REVIEW ARTICLE
Intertypic Recombinants of Herpes Simplex Viruses

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INTRODUCTION

Studies on recombinants between two closely related viruses have proved a useful approach for biochemical and antigenic analyses of virus genomes. For example, recombinants between adenovirus type 5 and the non-defective adenovirus 2-SV 40 hybrid, Ad2+NDI, have been used to map a number of gene functions and to correlate the adenovirus genetic and physical maps (Mautner et al. 1975; Williams et al. 1975). Analysis of recombinants between different influenza viruses (Palese, 1977) or reoviruses (Mustoe et al. 1978) has permitted correlation of polypeptides with specific RNA segments while T3 × T7 recombinants have been used to map most of the promoter sites of the major RNA species transcribed by T3 and T7 RNA polymerases on T3 and T7 DNA (Beier et al. 1977). Such analyses can be extended to any pair of genomes that recombine with each other.

The herpesvirus group contains more than 70 viruses which are classified together on the basis of similar appearance in the electron microscope but which show large variation in the extent of DNA sequence homology (Honess & Watson, 1977a). Thus the DNA of herpes simplex virus type 1 (HSV-1) and herpes simplex type 2 (HSV-2) show at least 47% homology (Kieff et al. 1972; Ludwig et al. 1972; Sugino & Kingsbury, 1976), HSV-1 and bovine mammillitis virus (BMV) about 14% (Sterz et al. 1974) and HSV-1 and pseudorabies virus (PRV) 8 to 10% (Ludwig et al. 1972). However, HSV-1 shows little, if any, sequence homology with human cytomegalovirus, Epstein Barr virus (Huang & Pagano, 1974), equine abortion virus (Ludwig et al. 1971), varicella zoster virus (Ludwig, 1972) or Marek’s disease virus (Bachenheimer et al. 1972). It seems clear, therefore, that many herpesviruses show too little sequence homology to allow recombination between them but it is also clear that some, such as HSV-1 and HSV-2, share extensive nucleic acid sequences.

In addition, Honess & Watson (1977a) have shown that some herpesviruses show significant serological relationships and have termed such a cluster of viruses a ‘seron’. Some of these viruses, such as HSV-1, HSV-2, BMV, SA8 and B-virus are linked by the more specific serological relationship of cross-neutralization of virus infectivity and have been grouped in a ‘neutroseron’. Others such as equine abortion virus and PRV show at least one precipitin line in common with HSV-1, HSV-2 and BMV but do not cross-neutralize with them (Killington et al. 1977); they are, therefore, more distantly related. Taken together with the nucleic acid sequence homology between these viruses, it is not surprising, therefore, that intertypic recombinants of HSV-1 and HSV-2 have been isolated. Recombination has also been sought between HSV and BMV (which cross-neutralize) or HSV and PRV (which do not cross-neutralize). The purpose of this review is an explanatory rather than an expository summary of the present position regarding the use of herpesvirus recombinants in the biochemical and antigenic analysis of the herpes simplex virus genome.
Isolation of herpesvirus recombinants

Surprisingly it is now 25 years since it was established that recombination could occur between strains of HSV (Wildy, 1955). Since then, recombination analysis and the construction of genetic maps has been carried out with \( ts \) mutants of HSV-1, HSV-2 and PRV. Timbury & Subak-Sharpe (1973) first showed that the shared nucleic acid homology between HSV-1 and HSV-2 is sufficient to allow genetic interactions between the viruses and, as confirmed by Esparza et al. (1976), that efficient complementation and recombination could occur in cells mixedly infected with a \( ts \) mutant of HSV-1 and a \( ts \) mutant of HSV-2 in many but not all combinations of different \( ts \) mutants. Kabuta (1974) and Yamamoto et al. (1975) have also demonstrated recombination between an HSV-1 strain which produces large syncytial plaques and an HSV-2 strain which produces non-syncytial plaques, the recombinants possessing characteristics intermediate between the parental viruses with respect to plaque size and morphology. Since syncytial and non-syncytial plaque morphology will be referred to throughout the review, it may be useful to define the terms at this stage. Syncytial plaque morphology (syn) is characterized by fusion of infected cells into polykaryocytes whereas non-syncytial plaque morphology (syn+) is characterized by rounding of individual cells.

The first HSV intertypic recombinants were isolated by Timbury & Subak-Sharpe (1973) by plaque purification of the progeny from an infectious centre assay of BHK 21 cells infected at 38 °C (non-permissive temperature) with a mixture of an HSV-1 (strain 17) and an HSV-2 (strain HG52) \( ts \) mutant. The vast majority of the progeny were parental \( ts \) mutants as one would expect from a complementation test but a small number were wild-type with respect to growth and were subsequently confirmed as intertypic recombinants following further plaque purifications.

Recombinants have also been isolated by plaque purification of the progeny from a mixed infection with a \( ts \) mutant of HSV-1 (strain HFEM) which produces syncytial plaques and a wild-type strain of HSV-2 (3345) which produces non-syncytial plaques (Halliburton et al. 1977). Infected cells were incubated at permissive temperature for 18 h to allow recombination to occur. The selection of putative recombinants then involved plaque purification of a syncytial wild-type virus from the infected cells plated out at non-permissive temperature on the assumption that some syn locus had been selected from the HSV-1 parent but that the type 1 DNA containing the mutation had been lost and replaced with the corresponding piece of type 2 DNA giving a type 1-type 2 intertypic recombinant. As with the isolation of recombinants by other groups, putative recombinants were always plaque purified three to six times before being characterized.

As an alternative selection, one of the parental viruses may be made resistant to phosphonoacetic acid (PAA; Overby et al. 1974) by growing it in the presence of increasing concentrations of PAA. Selection of recombinants then involves plaque purification of a PAA resistant (PAA\(^+\)) wild-type virus from a cross between a PAA\(^+\) \( ts \) mutant of HSV-1 and a PAA sensitive (PAA\(^-\)) wild-type HSV-2. Additional selection pressures are possible by crosses of the type PAA\(^+\) syn \( ts \) HSV-1 \( \times \) PAA\(^+\) syn\(^+\)ts\(^+\)HSV-2, an approach also used by Morse et al. (1977) with HSV-1 \( ts \) mutants of strain 17 or HFEM crossed with HSV-2 strain 186 or GP6 in VERO cells. Preston et al. (1978) have also isolated recombinants from a mixed infection with a \( ts \) mutant of type 1 (strain 17) and type 2 (strain HG52) at permissive temperature with or without plaque morphology and PAA resistance as additional selection pressures.

