The Isolation and Characterization of Mutants of Herpes Simplex Virus Type 1 that Induce Cell Fusion

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SUMMARY

Six cell fusion-causing syn mutants were isolated from the KOS (syn-101 to syn-106) and three from the HFEM (syn-107 to syn-109) strains of herpes simplex virus type 1 (HSV-1). The mutants were studied by complementation and recombination with syn-20 (a syncytial mutant of KOS) and ts-B5 (a syncytial mutant of HFEM). Some studies also employed MP, a syncytium-inducing strain isolated from the non-syncytial parent, mP. Complementation and recombination of syn-20 and ts-B5 indicated that these two mutants were altered in two different virus genes. The recombination frequency between syn-20 and ts-B5 was very similar to that observed between MP and ts-B5, indicating that syn-20 and MP may represent alterations in the same virus gene. syn-101, syn-103, syn-104 and syn-105 were tentatively assigned to the syn-20 complementation group, while syn-107 and syn-109 were tentatively assigned to the ts-B5 complementation group. syn-106 and syn-108 were excluded from the ts-B5 group. syn-102 could not be excluded from either complementation group. syn-101 induced markedly less fusion at 38 °C relative to 34 °C. At 34 °C the patterns of syn-101-infected cell peptides and glycopeptides, examined by SDS-gel electrophoresis, were normal, but at 38 °C the amount of glycopeptide gC was particularly reduced. syn-102 produced decreased amounts of glycoproteins, and a non-glycosylated peptide, probably ICP6, was absent from extracts infected with syn-106.

INTRODUCTION

Common laboratory strains of herpes simplex virus type 1 (HSV-1) cause the fusion of about 20% of infected human embryonic lung (HEL) cells in a monolayer culture (Read et al., 1980). Developing plaques in HEL cells are characterized by single cells, or small clumps of rounded cells, at the scalloped edges of the plaques, and fusion can be detected in a small fraction of these neighbouring cells upon microscopic examination. However, plaque-morphology mutants can be isolated that contain extensive regions of highly multinucleated cells (syncytia) within a stretched, but continuous, area of cytoplasm throughout the developing plaque. This striking feature of the mutants resulted in their designation as syn mutants (Brown et al., 1973).

The molecular mechanism of fusion is unknown, but the synthesis of virus-specified glycoproteins is required for fusion (see, for example, Knowles & Person, 1976). Fusion-causing mutants such as ts-B5 and MP exist that are deficient in the production of a single but different glycoprotein, gB for ts-B5 at the non-permissive temperature, and gC for MP (see, for example, Manservigi et al., 1977). The ability to unambiguously establish the
role of the missing glycoprotein in the fusion process is complicated by the finding that the same mutants result in the decreased processing of all major virus-specified glycoproteins (S. Person et al., unpublished results). Physical mapping studies of syncytial mutations revealed three genomic regions that affect fusion, and all were separable from each other by recombination (Ruyechan et al., 1979). One of the regions corresponded to a restriction fragment whose DNA sequences were also shown to specify gB, but none of the regions included DNA sequences that specify gC. These data for MP and subsequent data for ts-B5 (Honess et al., 1980) indicate that both mutants probably contain multiple mutations. It appears that studies of the fusion process are complicated by the lack of suitable mutants. Therefore, it is necessary to obtain and characterize a number of syncytial mutants that are isogenic, except for the fusion function. Previously, we reported a genetic study of a small collection of mutants, isolated from the KOS strain of HSV-1, that were selected for a range of fusion phenotypes (Read et al., 1980). In the present study, we report the isolation and partial characterization of a second group that were selected for extensive fusion production.

