. In these experiments, however, *ts*1204 was not prewarmed to 38.5° prior to virus infection. Recent tests revealed that a spontaneous mutant, *ts*1208, failed to complement *ts*1204 (Table 1), and recombined at low frequency with *ts*1204 (Table 2). On the basis of these results, *ts*1208 was placed in the same cistron as *ts*1204. In contrast to *ts*1204, this mutant did not have a penetration defect since virus prewarmed to 39.5° infected cells normally at the NPT. (39.5° was used as the restrictive temperature in these experiments because *ts*1208 was leaky at 38.5°). The mutant virus-infected cell polypeptides analysed by SDS polyacrylamide gel electrophoresis resembled wild-type virus-infected cell polypeptide profiles (data not shown). Electron microscopic studies of thin sections of *ts*1208-infected cells grown at the NPT, however, revealed that *ts*1208 produced more capsids than *ts*1204, although there were still fewer than seen in wild-type virus-infected cells. Again, only empty capsids were present in all *ts*1208-infected cells (Fig. 6c). Upon temperature shiftdown at 6 hr p.i. from 39.5 to 31°, the virus DNA was packaged and enveloped virions produced. *ts*1208 therefore has a *ts* lesion which affects assembly of functional capsids at the NPT. Interestingly, the capsids present in *ts*1204- and *ts*1208-infected cells at the NPT were different in appearance from those produced at the same temperature by *ts*1201, an unrelated mutant (Fig. 6d). The internal structure of the *ts*1201 capsids appeared to be less compact than the structure present in the capsids of other two mutants.

Physical map location of the ts1204 and ts1208 mutations. The *ts*1204 lesion had been previously mapped to DNA sequences within fragment *Kpn*I *t* (map units 0.322–0.344), by Matz *et al.* (1983). Using separated *Hpa*I fragments from cloned *Eco*RI *f* (plasmid pGX38) in marker rescue experiments, the map coordinates of this lesion were refined. The mutation mapped within a 400-bp region shared by *Hpa*I *i* and *Kpn*I *t* within *BamH*I *u* of HSV-1 (Fig. 7). The lesion in *ts*1208 also mapped within HSV-1 DNA fragment *Bam*HI *u*. When, however, cloned HSV-1 *Kpn*I fragments *t* and *m* were screened for the ability to rescue the *ts*1208 lesion, the mutation mapped within *Kpn*I *m* (Table 3). The *ts*1208 lesion is therefore situated to the left of the *ts*1204 mutation within BamHI *u* (Fig. 7).

ts1204 contains a single lesion. One explanation for the finding that *ts*1204 had defects in penetration of cells and in assembly of capsids is that the virus contained multiple mutations. In order to exclude this possibility, ts^+ progeny virus, isolated from marker rescue experiments in which cells were coinfected at 31° with *Bam*HI *u* and *ts*1204, was tested for the