Intertypic recombinants of HSV-1 and HSV-2 have also been generated by intertypic marker rescue. Knipe et al. (1978) rescued HSV-1 \( ts \) mutants of strain HFEM or 17 with XbaI restriction endonuclease fragments of HSV-2 strain G DNA. The two \( ts \) mutants
**Table 1. Method of selection and number of HSV intertypic recombinants isolated**

<table>
<thead>
<tr>
<th>Recombinant phenotype selected*</th>
<th>Marker rescue†</th>
<th>Reference</th>
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<tbody>
<tr>
<td>(ts^+)</td>
<td>(ts^+) syn</td>
<td>(ts^+) PAA</td>
</tr>
<tr>
<td>4</td>
<td>—</td>
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<td>—</td>
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<td>—</td>
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<td>—</td>
<td>1</td>
<td>7</td>
</tr>
</tbody>
</table>

* \(ts^+\), wild-type phenotype with respect to growth; syn, virus induces syncytial plaques due to fusion of infected cells; PAA*, resistance to phosphonoacetic acid.

† Intertypic marker rescue using intact DNA of HSV-1 \(ts\) mutant and fragments of HSV-2 wild-type DNA.

used, \(tsLB2\) of strain HFEM (Halliburton et al. 1977) and \(tsD\) of strain 17 (Brown et al. 1973) possess mutations in the gene coding for an infected cell polypeptide (ICP) termed ICP4 (Honess & Roizman, 1974) and were rescued only by \(XhoI\) fragment I from the S component of HSV-2 DNA, giving rise to intertypic recombinants in which the \(XhoI\)-fragment of HSV-2 DNA had replaced an identical amount of HSV-1 DNA in the right hand end of the S component or recombinants in which \(6 \times 10^6\) mol. wt. of the HSV-1 DNA sequences were replaced by \(11 \times 10^6\) mol. wt. of HSV-2 with insertion of \(5.5 \times 10^6\) mol. wt. into each of the left and right hand reiterated regions of S. Stow & Wilkie (1978) have also isolated intertypic recombinants from crosses involving \(ts\) mutants of strain 17 and unseparated fragments of wild-type HSV-2 DNA (strain HG52). In total, at least 113 HSV-1/HSV-2 intertypic recombinants have been isolated by these three groups of workers using these methods (Table 1).

In mixed infections involving \(ts\) mutants of HSV-1 (strain HFEM) and PRV, no evidence for recombination was obtained (Halliburton et al. 1977). This presumably reflects the much lower nucleic acid sequence homology between HSV-1 and PRV as compared to the more closely related HSV-1 and HSV-2. It should also be remembered that PRV does not cross-neutralize with HSV-1 (Honess & Watson, 1977a). In this respect it is of interest that in the Poxviridae the different genera are defined as groups of viruses which are capable of recombining with each other and which also cross-neutralize. It would therefore be of interest to test for recombination between HSV-1 or HSV-2 and other viruses with which they do cross-neutralize, e.g. BMV. \(Ts\) mutants of HSV-1 (PAA* or PAA) have been crossed with BMV with selection involving plaque morphology and/or PAA resistance. Mixed infections involving BMV and one \(ts\) mutant (out of three tested) have so far shown apparent recombination, but confirmation of this awaits isolation of recombinants showing phenotypic characteristics of BMV and HSV.

To date, therefore, among the herpesviruses, only HSV-1 and HSV-2 intertypic recombinants have been isolated and the remainder of this review will be concerned with the characterization of such recombinants starting with restriction endonuclease analysis of their DNAs and going on to the use of the recombinants in mapping HSV polypeptides, functions and markers.
Restriction endonuclease mapping of the sites of recombinational events

The genome of HSV-1 consists of a linear duplex DNA molecule of $95 \times 10^6$ to $100 \times 10^6$ mol. wt. Although HSV-2 DNA does not co-sediment with HSV-1 DNA in a cesium chloride density gradient due to a 2.2% difference in GC content (Goodheart et al. 1968; Ludwig et al. 1972; Halliburton et al. 1975), they do co-sediment in a neutral sucrose gradient. Even so, Morse et al. (1977) have suggested from summation of the mol. wt. of restriction endonuclease fragments that the mol. wt. of HSV-2 DNA may be $3 \times 10^6$ greater than that of HSV-1 DNA. Since it is clear, however, that HSV-1 and HSV-2 strains can differ slightly in the size of some fragments, this may not be a generalization.

HSV-1 DNA consists of two components, L and S, covalently linked and comprising 82 and 18% of the DNA respectively. The L component consists of a unique sequence (U_L) bracketed by inverted repeat sequences designated ab and a'b', each containing 6% of the total DNA whereas the S components consist of a unique sequence (U_S) bracketed by inverted repeat sequences designated ac and a'c', each containing 4.3% of total DNA, the overall sequence of components therefore being ab U_Lb'a'a'c'U_Sca (Sheldrick & Berthelot, 1974). The a sequence has been shown to be 400 to 600 base pairs in length and to contain a sequence d and its inverted repeat d' (Hyman et al. 1976; Wadsworth et al. 1976). Restriction endonuclease analysis of HSV-2 DNA confirms that it has a similar structure.

Sheldrick & Berthelot (1974) suggested that such a DNA structure could lead through recombination mechanisms to an inversion of the L relative to the S component resulting in a population of DNA molecules containing equimolar concentrations of four conformations of DNA differing solely in the relative orientation of L and S components. This structure has been confirmed by restriction endonuclease analysis of the DNA (Hayward et al. 1975; Wilkie & Cortini, 1976; Skare & Summers, 1977) and by partial denaturation analysis (Delius & Clements, 1976). Restriction endonuclease analysis of HSV-2 DNA has confirmed that it also consists of four populations differing in the orientation of L and S components. The restriction endonuclease maps of HSV-1 and HSV-2 DNA, however, differ markedly from each other and so analysis of the fragment profiles of recombinant DNAs can be used to determine the crossover points. The accuracy with which these can be determined is, however, largely related to the frequency of cleavage sites with the enzymes used and in some cases more than one interpretation may be possible, thereby affecting the position of the crossover by several million daltons, i.e. several genes.

The DNAs of the majority of the recombinants isolated from the different genetic crosses between HSV-1 and HSV-2 (Table 1) have been analysed with up to six restriction endonucleases, XbaI, HsuI (or its isochizomer HindIII), BglII, EcoRI, HpaI and KpnI and the crossover points in some of these are shown in Fig. 1 for one orientation of the L and S components (the prototype arrangement of Morse et al. 1977). In considering the crossovers found in recombinants the following points should be noted.