METHODS

Isolation of syn mutants. Growth media and procedures for HEL cell culture and for the preparation of virus stocks have been described previously (Person et al., 1976). Syn mutants were isolated from mutagenized cultures of KOS or HFEM essentially as described by Read et al. (1980). Monolayers of HEL cells that were about 75% confluent (6 x 10^4 cells/cm^2) were infected in 2-oz prescription bottles (21 cm^2 surface area) with KOS or HFEM at a multiplicity of infection (m.o.i.) of 10 and incubated at 34 °C. At 3 h post-infection the growth medium was removed and 3 ml of growth medium plus 4 to 8 μg/ml 5-bromodeoxyuridine, 20 to 40 μg/ml 2-aminopurine, or 1 μg/ml [3H]deoxycytidine was added from 3 to 24 h post-infection. At 24 h, fresh growth medium was added, the monolayer was disrupted by three cycles of freezing and thawing, clarified by centrifugation at 7500 g for 5 min, and stored at -75 °C as mutagenized virus stocks. These procedures reduced progeny virus titres to 1 to 10% of the unmutagenized control. syn-101, syn-102, syn-104, syn-105, and syn-106 were isolated from 2-aminopurine-mutagenized KOS stocks; syn-103 was isolated from a [3H]deoxycytidine-mutagenized KOS stock; syn-107, syn-108, and syn-109 were isolated from 5-bromodeoxyuridine-mutagenized HFEM stocks.

Mutagenized stocks were titrated and 50 p.f.u. and 6 x 10^4 cells were added to each well of a 96-well (1 cm^2 surface area/well) tissue-culture tray. After incubation for 48 h at 34 °C, the trays were scanned for plaques. Wells with plaques exhibiting syncytia were marked and titrated. Appropriate dilutions were made for each potential mutant so that 25 p.f.u., along with 6 x 10^4 cells, were added to each well of the 96-well trays. The contents of wells containing a syncytial plaque but no wild-type infection were picked and cloned twice by a similar procedure before preparation of a stock. Each mutant was isolated from a separate mutagenized stock. In this paper, m.o.i. is used to signify the number of adsorbed p.f.u. in 1 h at room temperature (Person et al., 1976).

Coulter counter fusion assay. The Coulter counter fusion assay was described previously (Person et al., 1976). Briefly, multiple cultures of HEL cells were infected simultaneously with one virus at an m.o.i. of 20, or with two viruses at an m.o.i. of 10 each unless otherwise noted. At various times after infection an uninfected cell culture was harvested, the number of small single cells (N) counted and scored as a fraction (N/N_0), N_0 being the average number of cells determined before the onset of fusion.

Recombination. HEL cell cultures were infected with one of the newly isolated mutants, and ts-B5, MP, or syn-20 at an m.o.i. of 5 for each virus. After incubation for 24 h, the viruses were harvested. The progeny of each pairwise cross were titrated and the resulting plaques were scanned to determine the frequency of non-syncytial plaques.
Metabolic labelling. HEL monolayers on 35-mm Petri dishes (9.6 cm² surface area) about 75% confluent (5 x 10⁴ cells/cm²) were infected with the indicated virus at an m.o.i. of 10. At 3 to 4 h post-infection, growth medium was decanted and the monolayers washed with tricine-buffered saline (Person et al., 1976) and replaced with 1 ml of growth medium containing 5 μCi [¹⁴C]glcN/ml or [³⁵S]methionine/ml (all from New England Nuclear). At 10 h post-incubation, monolayers were washed with tricine-buffered saline and cells were lysed in 0.2 ml electrophoresis sample solution (ESS) containing 0.05 M-Trizma-base adjusted to pH 7, 2% SDS, 5% β-mercaptoethanol, and 0.005% bromophenol blue.

SDS-gel electrophoresis. The method of Laemmli (1970) as modified by Manservigi et al. (1977) was used for SDS-polyacrylamide gel electrophoresis. The apparatus was as described by Knowles & Person (1976) except that En³Hance (New England Nuclear) was used essentially as described by the manufacturer to decrease the exposure time in the preparation of autoradiograms.