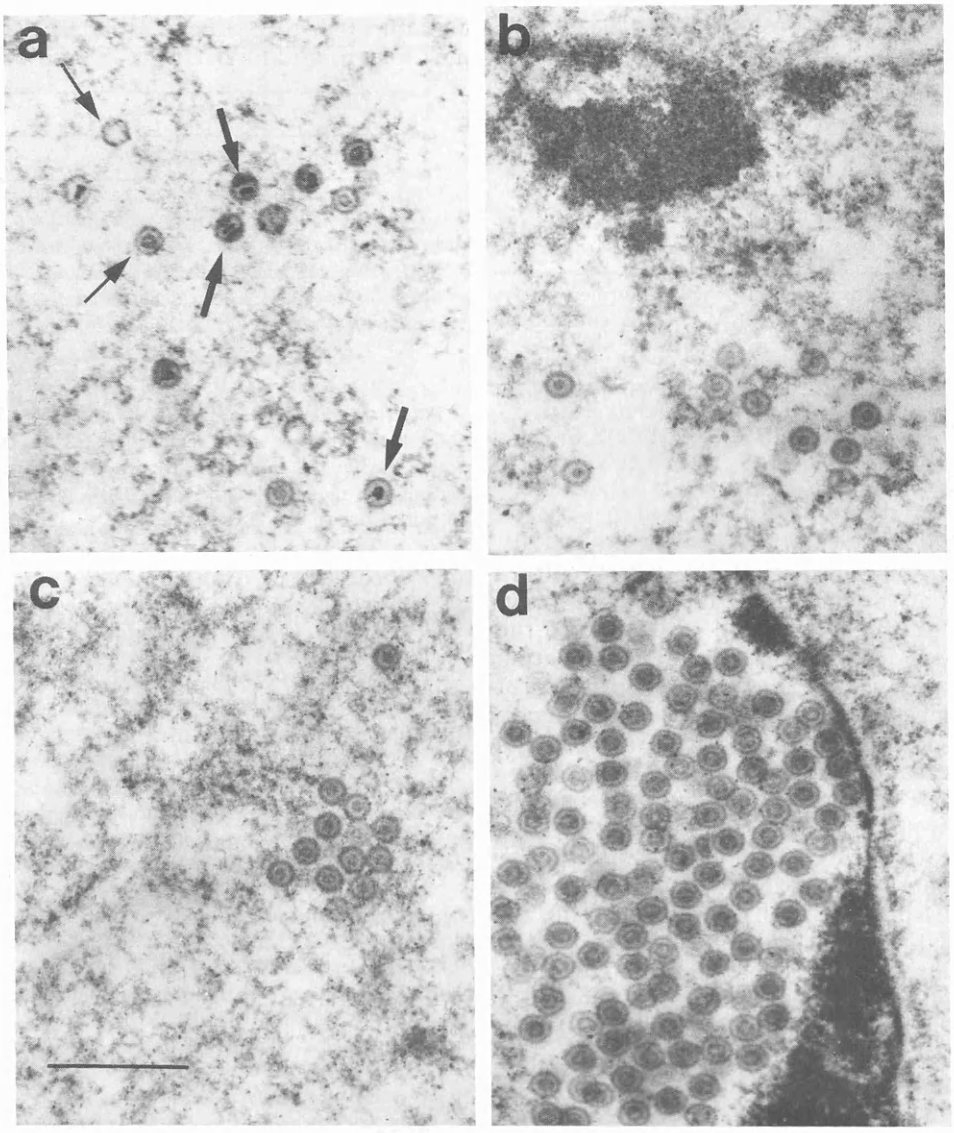

FIG. 6. Electron micrographs of thin sections HFL cells infected with 5 PFU of *ts*1204, *ts*1208, *ts*1201, or wild-type HSV-1. (a) Nucleus of a cell infected with wild-type HSV-1, harvested after 6 hr incubation at the NPT. (b) Nucleus of a cell infected with *ts*1204. After 1 hr incubation at the PT, cells were transferred to the NPT and harvested at 6 hr p.i. (c) Nucleus of a cell infected with *ts*1208 at the NPT. Cells were harvested after 6 hr incubation at the NPT. (d) Nucleus of a cell infected with *ts*1201 at the NPT. Cells were harvested at 10 hr p.i. Broad arrows indicate full capsids, narrow arrows indicate empty capsids. The bar represents 0.5 μM.

ability to infect cells at the NPT. A ts^+ revertant of *ts*1204 was also screened. Both types of virus gave similar results to wild-type virus in indirect immunofluorescence assay using MA1147. In addition, the relative efficiency of plaque formation $(\text{e.o.p.})^{\text{NPT/PT}}$ of the marker-rescued ts^+ virus was comparable to wild-type virus (data not shown). *ts*1204 therefore appeared to contain a single *ts* mutation.

TABLE 1

COMPLEMENTATION BETWEEN *ts*1204, 1208, 1201, AND A[a]

Mutant	*ts*1204	*ts*1208	*ts*1201	*ts*A[b]
*ts*1204	1	1.56	24.32	101.6
*ts*1208		1	42.7	88
*ts*1201			1	98.4
*ts*A				1

[a] Complementation tests were performed as described by Brown *et al.* (1973). Complementation indices greater than 4 were considered positive.

[b] *ts*A has a temperature-sensitive mutation which maps in the glycoprotein gB gene. (V. G. Preston, personal communication).

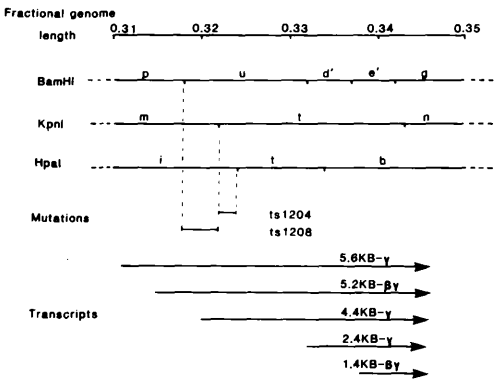

FIG. 7. Marker rescue of *ts*1204 and *ts*1208. The solid lines represent the regions of HSV-1 DNA which rescue the mutants. Arrows indicate transcripts identified by Holland *et al.* (1984), which map in this region of the HSV-1 genome.