(1) Some putative recombinants (e.g. RS10 and RB7a, Fig. 1) have no apparent crossovers. Recombinants RS10, RS12 and RS14 from this laboratory, Dxt1(15) and Bxt1(26) of Preston et al. (1978), and four obtained in intertypic marker rescue experiments by Knipe et al. (1978), have physical maps identical to that of the DNA of HSV-1 and five recombinants from this laboratory are identical to their type 2 parents. These viruses may therefore be revertants of the parental ts mutants, variants of the parental wild-type virus (if a wild-type virus was used in the cross) or true recombinants with undetected small crossovers. Such 'hidden' crossovers could easily exist. Although analysis with XbaI, HsuI, BglII, EcoRI and HpaI involve about 56 cleavage sites in HSV-1 DNA and about 40 in
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Fig. 1. Summary of the genome structures of 9 intertypic recombinants. The upper and lower lines in each pair marked at the left with 1 and 2 represent HSV-1 and HSV-2 DNA sequences respectively. The heavy lines represent the DNA sequences present in each recombinant. The dash ending in a knob indicates the presence of a restriction enzyme cleavage site characteristic of the parental DNA. The dash lacking a knob represents the absence of a cleavage site characteristic of that parental DNA. The arrows indicate specific cleavage sites mentioned in the text.

HSV-2 DNA, there can still be regions of a recombinant DNA up to several million daltons in size in which there are no cleavage sites with any of the five enzymes. A double crossover within such regions would not be detected. Certainly such small crossover must exist in some cases. For example, RB20 which, like RB74 (Fig. 1) has no detectable crossover to HSV-1, has been shown to be neutralized by both type 1 and type 2 specific (absorbed) antisera and to induce both type 1 and type 2 polypeptides (Halliburton et al. 1977). Type 1 and type 2 characteristics have, in fact, been detected in most of the recombinants with no detectable crossovers.

(2) Of the 99 recombinants for which restriction enzyme analysis is available, only 19.2% have one crossover, 42.4% have two, 6.1% have three, 12.1% have four, 3.0% have five, 2.0% have six and the remainder show no detectable crossover. It seems certain, therefore, that in addition to the crossover event which removes the ts lesion, other independent crossover events can also occur.

(3) Double crossovers are sometimes detected only because they bracket a cleavage site. For example, RB28 (Fig. 1) picks up an HSV-2 cleavage site at map unit (in terms of fractional distance) 0.40 on digestion with HsuI and RB50 has lost an unexpected cleavage
Fig. 2. Histogram showing the relative frequency with which crossovers have been detected in intertypic recombinants in each 2.5% of the HSV-1 genome.

site at map unit 0.577 on digestion with *HpaI*. In neither of these examples is the double crossover detected on digestion of the DNA with any other enzyme. Depending on the position of other cleavage sites to the right and left, such events can introduce a degree of uncertainty in the amount of heterologous DNA and therefore in the number of genes picked up by the double crossover. The fact that such double crossovers are detected and indeed can only be detected when cleavage sites are involved, increases the probability that the 'hidden' crossovers mentioned above do also exist in areas of the genome not involving cleavage sites.

(4) Recombinants can be isolated with crossovers unrelated to the sites of the *ts* lesions: recombinant 3134 (*tsB* × *tsI*) of Wilkie *et al.* (1978) is still temperature sensitive and therefore the insertion of HSV-2 DNA sequences around map position 0.42 need not be related to the lesion in *tsB* of HSV-1. Some of the recombinants of Morse *et al.* (1977) are also still *ts* but this cannot be readily related to the crossovers. In this laboratory, growth of the infected cells for plaque purifications of all recombinants was performed at non-permissive temperature and so none of the recombinants is temperature sensitive.

(5) Crossovers have been detected in every region of the genome, U_L, U_S and each repetitive sequence bracketing both the L and S regions. From an analysis of 28 intertypic recombinants, Morse *et al.* (1977) suggested that there were preferential crossover sites in particular between map units 0.40 to 0.45 and 0.60 to 0.70. Fig. 2 shows a histogram of the proportion of crossover sites detected in each 2.5% of the genome from an analysis of 79 recombinants. Areas of the genome showing most frequent crossover events lie between map units 0.225 and 0.250, 0.40 and 0.45, 0.825 and 0.850 and 0.650 and 0.675 but there are no obvious preferential hot spots for recombinational events. The most frequent crossover events per 5% of the genome occur between map unit 0.40 and 0.45, 0.20 and 0.25 and map unit 0.65 and 0.70 in agreement with Morse *et al.* (1977), but this is solely because of an increased frequency of such events in the recombinants of Morse *et al.* (1977) and is not reflected in analysis of the recombinants of Halliburton *et al.* (1977, 1980), Preston *et al.* (1978) or Marsden *et al.* (1978). The frequency of crossover events per 10% of the genome is relatively constant between map unit 0.10 and 0.90 but much lower between map units 0 and 0.10 and between 0.90 and 1. There have been no crossover events detected between 0 and 0.025, 0.050 and 0.075 or between 0.875 and 0.900 map units. Fewer crossover events have been detected in the S region relative to L considering the proportions of the genome comprising S and L.

(6) So far, all workers have displayed the sequence arrangements for recombinants in one arrangement of L and S such that the number of crossover events is minimized. Morse *et al.* (1977) suggested that such a manner of displaying the data argued that only one
arrangement of the parental DNA participates in recombination. The argument is not affected by recombinants showing an even number of crossover events but is affected by the 15 recombinants showing an odd number of crossover events. Wilkie et al. (1978) have isolated an interesting recombinant, BX1(28), which appears to contain a high proportion of one orientation of L frozen in the orientation which is, in fact, the one normally adopted for presentation of all results. However, by finer mapping with additional restriction endonucleases with cleavage sites in the repeat regions, N. M. Wilkie, N. D. Stow & A. J. Davison (personal communication) have detected additional crossovers in recombinants with a previously detected odd number of crossovers so that there is currently no strong evidence that only one orientation of the genome participates in recombination.

Mapping of HSV polypeptides, functions and markers

Mapping of HSV polypeptides

In the mapping of HSV polypeptides there are two stages: first the identification of the polypeptide of a polyacrylamide gel electrophoretic separation of recombinant infected cell polypeptides as type 1 or type 2, and second, correlation of these results with the physical maps of the recombinants obtained by restriction endonuclease analyses of their DNAs. The overall composition of HSV-1 and HSV-2 polypeptide profiles is similar but identification of the polypeptides of recombinants relies very largely on the fact that about half the HSV-1 and HSV-2 polypeptides differ slightly in mobility on the gels (Cassai et al. 1975; Halliburton et al. 1977). Additional evidence for equivalence of HSV-1 and HSV-2 polypeptides can be obtained from analysis of a number of other properties such as (1) kinetics of synthesis; (2) relative nuclear and cytoplasmic distribution; (3) amount of each polypeptide; (4) relative labelling with different amino acids; (5) behaviour in pulse-chase experiments; (6) glycosylation; (7) phosphorylation and (8) sulphation. Most studies have taken into account points (1), (5), (6) and (7) only. Certainly identification of HSV-1 and HSV-2 functionally equivalent polypeptides merely on the basis of the major single criterion of similar but not identical mobility on polyacrylamide gel does give some cause for concern.