RESULTS

Complementation test between syn-20 and ts-B5

Complementation is tested by mixedly infecting cells with two mutant viruses using conditions such that the mutant gene products are inactive. If the mutations are in different genes each virus will produce a gene product whose activity is missing from the other and the wild-type phenotype may be observed. If two syn mutants are used, each of which expresses a syncytial phenotype in single infection, the appearance of the non-syncytial phenotype in the mixed infection would be an indication of complementation. A requirement for a meaningful complementation test is that the mutants not be dominant in a mixed infection with non-syncytial viruses. For ts-B5 in single or mixed infections with either HFEM or KOS, extensive fusion was observed at 34 °C (Fig. 1, 2). This indicates that ts-B5 is at least co-dominant to wild-type in HEL cells at the permissive temperature. At the non-permissive temperature (38 °C), ts-B5 caused no fusion in single infections (Fig. 1), while in mixed infections with non-syncytial viruses (KOS or HFEM) the small amount of fusion characteristic of the non-syncytial virus (see, for example, Read et al., 1980) was observed (Fig. 2). To emphasize that ts-B5 at 38 °C does not inhibit the small but detectable fusion produced by non-syncytial viruses, fusion kinetics at 38 °C were obtained for HFEM and for HFEM plus ts-B5 and were plotted on the same graph (Fig. 3). ts-B5 is not an immediate descendant of the HFEM strain used in these studies (both were obtained from Dr P. G. Spear, University of Chicago, U.S.A.). Although the HFEM strain used here is syncytial for Vero cells (Manservigi et al., 1977) it is non-syncytial for HEL cells.

syn-20 produced extensive fusion at 34 °C and 38 °C in single infections of HEL cells (Fig. 4) and it has been shown previously that it is recessive to KOS in mixed infections (Read et al., 1980). Therefore, a meaningful complementation test can be performed by using mixed infections of ts-B5 and syn-20 at 38 °C. Extensive fusion was observed at 34 °C and an amount of fusion characteristic of HFEM or KOS alone was observed at 38 °C (Fig. 5). The two mutants complemented each other at 38 °C and, therefore, the mutations in ts-B5 and syn-20 were assigned to different complementation groups.

Complementation and recombination studies of newly isolated mutants

When we began these experiments all of the syn mutants used in this study were tested for complementation in all possible pairwise mixed infections with themselves and with syn-20, ts-B5 and MP. In addition, monolayers were visually scored for fusion phenotype using different cell types at both the permissive and non-permissive temperatures (data not shown). Numerous ambiguities resulted regarding the assignment of mutants to complementation groups. The ambiguities may be due to the necessarily complex nature of cell fusion. Fusion
Fig. 1. Kinetics of cell fusion of ts-B5-infected HEL cells. Cells were infected with ts-B5 at an m.o.i. of 20. After adsorption (zero time on the abscissa) infected cells were incubated at 34 °C (○) or 38 °C (●) and harvested at the indicated times to determine the number of cells remaining unfused (N). The fraction of cells remaining unfused (N/N₀) is plotted as a function of time after infection.

Fig. 2. Kinetics of cell fusion of HEL cells mixedly infected with ts-B5 and HFEM (○, ○) or ts-B5 and KOS (▲, ▲). Equal volumes of virus suspensions at 2 × 10⁸ p.f.u./ml were mixed and 0-2 ml used to infect cells to give an adsorbed m.o.i. of 10 for each virus. Infected cells were incubated at 34 °C (○, ▲) or 38 °C (○, ▲). The fraction of cells remaining unfused is plotted as a function of time after infection.

Fig. 3. Kinetics of cell fusion of HEL cells infected with HFEM (●) or with ts-B5 plus HFEM (○) at 38 °C. Cells were infected with a total m.o.i. of 20 (m.o.i. of 10 for each virus in the mixed infections). The fraction of cells remaining unfused is plotted as a function of time after infection.

requires incorporation of virus-specified glycoproteins into plasma membrane domains. Side-by-side interactions of the molecules involved in fusion, and interactions of fusion-associated molecules with submembrane–cytoskeleton components and with receptors on neighbouring cells may alter the fusion response. Complexity of the fusion process viewed at this level may be responsible for the variety of co-dominance and cell-type effects that are found for different syn mutants (see, for example, Bzik & Person, 1981). For these reasons we chose two test mutants that we could verify as recessive in mixed infections of HEL cells with wild-type strains, and which in mixed infections with each other gave an unambiguous complementation result using a quantitative assay, syn-20 and ts-B5 incubated at the non-permissive temperature satisfied these requirements and were subsequently used in all of the experiments.