DISCUSSION

We have identified a mutant, *ts*1204, which fails to penetrate tissue culture cells at the NPT. Treatment of these mutant-infected cells with PEG enabled the virus to infect cells at the NPT, indicating that this mutant has a defect in fusion of the virus envelope with the cell membrane. The failure of *ts*1204 to penetrate cells appears to be independent of the cell type used. The same phenotype was observed when either BHK clone 13 cells or HFL cells were infected with the mutant (data not shown). Our experiments indicate that *ts*1204 binds to cell receptors specific for HSV-1. This conclusion is based on the finding that cells infected at high m.o.i. of *ts*1204 (200 PFU per cell) at the NPT could be superinfected with wild-type HSV-2 but not HSV-1. These results are in agreement with previous work by Vahlne *et al.* (1979), who showed that HSV-1 and HSV-2 attached to different cellular receptors. Our data also indicate that *ts*1204 virions bind very tightly to cellular receptors at the NPT and cannot be removed by competition with wild-type HSV-1 or by extensive washing of the cell monolayer.

TABLE 2

RECOMBINATION BETWEEN *ts*1204, 1208, 1201, AND A[a]

Mutant	*ts*1204	*ts*1208	*ts*1201	*ts*A
*ts*1204	<0.001	0.1	13.58	52.4
*ts*1208		<0.001	11.9	85.3
*ts*1201			<0.001	16.1
*ts*A				<0.001

[a] Recombination frequencies (%) were calculated as described by Brown *et al.* (1973).

TABLE 3

SUMMARY OF MARKER RESCUE RESULTS

Mutant	DNA fragment[a]		e.o.p.$^{NPT/PT}$ $\times 10^{-3}$[b]
*ts*1204	None		<0.001
	*Kpn*I	t	2.8
	*Kpn*I	m	<0.001
	*Bam*HI	u	1.4
	*Hpa*I	a	9.8
	*Hpa*I	b	<0.001
*ts*1208	None		<0.001
	*Kpn*I	t	<0.001
	*Kpn*I	m	5.4
	*Bam*HI	u	10

[a] Cloned *Eco*RI *f*, digested with *Hpa*I, gave two fragments; a which contained sequences from *Hpa*I *i*, *b* and pAcyc184, and b which contained sequences from *Hpa*I *t* only. These fragments were purified and used in marker rescue experiments. The other DNA fragments were available as cloned DNAs and were digested with the appropriate restriction endonuclease prior to transfection.

[b] The relative efficiency of plating (e.o.p.$^{NPT/PT}$) was calculated from the yield of progeny virus at the PT and NPT from the transfected cells.

vestigation is required to determine the polypeptide involved and its precise function.

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REFERENCES

ATKINSON, M. A., BARR, S., and TIMBURY, M. C. (1978). The fine structure of cells infected with temperature-sensitive mutants of herpes simplex virus type 2. *J. Gen. Virol.* **40**, 103–119.

BATTERSON, W., FURLONG, D., and ROIZMAN, B. (1983). Molecular genetics of herpes simplex virus. VIII. Further characterisation of a temperature-sensitive mutant defective in release of viral DNA and in other stages of the viral reproductive cycle. *J. Virol.* **45**, 397–407.

BECKER, Y., DYM, H., and SAROV, I. (1968). Herpes simplex virus DNA. *Virology* **36**, 184–192.

BRAUN, D. K., ROIZMAN, B., and PEREIRA, L. (1984). Characterisation of post-translational products of herpes simplex virus gene 35 binding to the surfaces of full capsids but not empty capsids. *J. Virol.* **49**, 142–153.

BROWN, S. M., RITCHIE, D. A., and SUBAK-SHARPE, J. H. (1973). Genetic studies with herpes simplex virus type 1. The isolation of temperature-sensitive mutants, their arrangement into complementation groups and recombination analysis leading to a linkage map. *J. Gen. Virol.* **18**, 329–346.

BZIK, D. J., FOX, B. A., DELUCA, N. A., and PERSON, S. (1984). Nucleotide sequence specifying the glycoprotein gene, gB, of herpes simplex virus type 1. *Virology* **133**, 301–314.

DELUCA, N., BZIK, D. J., BOND, V. C., PERSON, S., and SNIPES, W. (1982). Nucleotide sequences of herpes simplex virus type 1 (HSV-1) affecting virus entry, cell fusion, and production of glycoprotein gB (VP7). *Virology* **122**, 411–423.

DALES, S., and SILVERBERG, H. (1969). Viropexis of herpes simplex virus by HeLa cells. *Virology* **37**, 475–480.