A comparison of type 1 (strain HFEM) and type 2 (strain 3345) polypeptides is illustrated in Fig. 3 which shows an autoradiogram of infected cell polypeptides labelled with 3H-amino acids from 4 to 8 h after infection. Some bands, such as the major capsid polypeptide, ICP5, are readily identifiable and the corresponding HSV-1 and HSV-2 polypeptides show quite a marked mobility difference. Other bands are not as easily identified or differentiated. Fig. 3 also shows the corresponding profiles for intertypic recombinants RB71, RB73, RB74, RB75, RB76 and RB77, which were all isolated from a cross between ts LB7 of strain HFEM and wild-type strain 3345 (Halliburton et al. 1980). An examination of the profiles further illustrates the identification of HSV-1 and HSV-2 polypeptides specified by the recombinants leading to a mapping of their templates. Thus the polypeptide profile of RB74 is identical to that of its HSV-2 parent, 3345, as indeed is its physical map (Fig. 1). However, this recombinant is neutralized by both type 1- and type 2-specific (absorbed) antisera and therefore specifies both type 1- and type 2-specific sites involved in neutralization. The profile for RB75 is in fact very similar to that of uninfected cells, the recombinant inducing very few virus-specified polypeptides at this time interval after infection and apparently also failing to switch off host protein synthesis efficiently. The polypeptides of the other recombinants of the RB7 series are, however, largely of the same mobility as those of their type 1 parental virus, strain HFEM, with a small number of exceptions, namely ICP7, 21, 43, 44 and 47. For RB71, RB73, RB75 and RB76, ICP 7 is closer in mobility to that of 3345 although ICP7 of RB75 does not seem to be identical to that of 3345 having a mobility slightly faster than that of RB76. RB71 specifies ICP21 of HSV-2; RB73, RB75 and RB76 specify ICP21 of HSV-1. Quite clearly, RB71 and RB73 specify ICP43, 44 and
Fig. 3. Autoradiogram of polypeptides induced by HSV-1 (HFEM), HSV-2 (3345) or series RB7 intertypic recombinants. Polypeptides were labelled with a mixture of $^{14}$C-leucine, valine and isoleucine from 4 to 8 h after infection and were separated by electrophoresis on a 9·25 % polyacrylamide gel under denaturing conditions. Polypeptide ICP numbers are according to Honess & Roizman (1974).

47 of HSV-2 whereas RB7$\text{a}$ and RB7$\text{b}$ specify ICP43, 44 and 47 of HSV-1. This is summarized in Table 2. These five recombinants therefore differ slightly from each other in a small number of the polypeptides they specify. Correlation of these results with the physical maps of the recombinants allows the mapping of the templates for these polypeptides shown in Fig. 4 which also shows the map positions of the templates of other polypeptides obtained from a similar analysis of other recombinants taking into account
Table 2. HSV-1- and HSV-2-infected cell polypeptides

<table>
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<tr>
<th>Polypeptide number</th>
<th>Intertypic recombinant*</th>
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<tr>
<td></td>
<td>RB7₁</td>
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<tr>
<td>ICP7</td>
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<tr>
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<tr>
<td>All other ICPs</td>
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</table>

* Polypeptides identified as HSV-1 are labelled as 1, those identified as HSV-2 as 2.
† Identifies a polypeptide with a mobility close to, but not identical with that of HSV-2.

Kinetic studies and some of the other means of identifying HSV-1 and HSV-2 polypeptides mentioned above.

Comparison of these results with a similar analysis by Morse et al. (1978a) and Marsden et al. (1978) shows good agreement in the map positions for some templates but lack of agreement for others. For ICP₄, 5, 6, 8, 27 and 44 the agreement is good but for all
others the map positions do not entirely agree or do not agree at all. Two obvious reasons which make comparisons of this sort of data between groups difficult are (1) the gel system used and (2) the polypeptide nomenclature used. Thus Halliburton et al. (1977) used linear gels measuring 18 × 16 cm whereas Morse et al. (1978a) used linear gels measuring 25 × 30 cm, which does alter the resolution of some areas of the gel, and Marsden et al. (1978) generally used gradient gels which markedly alter these profiles. In addition, there is no general agreement on polypeptide nomenclature with some groups adopting a number system such as the one used in Fig. 3 and others referring to polypeptides by estimated mol. wt. on SDS-polyacrylamide gels. These two points, taken together with the fact that not all of the polypeptides specified by recombinants are identified as type 1 or type 2 by anything like all the criteria listed earlier and therefore may not always be correctly identified, results in great difficulty and uncertainty in relating the results of one group with those of another.

Certainly the identification of functionally equivalent polypeptides is strengthened if:

(a) possession of the polypeptide of one serotype by a recombinant is associated with exclusion of the putative functionally related polypeptide of the other serotype. Unfortunately, exceptions to this rule do exist. The recombinant D1E1 of Morse et al. (1978a) specifies the ICP4 of both HSV-1 and HSV-2, as do eight recombinants isolated by marker rescue of ts mutants of HSV-1 by the XbaI-I fragment of HSV-2 (Knipe et al. 1978). In addition, some recombinants have been shown to specify a small number of altered polypeptides with mobility different from that of the HSV-1 or HSV-2 parents (Marsden et al. 1978; Morse et al. 1978a; Wilkie et al. 1978; RB7b, Table 2).

(b) Independent mapping of the infected cell polypeptide of HSV-1 or HSV-2 results in the same map position. This of course assumes that genes coding for functionally equivalent polypeptides are collinear on the HSV-1 and HSV-2 genomes. Although apparently true for most functions, this is not likely to hold for all functions and indeed in Ruyechan et al. (1979) and in this report it is shown that the templates specifying glycoprotein C of HSV-1 and HSV-2 are not collinear. Mapping of the templates for polypeptides therefore requires clarification.

Mapping of virus glycoproteins

Polyacrylamide gel electrophoresis of infected cell extracts labelled with, for example, 14C-glucosamine has established the existence of at least five major glycoprotein species (labelled gA, gB, gC, gD and gE) plus several minor components (Fig. 7). Some of the minor glycosylated species have been shown to be precursors of four of the major glycosylated proteins (gA, gB, gC and gD) present in virus particles and in infected cells (Spear, 1976). Three of these HSV-1 glycoproteins (gA, gD and gB or gC) are antigenically related to HSV-2 glycoproteins (Spear, 1978). The nomenclature used in this review is, as decided by interested parties at the fourth Cold Spring Harbour meeting on Herpesviruses (1979), that unique glycoproteins will be identified by a letter of the alphabet preceded by a lower case g; precursors of any glycoprotein will be identified by a lower case p before the identifying letter followed by the estimated molecular weight of the precursor in brackets.