The newly isolated mutants, syn-101 to syn-109, were assayed for their ability to complement ts-B5 and syn-20 at 38 °C. The extent of fusion was determined between 5 and 10 h post-infection since the decrease in the number of single cells had been found to be
Fusion-inducing mutants of HSV-1

1.0
0.8
0.6
0.4
0.2
0.1

Fraction of cells not fused (N/N₀)

Time after infection (h)

Fig. 4

Fig. 5

Table 1. Complementation test of mutants with syn-20 and ts-B5*

<table>
<thead>
<tr>
<th>syn mutant</th>
<th>101</th>
<th>102</th>
<th>103</th>
<th>104</th>
<th>105</th>
<th>106</th>
<th>107</th>
<th>108</th>
<th>109</th>
<th>20</th>
<th>ts-B5</th>
</tr>
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<tbody>
<tr>
<td>syn-20</td>
<td>9(2)</td>
<td>5</td>
<td>2</td>
<td>9(3)</td>
<td>3</td>
<td>2</td>
<td>5(2)</td>
<td>2</td>
<td>4</td>
<td>2</td>
<td>&gt;10²(3)</td>
</tr>
<tr>
<td>+ ts-B5</td>
<td>1</td>
<td>5</td>
<td>3</td>
<td>5</td>
<td>4</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>20</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>+ parent</td>
<td>6</td>
<td>6</td>
<td>5</td>
<td>18</td>
<td>7</td>
<td>5</td>
<td>6</td>
<td>14</td>
<td>9</td>
<td>20</td>
<td>20</td>
</tr>
</tbody>
</table>

* HEL cells were infected at a total m.o.i. of 10 (m.o.i. of 5 for each virus in the mixed infections) and incubated at 38 °C. From 5 to 10 h after infection cells were harvested and the fraction of cells remaining as small single cells was determined as usual. The rate of fusion or the time in hours to reduce the number of single cells to 1/e of its original value is shown. The numbers in parentheses are the corresponding times at 34 °C for mutants that were temperature-sensitive for fusion. Data for mixed infections with parental types are shown for comparison. Data are an average of at least two experiments, and the maximum variation in the 1/e time was always less than 2x. The word parent refers to KOS for syn-101 to syn-106, and to HFEM for syn-107 to syn-109.

exponential during that time interval (see Fig. 4, 6). The time to reduce the number of single cells to 1/e of its starting value (an average value of one fusion event per cell) was determined and is shown for each mutant in single and mixed infections (Table 1). Data are also given for mixed infections with the parent virus. Times of 2 to 5 h were generally observed for the mutants in single infections. The longer times for syn-101, syn-104 and syn-107 reflect a temperature sensitivity for fusion, and the 1/e times for these mutants at 34 °C are shown in parentheses. Complete fusion kinetics curves at the two temperatures are shown for syn-101 (Fig. 6). Mutants that are not temperature-sensitive showed a small increase in fusion at 38 °C relative to 34 °C (see Fig. 4 for syn-20). We somewhat arbitrarily took an increase of at least twofold in the 1/e times by either ts-B5 or syn-20 as evidence of complementation. By this criterion, syn-103 and syn-105 were assigned to the syn-20 complementation group and syn-109 to the ts-B5 complementation group. In repeated experiments the presence of wild-type virus increased, rather than decreased, the fusion in mixed infections with syn-101 at 38 °C.
Fig. 6. Kinetics of cell fusion of syn-101 at 34 °C (●) and 38 °C (▲). HEL cells were infected at an m.o.i of 10. The fraction of cells remaining unfused is plotted as a function of time after infection.

Table 2. Recombination test of syn mutants with syn-20 and ts-B5*

<table>
<thead>
<tr>
<th>Mutant</th>
<th>syn-20</th>
<th>ts-B5</th>
</tr>
</thead>
<tbody>
<tr>
<td>101</td>
<td>2(3/395)</td>
<td>7(12/329)</td>
</tr>
<tr>
<td>102</td>
<td>0(0/357)</td>
<td>1(3/485)</td>
</tr>
<tr>
<td>104</td>
<td>0(0/304)</td>
<td>5(22/842)</td>
</tr>
<tr>
<td>106</td>
<td>0(0/1521)</td>
<td>8(3/71)</td>
</tr>
<tr>
<td>107</td>
<td>12(6/96)</td>
<td>3(2/141)</td>
</tr>
<tr>
<td>108</td>
<td>0(0/1612)</td>
<td>7(16/427)</td>
</tr>
<tr>
<td>20</td>
<td>—</td>
<td>11</td>
</tr>
<tr>
<td>ts-B5</td>
<td>11(92/1736)</td>
<td>—</td>
</tr>
</tbody>
</table>