EPSTEIN, A. L., JACQUEMONT, B., and MACHUCA, I. (1984). Infection of a restrictive cell line (XC cells) by intratypic recombinants of HSV-1: Relationship between penetration of the virus and relative amounts of glycoprotein gC. *Virology* **132**, 315–324.

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.

FURLONG, D., SWIFT, H., and ROIZMAN, B. (1972). Arrangement of herpesvirus deoxyribonucleic acid in the core. *J. Virol.* **10**, 1071–1074.

GIBSON, W., and ROIZMAN, B. (1972). Proteins specified by herpes simplex virus. VIII. Characterisation and composition of multiple capsid forms of subtypes 1 and 2. *J. Virol.* **10**, 1044–1052.

HEILMAN, C. J., ZWEIG, W., STEPHENSON, J. R., and HAMPAR, B. (1979). Isolation of a nucleocapsid polypeptide of herpes simplex virus types 1 and 2 possessing immunologically type-specific and cross-reactive determinants. *J. Virol.* **29**, 34–42.

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.

HOLLAND, L. E., SANDRI-GOLDIN, R. M., GOLDIN, A. L., GLORIOSO, J. C., and LEVINE, M. (1984). Transcriptional and genetic analysis of the herpes simplex virus type 1 genome: Coordinates 0.29 to 0.45. *J. Virol.* **49**, 947–959.

HOLLAND, T. C., SANDRI-GOLDIN, R. M., HOLLAND, L. E., MARLIN, S. D., LEVINE, M., and GLORIOSO, J. C. (1983). Physical mapping of the mutation in an antigenic variant of herpes simplex virus type 1 by use of an immunoreactive plaque assay. *J. Virol.* **46**, 649–652.

LITTLE, S. P., JOFRE, J. T., COURTENEY, R. J., and SCHAFFER, P. A. (1981). A virion-associated glycoprotein essential for infectivity of herpes simplex virus type 1. *Virology* **115**, 149–160.

LONBERG-HOLM, K., CROWELL, R. L., and PHILLIPSON, L. (1976). Unrelated animal viruses share receptors. *Nature (London)* **259**, 679–681.

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.

MATZ, B., SUBAK-SHARPE, J. H., and PRESTON, V. G. (1983). Physical mapping of temperature-sensitive mutations of herpes simplex virus type 1 using cloned restriction endonuclease fragments. *J. Gen. Virol.* **64**, 2261–2270.

PALFREYMAN, J. W., HAARR, L., CROSS, A., HOPE, R. G., and MARSDEN, H. S. (1983). Processing of herpes simplex virus type 1 glycoproteins: Two-dimensional gel analysis using monoclonal antibodies. *J. Gen. Virol.* **64**, 873–886.

PRESTON, C. M. (1979). Abnormal properties of an immediate early polypeptide in cells infected with the herpes simplex virus type 1 mutant *tsK*. *J. Virol.* **32**, 357–369.

PRESTON, V. G. (1981). Fine structure mapping of herpes simplex virus type 1 temperature sensitive mutations within the short repeat region of the genome. *J. Virol.* **39**, 150–161.

Saturation binding of *ts*1204 to HSV-1 specific cell receptors on HFL cell monolayers was obtained using a m.o.i. of 200 PFU per cell (approximately 4×10^3 particles per cell). Therefore, there appears to be roughly greater than 10^3 HSV-1 receptors on an HFL cell in monolayer. This figure is similar to estimates of receptors for HSV-1 and HSV-2 (Vahlne *et al.*, 1979), and other viruses (Lonberg-Holm *et al.*, 1976). One interesting finding we obtained was that saturating amounts of the HSV-1 mutant *ts*K, which penetrates cells normally, did not prevent subsequent infection by wild-type HSV-1, 1 hr later, at the NPT. This result indicates either that cell surface receptors are reusable or rapidly replaced.

Analysis of virus-induced polypeptides in *ts*1204-infected cells grown at the NPT supported the conclusion that the mutant was unable to penetrate cells at this temperature. The pattern of polypeptide synthesis obtained from these cells was similar to the mock-infected cell polypeptide profile except that some inhibition of host polypeptide synthesis was observed. Although a virion polypeptide has been implicated in this process (Fenwick and Walker, 1978), we do not know whether, in this case, the inhibition was caused by a specific structural protein or induced as a consequence of alterations to the cellular membrane by virions unable to penetrate.