Analysis of cell extracts infected with recombinants has allowed the map positions of gA, gB, gC and gD shown in Fig. 5 to be determined. Several recombinants specify glycoproteins of aberrant mobility (shown by arrows in Fig. 6). The reason for glycoproteins of mobility different from that of either parental virus cannot be explained by the crossovers possessed by the recombinants and they may well arise because possession of certain combinations of type 1 and type 2 genes results in altered processing of some glycoproteins. It is of interest, for example, that recombinant RB7 possesses type 2 DNA only from map positions 0.824 to 0.837 (Fig. 1), correlating with the map position of the template for gD, yet shows altered mobility of most other glycoproteins. This may suggest the presence
of a locus affecting control of processing of glycoproteins. Ruyechan et al. (1979) have mapped a locus (Cr) which determines the accumulation of gC at map unit 0.70 to 0.83.

Comparison of the map positions of the templates for the major glycoproteins with the results of other groups shows areas of agreement and of disagreement. Marsden et al. (1978) and Wilkie et al. (1978) have mapped the templates specifying glycoproteins of mol. wt. 122, 117, 92, 63 and 51 K. Glycoproteins 122 and 117 correlate with glycoprotein B and its precursor respectively; 51 correlates with the precursor of glycoprotein D but there is no known correlation between glycoproteins 92 and 63 and those found by other groups (H. S. Marsden, personal communication). The capital letters for glycoprotein designation used by Ruyechan et al. (1979) and in this study are the same. The map positions for the templates specifying gA and gB obtained by Ruyechan et al. (1979) overlap with those in the present study and also with those obtained for glycoproteins 117 and 122 by Wilkie et al. (1978). However, the latter results and those from this laboratory just fail to overlap. Overall, the templates specifying these two glycoproteins do appear to map at the same position between map unit 0.260 and 0.420 and the HSV-1 and HSV-2 species are collinear. Results from all those groups show overlapping map positions for the templates specifying gD, maximal boundaries being 0.824 to 0.945 map unit and the HSV-1 and HSV-2 gDs being collinear. Taking the results of all three groups, minimal
coordinates were 0.900 to 0.937 map unit but the recombinant then being used to delineate the left hand boundary of the template is D1E1 of Ruyechan et al. (1979) which does show an aberrant mobility of gD in that it is classified as type 1 yet has a faster mobility than that of type 1 gD specified by other recombinants isolated from the same cross. Indeed, its mobility seems closer to that of gD of its type 2 parental strain which would then alter the probable coordinates for gD to 0.840 to 0.900 map unit. This conclusion is strengthened by the fact that the Band II type 1-specific antigenic site involved in neutralization of virus infectivity (Watson & Wildy, 1969; Sim & Watson, 1973) has been correlated with gD (see below) yet recombinant D1E1 is not neutralized by Band II type 1-specific (absorbed) antiserum which would suggest that either it specifies an altered gD affected by the crossover event, or a type 2 gD. In general, Ruyechan et al. (1979) ignored the fact that many
Fig. 7. Autoradiogram of glycoproteins induced by three different HSV-1 strains in four different cell lines labelled with $^{14}$C-glucosamine from 3.5 to 10 h after infection and separated by electrophoresis on a 9% polyacrylamide gel under denaturing conditions. The nomenclature at the left relates specifically to the HFEM profile in BHK cells.
of their recombinants specified glycoproteins of mobility different from that of the type 1 or type 2 parental strains whereas in this laboratory, such recombinants were not used in mapping the templates for glycoproteins.

In the mapping of the templates for gC, advantage was taken of the crossovers in recombinants which fail to induce any gC even though both parental viruses used in the cross from which such recombinants were isolated did induce gC (e.g. RB2s and RB2s, Fig. 6). This leads one to conclude firstly, that glycoprotein C is not essential for the production of infectious progeny virus and, secondly, that the templates for HSV-1 and HSV-2 gC are probably not collinear since the crossover event can delete both templates. Due to a lack of convenient crossovers in the necessary position, the template for HSV-1 gC is mapped with the rather large coordinates of 0.637 to 0.824 map unit, gC of HSV-2 mapping at 0.690 to 0.710 map unit. These map positions do not totally agree with the findings of Ruyechan et al. (1979) who have also, however, concluded that the templates specifying gC of both HSV-1 and HSV-2 are not collinear since they have three recombinants which specify gC of both HSV-1 and HSV-2, resulting in map positions of 0.530 to 0.645 map unit for gC of HSV-1 and 0.645 to 0.690 map unit for gC of HSV-2 (Fig. 5). This map position for gC of HSV-1 overlaps with that from this laboratory but considerably narrows the map coordinates, the studies taken together suggesting a position of 0.637 to 0.645 map unit for HSV-1 gC. The map positions for the templates for gC of HSV-2 do not overlap and currently cannot be reconciled. It is of interest that Ruyechan et al. (1979) made use of some recombinants in their mapping of gC which specify gC even though they were isolated from a cross between a ts mutant of strain HFEM and the wild-type strain GP6, neither of which, in their hands, specified gC. In this laboratory, strain HFEM does appear to specify gC but this illustrates a major problem in glycoprotein mapping in as much as it is very difficult to identify unambiguously even the major glycoproteins. This problem is greatly compounded by three other points: (1) different virus strains (particularly HSV-1 strains) sometimes show markedly different glycoprotein profiles in the same cell line (compare strains HFEM, 17 and KOS in HEp2 cells, Fig. 7); (2) the same virus strain can also show markedly different glycoprotein profiled in different cell lines (Fig. 7); (3) the gel system used to resolve glycoproteins can also significantly affect the mobility of glycoproteins, differences being obtained depending on whether bisacrylamide or diallyltartardiamide is used as cross-linker. Also, the use of gradient gels can show quite different glycoprotein mobilities from linear gels, e.g. gC of strain 17 grown in BHK cells separated on a linear gel has an estimated mol. wt. of 126000 but on a gradient gel, it has an estimated mol. wt. of 110000 and migrates faster, rather than slower, than gB and gA (H. S. Marsden, personal communication). These differences must be borne in mind in such studies. Further analysis of HSV glycoproteins is needed to clarify the situation with gC and to identify and map any minor glycosylated species.

Antigenic sites on recombinant virus particles

Although the precise mechanism of neutralization of HSV is not well understood, it is clearly established that the structural antigens involved in neutralization may be classified as type-specific or type-common, that is cross-reacting (Sim & Watson, 1973). Clearly the recombinants offer no special advantage over wild-type strains for the study of type-common antigenic sites but with appropriate antisera, it should be possible to identify type-specific antigenic sites on recombinant virus particles and by correlating this with the physical maps of the recombinants, map the functions and hopefully correlate them with specific glycoproteins.