* HEL cells were mixedly infected at an adsorbed m.o.i. of 5. 24 h after infection progeny virus were harvested and the number of wild-type plaques and the total number of plaques were determined and are shown in parentheses. The reversion frequency for syn-20 is \( \leq 8.3 \times 10^{-6} \) (Read et al., 1980) and is \( \leq 5 \times 10^{-4} \) for other mutants. The recombination frequencies are in percentage form and were not corrected for spontaneous reversion. In a recombination test with MP all of the mutants isolated from the KOS strain gave no recombination, syn-107 gave 12% (17/112), syn-20 gave 0% (0/1400), and ts-B5 gave 11% (50/885).

The six mutants that were not assigned to a complementation group were tested for recombination with ts-B5 and syn-20 (Table 2). Schaffer et al. (1974) have shown that a recombination value of up to 3% may indicate that the two mutations are in the same gene, whereas larger values indicate that the mutations are probably in different genes. Recombination values as high as 2% between other syn mutants were previously taken as being in the same gene (Read et al., 1980), and a value as high as 5.5% was reported for the two mutations in ts-B5 which we show are in the same gene (see Discussion). Using recombination values of less than 5% as being in the same gene, in combination with the complementation data from Table 1, we assigned syn-101 and syn-104 to the syn-20 complementation group and syn-107 to the ts-B5 complementation group. syn-106 and syn-108 were excluded from the ts-B5 complementation group, and syn-102 was not excluded from or included in either complementation group.

syn-101, syn-107 and syn-109 caused the fusion of monolayers of HEp-2 cells. The other mutants and ts-B5 and syn-20 did not cause the fusion of HEp-2 cells. In mixed infections of HEp-2 cells with the mutants and either syn-20 or ts-B5, extensive fusion was never observed (data not shown).
**Fusion-inducing mutants of HSV-1**

![Image](image.png)

**Fig. 7.** Mobilities of SDS-peptides of metabolically labelled infected cells. $[^{35}S]$methionine was added to infected cells from 4 to 10 h after infection. The cells were lysed in an SDS-sample solution, separated using SDS-gel electrophoresis and visualized by fluorography. Lane 1, radioactively labelled mol. wt. ($\times 10^3$) standards; lane 2, syn-101 at 34 °C; lane 3, syn-101 at 38 °C; lane 4, syn-102; lane 5, syn-106; lane 6, KOS. The incubation temperature was 34 °C unless otherwise indicated. syn-101-infected cells were also incubated and labelled in the presence of 1 μg/ml tunicamycin, an inhibitor of glycosylation (see, for example, Pizer *et al.*, 1980), at 34 °C (lane 7) and 38 °C (lane 8).

**Fig. 8.** Mobilities of SDS-glycopeptides of metabolically and surface-labelled infected cells. For metabolically labelled cells $[^{14}C]$glucosamine was present in the growth medium from 4 to 10 h post-infection. After labelling, cells were lysed in an SDS-sample solution, the glycopeptides separated using SDS-gel electrophoresis and visualized by fluorography. Lane 1, mock-infected cells; lane 2, syn-101 at 34 °C; lane 3, syn-101 at 38 °C; lane 4, KOS; lane 5, syn-102; lane 6, syn-106. The incubation temperature was 34 °C unless otherwise indicated. The mobilities of the major glycoproteins gB, gC and gD are indicated. gC contains relatively little methionine label and is difficult to visualize using $[^{35}S]$methionine as a label.

The recombination data between *ts*-B5 and MP and between *ts*-B5 and syn-20 are consistent with the mutations in *ts*-B5 and MP being in different genes, and with the mutations MP and syn-20 being in the same gene (Table 2).