Provided *ts*1204 was allowed to enter cells by a brief incubation at the PT before shiftup to the NPT, a second phenotypic defect became evident. In comparison with wild-type HSV-1 infected cells, fewer capsids were produced, and none contained DNA. Another mutant, *ts*1208, which did not complement *ts*1204, also failed to package DNA, but unlike *ts*1204, this mutant penetrated cells normally at the NPT. *ts*1208-infected cells produced fewer capsids than wild-type HSV-1-infected cells at the NPT, although there were consistently more present than in *ts*1204-infected cells. In this respect, *ts*1204 and *ts*1208 differed from *ts*1201, an unrelated mutant, which made considerably more capsids at the restrictive temperature than wild-type virus (Preston *et al.*, 1983). Although the *ts*1208 defect could be overcome by shifting the virus-infected cells to the PT, it is not clear whether the empty capsids produced at 39.5° are able to package DNA subsequently at 31°.

Analysis of ts^+ virus, generated by marker rescue of *ts*1204 with cloned *Bam*HI *u* from wild-type virus, and a ts^+ revertant of *ts*1204, suggest that *ts*1204 has a single mutation that affects the ability of the mutant to penetrate cells and assemble functional capsids at the NPT. Marker rescue and recombination experiments indicate that the *ts*1204 lesion mapped close to the *ts*1208 lesion. Both these mutations might lie within a multifunctional gene. Alternatively, *ts*1204 might contain a single mutation within the coding regions of two overlapping genes and, as a consequence, have *ts* defects in two gene products. In this situation, *ts*1208 would have a mutation affecting the function of one of these genes only.

Holland *et al.* (1984) have mapped five mRNAs within *Bam*HI *u*. Three of these, 5.6-, 5.2-, and 4.4-kb mRNAs hybridise to the region of *Bam*HI *u* where the *ts*1204 and *ts*1208 lesions lie (Fig. 7). Further investigation is required to determine which mRNA encodes the *ts* gene products. It is of interest to note that mutants of HSV-1 strain $17syn^+$, which have deletions in the 5′ regulatory and coding regions of the thymidine kinase gene, have a syncytial plaque morphology (Sanders *et al.*, 1982). These deletions map within DNA sequences encoding the 5′ regions of the 5.6-kb and possibly the 5.2-kb mRNAs. More work is needed, however, to show whether these deletions affect the gene in which the *ts*1204 and *ts*1208 mutations map.

To date no polypeptide has been assigned to the region of *Bam*HI *u* in which the *ts*1204 and *ts*1208 defects have been located. Since *ts*1204 virions which failed to penetrate cells at 38.5° were fully infectious at 31°, the mutation probably lies within a structural gene. We cannot, however, exclude the possibility that there was incorrect processing of polypeptides at 31° which caused a structural polypeptide to become thermolabile. Further in-

PRESTON, V. G., COATES, J. A. V., and RIXON, F. J. (1983). Identification and characterisation of a herpes simplex virus type 1 gene product required for encapsidation of virus DNA. *J. Virol.* **45**, 1056–1064.

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.

ROIZMAN, B., and FURLONG, D. (1974). The replication of herpesviruses. *In* "Comprehensive Virology" (H. Frenkel-Conrat and R. R. Wagner, eds.), Vol. 3, pp. 229–403. Plenum, New York/London.

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.* **29**, 677–697.

SANDERS, P. G., WILKIE, N. M., and DAVISON, A. J. (1982). Thymidine kinase deletion mutants of herpes simplex virus type 1. *J. Gen. Virol.* **63**, 277–295.

SARMIENTO, M., HAFFEY, M., and SPEAR, P. G. (1979). Membrane proteins specified by herpes simplex viruses. III. Role of glycoprotein VP7 (B_2) in virion infectivity. *J. Virol.* **29**, 1149–1158.

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.

SHULMAN, M., WILDE, C. D., and KOHLER, G. (1978). A better cell line for making hybridomas secreting specific antibodies. *Nature (London)* **276**, 269–270.

STOW, N. D., MCMONAGLE, E. C., and DAVISON, A. J. (1984). Fragments from both termini of the herpes simplex virus type 1 genome contain signals required for the encapsidation of viral DNA. *Nucl. Acids Res.* **11**, 8205–8220.

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.

VAHLNE, A., SVENNERHOLM, B., and LYCKE, E. (1979). Evidence of herpes simplex virus type-selective receptors on cellular plasma membranes. *J. Gen. Virol.* **44**, 217–225.

VLAZNY, D. A., KWONG, A., and FRENKEL, N. (1982). Site specific cleavage/packaging of herpes simplex virus DNA and the selective maturation of nucleocapsids containing full-length viral DNA. *Proc. Natl. Acad. Sci. USA* **79**, 1423–1427.