Neutralization of 43 intertypic recombinants with type 1- and type 2-specific (absorbed) antisera, and two type 1-specific antisera of more restricted specificity, anti-VP7/8 (Powell
Table 3. Neutralization of recombinant viruses by type I-specific (absorbed), type 2-specific (absorbed), Band II type I-specific (absorbed) and VP7/8 antisera*

<table>
<thead>
<tr>
<th>Type I-specific antiserum</th>
<th>Type 2-specific antiserum</th>
<th>Band II type I-specific antiserum</th>
<th>VP7/8 antiserum</th>
<th>Number of recombinants</th>
</tr>
</thead>
<tbody>
<tr>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>13</td>
</tr>
<tr>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>4</td>
</tr>
<tr>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>25</td>
</tr>
<tr>
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<td>+</td>
<td>+</td>
<td>+</td>
<td>1</td>
</tr>
</tbody>
</table>

* A plus sign indicates positive neutralization, a minus sign no neutralization relative to pre-incubation of the virus with pre-immune serum.

et al. 1974) and anti-Band II type I-specific (absorbed) antiserum (Watson & Wildy, 1969; Sim & Watson, 1973) has shown that they fall into four groups (Table 3). Thirty per cent of the recombinants specify only type I-specific antigenic sites involved in neutralization since they are neutralized by type I-specific (absorbed), Band II type I-specific (absorbed) and VP7/8 antiserum but not by type 2-specific (absorbed) antiserum. These include recombinants like RS16 which show only type I DNA on restriction endonuclease analysis (Fig. 1) but also recombinants like RB52, RB7a and RB77 which possess type 2 as well as type I DNA and in the case of RB7b, as much as 31.9% of the genome is type 2. The regions of type 2 DNA possessed by these recombinants do not, therefore, contain genes specifying antigens involved in neutralization. About 10% of the 43 recombinants are neutralized only by type 2-specific antiserum. Again, these include recombinants for which there is no evidence of crossovers in the DNA but also C1D and C2D of Morse et al. (1977) which possess 6 and 14% type I DNA respectively, areas of the genome which cannot, therefore, specify antigens involved in neutralization.

The majority of the recombinants (26 of 43) are neutralized by type I- and by type 2-specific antiserum and therefore possess both type I- and type 2-specific antigenic sites involved in neutralization. Surprisingly, only one of these recombinants, 2sa of Timburry & Subak-Sharpe (1973), is neutralized by both Band II type I-specific (absorbed) and VP7/8 antiserum; the remaining 25 recombinants are neutralized by VP7/8 antiserum but not by Band II type I-specific (absorbed) antiserum. The template for Band II type I-specific antigen has, however, been mapped by identification of the region of type I DNA common to the 14 recombinants neutralized by Band II type I-specific (absorbed) antiserum as 0.830 to 0.925 map unit. The right hand end of this template is confirmed by studies on the neutralization of recombinant D1E1 of Morse et al. (1977). The recombinant possesses type I DNA only from 0.925 to 1.000 map unit but is not neutralized by Band II type I-specific (absorbed) antiserum. On the other hand, the fact that RB77 is neutralized by type 2-specific (absorbed) antiserum yet only contains type 2 DNA from 0.83 to 0.93 map unit (Fig. 1) shows that there is also a template for a type 2-specific antigen(s) at this position on the genome.

Additional neutralization studies with general type I or type 2 antiserum absorbed with recombinants which possess both type I- and type 2-specific antigenic sites have established that there are qualitative differences in the particular antigenic determinants involved in neutralization present in these recombinants. Thus Halliburton et al. (1977), in such a study with four recombinants, showed that they not only differed from each other with respect to the type-specific antigenic sites but also used the results to establish the presence of at least one type I-specific and three type 2-specific antigenic sites involved in neutralization. This study has now been extended to include 13 recombinants and by correlating the results with the physical maps of the recombinants it has been possible to
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establish other areas of the genomes of HSV-1 and HSV-2 that do and some areas that
do not possess templates specifying antigens involved in neutralization (Fig. 5).

It is of interest to try to correlate these antigenic sites with specific glycoproteins and
Fig. 5 allows a comparison with the map positions of the major glycoproteins. The Band II
type 1-specific antigenic sites and gD (mol. wt. 59,000) map at the same position and
since gD is precipitated by Band II antiserum, this establishes a possible association between
the two. Cohen et al. (1978) have also shown an association between their CP-1 antigen,
which shares immunological properties with Band II antigen (Cohen et al. 1972; Powell &
Watson, 1975), and a 59,000 mol. wt. glycoprotein. The type 2 gD may correspondingly
correlate with a type 2-specific antigen involved in neutralization mapping at the same
position. At this stage, other type 1- and type 2-specific antigenic sites cannot be correlated
as strongly with specific glycoproteins and, indeed, some of the antigenic sites map at
positions on the type 1 and type 2 genomes at which no glycoproteins have been mapped.
It may be that some of the minor glycoprotein species so far ignored are involved in
neutralization. It is, however, very clear that straightforward correlation of glycoproteins
and antigenic sites involved in neutralization is not as easy as one might have hoped.
For example, the glycoprotein profile for RB20 is identical to that of its type 2 parent and
yet RB20 specified type 1 as well as type 2-specific antigenic sites involved in neutralization
(Halliburton et al. 1977).

Inhibition of host protein and DNA synthesis

Infection of cells with HSV results in an inhibition of host cell protein, DNA and RNA
synthesis by mechanisms unknown (Ben-Porat & Kaplan, 1973). The inhibition of host
protein synthesis has been reported to require virus protein synthesis (Sydiskis & Roizman,
1967; Ben-Porat et al. 1971) but alternatively, Fenwick & Walker (1978) have shown that
host protein synthesis is still inhibited following infection with u.v. inactivated virus,
suggesting that the inhibition is due to a constituent of the infecting virus particle. Similar
considerations apply to the inhibition of host cell DNA synthesis, the mechanism involving
either an early virus-specified protein (Ben-Porat & Kaplan, 1965) or a structural component
of the virus (Fenwick & Walker, 1978).

Since the inhibition of host protein synthesis following infection with most HSV-2
strains is more rapid and efficient than that after infection with most HSV-1 strains (Powell
& Courtney, 1975; Pereira et al. 1977), it is possible by analysis of polypeptide profiles of
cells labelled at early times after infection with recombinants to classify the recombinants
as type 1 or type 2-like with respect to inhibition of host cell protein synthesis. Thus seven
interotypic recombinants rapidly and efficiently switch off host cell protein synthesis in
a manner analogous to their type 2 parental strain. Correlation of this result with the
physical maps of their DNAs allows mapping of the 'switch-off function' to 0.69 to 0.75
map unit. In a similar study, however, Morse et al. (1978a) mapped such a function to
0.52 to 0.59 map unit. It may be, therefore, that inhibition of host protein synthesis results
from the action of the products of more than one gene.

Fenwick et al. (1979) have shown that HSV-2 also inhibits host cell DNA synthesis
more rapidly than HSV-1 and have also mapped this accelerated inhibition by HSV-2 to
0.52 to 0.59 map unit. They therefore find that a function involved in the inhibition of host
cell protein synthesis and a function involved in cessation of host cell DNA synthesis map
to the same region of the genome and have tentatively associated one or both of these
functions with ICP10 which also maps to this region. Again the situation may be more
complex than this as Halliburton & Timbury (1976) have shown that the inhibition of
host cell DNA synthesis involves the expression of more than one virus-specified function
since each of two ts mutants of HSV-2 which complement one another fail to switch
off host cell DNA synthesis at the non-permissive temperature.
Mapping of thymidine kinase

The thymidine kinase induced in lytic infection by intertypic recombinants can be identified as type 1 or type 2 by studies on the thermolability of enzyme activity, by neutralization with type 1 or type 2 HSV antiserum and by agar gel immunodiffusion with type 1 or type 2 thymidine kinase antiserum. Halliburton et al. (1980) have identified the thymidine kinase induced by 36 intertypic recombinants using these methods and by correlating the results with restriction endonuclease analysis of the DNA of the recombinants have mapped the HSV-1 thymidine kinase gene at 0.300 to 0.309 map unit and the HSV-2 gene at 0.295 to 0.315 map unit.

All other available data are now in agreement with these map positions. Thus Stow et al. (1978), in marker rescue experiments with intact HSV-1 DNA and XbaI fragments from a thymidine kinaseless mutant mapped the HSV-1 thymidine kinase gene between 0.283 and 0.45 map unit. Wigler et al. (1977) showed that a 3.4 kilobase BamI fragment from the left hand end of these map units could transform thymidine kinaseless L cells to a TK+ phenotype and that HSV-1 thymidine kinase activity was expressed by the transformants. In an analysis of a small number of intertypic recombinants, Morse et al. (1978a) suggested that the type 1 thymidine kinase gene mapped between 0.27 and 0.35 map unit and suggested a possible correlation with ICP35 (mol. wt. 42000) which mapped in the same position. Maitland & McDougall (1977) originally mapped HSV-2 thymidine kinase at 0.532 to 0.646 map unit since HSV-2 DNA fragments from these coordinates could transform LMTK- cells to a TK+ phenotype, the transformants specifying a thymidine kinase identical to that of HSV-2 as judged by thermolability of enzyme activity and mobility on polyacrylamide gels. Clearly such a map position does not agree with the findings of Halliburton et al. (1980) for HSV-2 thymidine kinase. However, J. K. McDougall, E. J. Tollentino & T. H. Massey (personal communication) having re-examined the situation, now consider the map position of HSV-2 thymidine kinase to be 0.299 to 0.339 map unit. There is, therefore, very good agreement on the map position of HSV-1 (0.300 to 0.309 map unit) and HSV-2 (0.299 to 0.315 map unit) thymidine kinase genes and the two are collinear.

Mapping of ts mutants

All groups have made use of ts mutants in the isolation of intertypic recombinants selecting wild-type recombinants which lacked the region of the genome containing the mutation. Therefore, if several recombinants have been isolated from a particular cross, it may be possible to obtain a physical location for the region of the genome containing the mutation by identifying a common sequence missing from each recombinant. Fig. 4 shows the probable map positions for tsLB5 and tsLB7 obtained in this manner, plus the physical map position for tsLB2 obtained by marker rescue by Knipe et al. (1978). In addition, Fig. 4 shows the map locations of eight other ts mutants obtained from analysis of intertypic recombinants (Wilkie et al. 1978) and in most cases confirmed by marker rescue experiments (Stow et al. 1978; Stow & Wilkie, 1978). Fig. 4 also shows the position for one other ts mutant obtained by analysis of intertypic recombinants by Morse et al. (1978a).

Attempts to correlate the physical map positions of the ts mutants with genetic maps obtained by recombination analyses have revealed several anomalies in that recombination frequencies do not always reflect physical map distances accurately (Wilkie et al. 1978). In addition, from such genetic studies it should be possible to comment on whether or not all four genome arrangements can take part equally in intergenic recombination since mutants like tsLB2, tsD or tsK, which map in S, should have the same recombination
frequency with any *ts* mutant mapping in L if all four arrangements do take part equally. *TsLB2*, *tsD* and *tsK* can in fact be ordered into a linear map with mutants in L (Brown & Jamieson, 1978; R. W. Honess, A. Buchan, I. W. Halliburton & D. H. Watson, personal communication), a finding which would argue that all four conformations do not enter equally into recombination.

It is of interest also to correlate the physical mapping of *ts* mutants with the mapping of the polypeptide specified by the gene containing the *ts* mutation if this can be identified. For *tsLB2* and *tsD* this has been identified as ICP4 (Knipe *et al.* 1978). Mapping of ICP4 by analysis of polypeptides specified by HSV-1 × HSV-2 intertypic recombinants is in direct agreement with the physical map positions of *tsLB2* and *tsD* (Fig. 4).

*Mapping of the syn loci*

In general terms, the intertypic recombinants express either a syncytial or a non-syncytial plaque morphology phenotype. Morse *et al.* (1978b) have mapped the template responsible for the syn phenotype between 0·30 and 0·42 map unit by correlating the plaque morphology of the recombinants with the physical maps of their DNAs obtained by restriction endonuclease analysis. However, the situation is clearly not as simple as this. In the isolation of the recombinants of Halliburton *et al.* (1977, 1980), syn morphology was generally used as a selection marker in that 29 of 31 recombinants were isolated as syncytial wild-type viruses from crosses between a syncytial HSV-1 *ts* mutant and a non-syncytial HSV-2 wild-type virus. As can be seen from the physical maps of the DNA of some of the recombinants (Fig. 1), there is clearly no region of HSV-1 or HSV-2 DNA in common. Indeed, as has already been stated, some recombinants possess only type 1 DNA and some possess only type 2 DNA, yet all 29 express a syn phenotype. It must be stated, however, that morphologically, the syn phenotype does differ markedly but reproducibly between recombinants, there being at least four distinguishable syn morphologies. It would seem therefore that if these recombinants do possess some characteristic from HSV-1 associated with syn morphology, then the expression of syn can be modified by some of the products of other type 1 or type 2 genes possessed by the recombinants. In other words, there is more than one gene product involved in the formation of polykaryocytes. Indeed, R. W. Honess, A. Buchan, I. W. Halliburton & D. H. Watson (personal communication) have concluded from recombinational analysis that there are at least two loci involved in syn formation. Ruyechan *et al.* (1979) have mapped the location of three mutations at different loci which affect the formation of polykaryocytes (Fig. 4) and Yamamoto & Kabuta (1977) have mapped four and possibly six cistrons associated with cell fusion by recombination analysis with non-fusing mutants.

*Resistance to phosphonoacetic acid (PAA')*

Phosphonoacetic acid selectively inhibits the synthesis of herpes simplex virus DNA in infected cells and the activity of virus DNA polymerase *in vitro* (Honess & Watson, 1977b). It has been used as a selective marker in the isolation of intertypic recombinants by Morse *et al.* (1977), Marsden *et al.* (1978) and in this laboratory. Morse *et al.* (1978a) have mapped the PAA resistance marker to 0·43 to 0·52 map unit by analysis of HSV-1 × HSV-2 intertypic recombinants and Knipe *et al.* (1979) have confirmed and narrowed these map coordinates to 0·45 to 0·52 by two techniques of marker transfer. Studies in this laboratory are consistent with this map position but do not allow such fine mapping. Chartrand *et al.* (1979) have also mapped PAA' mutations of HSV-1 and HSV-2 and found them to be collinear but at a position at least 3·8 kilobase pairs to the left of that reported by Morse *et al.* (1977) and Knipe *et al.* (1978). This may indicate that more than one locus is involved in DNA polymerase activity.
Do naturally occurring intertypic recombinants exist?

In view of the 'ease' with which it has been possible to isolate HSV-1 × HSV-2 intertypic recombinants in the laboratory, there seems no reason per se why they should not arise in nature. So far, however, there is no convincing evidence that any have been isolated. Certainly it has to be remembered that in the laboratory, recombinants are isolated from a mixed infection at a reasonably high multiplicity of infection, a situation that is not as likely to arise in vivo.

One of the main problems, however, is how one would recognize a naturally occurring recombinant as such. It is clear that characterization of polypeptides, glycoproteins or antigenic sites involved in neutralization of an isolate would not necessarily characterize it as a recombinant virus. In one of the most comprehensive studies of polypeptide variation of HSV strains, Pereira et al. (1976) showed, in an analysis of the structural polypeptides of 53 strains of HSV-1, that there were at least seven polypeptides that could vary in mobility on polyacrylamide gel electrophoresis and the 53 strains could be classified into 19 groups on the basis of such polypeptide mobility differences. Without knowledge of the corresponding polypeptide profiles for the parental strains from which any putative naturally occurring intertypic recombinant arose (which it would be unlikely to obtain with any confidence) it would not be possible to distinguish easily between an isolate being another strain and being a recombinant.

Similarly, the mobility of glycoproteins of different HSV-1 or HSV-2 strains can clearly differ, sometimes markedly (Fig. 7) and an extension of such a study to include 32 isolates from a diagnostic virology laboratory has confirmed that HSV-1 isolates, defined as HSV-1 on the basis that they are neutralized by type 1-specific but not by type 2-specific antiserum, has confirmed variability, particularly of HSV-1 glycoprotein profiles.

Neutralization by type 1 or type 2 general antisera intermediate between that of the type 1 or type 2 parental viruses has been used as evidence of a recombinant phenotype (Timbury & Subak-Sharpe, 1973). Roizman et al. (1970), however, showed that strain MP exhibited neutralization kinetics intermediate between those of HSV-1 and HSV-2 strains but it is now clearly established that strain MP is a type 1 virus possessing only type 1-specific antigenic sites involved in neutralization since it is neutralized by type 1-specific but not by type 2-specific antiserum. Certainly neutralization of a virus isolate by type 1 and by type 2-specific antiserum would be better evidence of a possible recombinant phenotype but, so far, studies of over 60 HSV isolates in this laboratory have not revealed such a phenotype. In any case, it is clear that not all recombinants necessarily possess both type 1- and type 2-specific antigenic sites involved in neutralization (Table 3).

Restriction endonuclease analysis of the DNA of a virus isolate seems likely to be one of the best criteria of a recombinant virus. It is clear that many HSV isolates vary in restriction endonuclease profile with at least one enzyme but more drastic variation might be indicative of a recombinant virus. On the other hand, recombinants can arise which show no crossovers detectable even on analysis of digests with each of five restriction endonucleases (e.g. RSI0 and RB74, Fig. 1). It may be, therefore, that the best approach would be a combination of, for example, restriction endonuclease analysis and neutralization studies with type 1-specific (absorbed) and type 2-specific (absorbed) antisera. The method used by Lonsdale (1979) does not require even partial purification of the virus DNA and is reasonably rapid although no more so than neutralization of virus infectivity. These types of approach are now being applied in several laboratories (Buchman et al. 1978; Linnemann et al. 1978; Lonsdale et al. 1979) including this one and besides screening for naturally occurring intertypic recombinants they will allow the study of whether particular virus strains are associated with specific clinical infections.
CONCLUSIONS

There can be no doubt that herpes simplex virus intertypic recombinants have proved to be of great use in the mapping of biochemical and antigenic characteristics of the viruses. Clearly the approach is limited to mapping genes whose products can be identified as type 1 or type 2 by their mobility on polyacrylamide gels, serological characteristics or by some other means. Polypeptides or functions showing type common characteristics will have to be mapped by other methods but in theory at least, anything that can be differentiated between HSV-1 and HSV-2 can be mapped. Some distinguishable characteristics such as pock size on the chorioallantoic membrane or virulence in mice may, however, be resulting from the interaction of more than one gene product and may prove difficult to map.

With a very few exceptions (e.g. glycoprotein C), the evidence to date suggests that the linear order of genes on HSV-1 and HSV-2 genomes must be at least grossly similar and most equivalent gene products functionally equivalent. Although recombinants have been isolated with crossovers in every region of the genome (Fig. 2) it is very likely that all possible combinations of type 1 and type 2 genes have not been observed. It would be interesting to evaluate whether possession of any one region of type 1 or type 2 DNA is always associated with possession of other regions of the same genome. It is also of interest to wonder if there are lethal gene combinations, i.e. if any particular crossovers result in functionally unacceptable combinations of type 1 and type 2 genes.

In general, considering the uncertainties involved, there is good agreement from different laboratories on the mapping of many functions. Perhaps the greatest uncertainties arise with mapping polypeptides, one of the major problems being that there is no universally accepted polypeptide nomenclature and of the 50 or so polypeptides so far identified there is probably only general agreement on unambiguous identification of at the very most about 20. When two laboratories can supposedly be mapping the same polypeptide and yet have little confidence that it is indeed the same, then the situation is rather unsatisfactory. An additional very large gap in our current knowledge of herpesviruses lies with correlation of polypeptide bands on an acrylamide gel with function. Certainly further finer mapping of polypeptides and functions using intertypic recombinants will allow more correlation of polypeptides and glycoproteins with functions and at least suggest associations which can then be confirmed by other means.

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Review: Herpesvirus recombinants


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