**Glycopeptides and peptides synthesized in mutant-infected cells**

Infected cells were radioactively labelled, the glycopeptides and peptides were separated using SDS-gel electrophoresis, and visualized by fluorography. syn-101 gave relatively normal peptide and glycopeptide profiles at 34 °C (Fig. 7 and 8, lane 2) and less of all the glycopeptides at 38 °C (twice as much sample was applied to lane 3 relative to lane 2 in Fig. 8). At 38 °C there was an extreme decrease in the amount of gC (Fig. 8, lane 3) as well as two adjacent peptides of apparent mol. wt. slightly greater than 69 $\times 10^3$ (Fig. 7, lane 3). The small decrease in the mobilities of glycopeptides at 38 °C was reproducible, but was also observed for KOS at 38 °C (not shown). syn-102 showed a decrease in the amount of gC and perhaps gB production (Fig. 8, lane 5) and in some experiments showed an increase in the mobility of glycoproteins (Fig. 7, lane 4). Although the glycopeptide profile of syn-106 is normal (Fig. 8, lane 6), repeated experiments showed a complete lack of a non-glycosylated
peptide, perhaps infected-cell peptide 6 (Honess & Roizman, 1973) of apparent mol. wt. 136 × 10^3 (Fig. 7, lane 5). The glycopeptide and peptide profiles of the other mutants which produce extensive cell fusion were indistinguishable from each other and from the parent viruses (data not shown).

DISCUSSION

The complementation and recombination data presented here are consistent with the mutations in ts-B5 and syn-20 being in two different genes. These data form the first rigorous demonstration for genetic complementation between two HSV-1 mutants that affect cell fusion. In single infections of HEL cells, ts-B5 caused fusion at 34 °C but not at 38 °C. In cells mixedly infected with ts-B5 and either HFEM or KOS at 38 °C, the small amount of fusion characteristic of the non-syncytial strains was observed. The same amount of fusion was observed at 38 °C for cells mixedly infected with ts-B5 and syn-20 at 38 °C. Therefore, it cannot be argued that ts-B5 merely blocks fusion at the restrictive temperature. Although mixed infections of HEp-2 cells with ts-B5 and MP at 38 °C were previously reported to give no fusion (Manservigi et al., 1977), one cannot conclude unequivocally that complementation had resulted, because ts-B5 did not induce fusion in single infections of HEp-2 cells at any temperature. It would have been informative to use MP in these experiments because it is known to be altered in a different gene than is ts-B5 (Ruyechan et al., 1979). However, it could not be used because of its co-dominance in HEL-infected cells (Bzik & Person, 1981). For example, extensive fusion was observed between ts-B5- and MP-infected HEL cells at 38 °C (data not shown).

Yamamoto & Kabuta (1976) examined recombination between a syn and syn + virus of HSV-1 and frequently observed progeny plaques of intermediate size and, apparently, fusion characteristics. The syn phenotype was inferred to be due to a double-mutation in widely separated cistrons with the intermediate plaque-producing recombinants containing either of the single mutations. However, since syn + progeny were only rarely produced from crosses between recombinant viruses of the intermediate-size type, the data are also compatible with the two mutations being within a single cistron. Complementation experiments between the intermediate plaque-size mutants were not reported. However, in a provocative later set of experiments, the same authors performed complementation tests between syn + revertants isolated from a single syn mutant of HSV-1 (Yamamoto & Kabuta, 1977). The syn phenotype was observed in a number of cases and, barring possible complications, the results represent an example of intergenic complementation between the original syn mutation and a second suppressor mutation which could be in any one of several cistrons. These cistrons, when altered by mutation, suppress fusion in an existing syn mutation. Unfortunately, the syn mutant was the one used in the previous study and, therefore, apparently contains multiple syn mutations.

In a recent publication Little & Schaffer (1981) scored complementation using the plaque morphology of single plaques arising from infectious centres mixedly infected with two syn mutants. Plaques with a syn + phenotype were observed using syn mutants 78R and 804, derived from the KOS strain of HSV-1. However, only 76% of the plaques showed this phenotype in Vero cells, perhaps due to varying multiplicities in secondary and tertiary rounds of infection required to produce plaques, but no syn + plaques were observed for a test cross between strains HFEM and MP that have been shown by physical mapping to contain syn alteration in different genes (Ruyechan et al., 1979). Co-dominance of syn mutants was not considered in these studies. The results of marker transfer experiments appear to support the conclusion of the complementation experiments (Little & Schaffer, 1981). However, these experiments need to be repeated using clones, rather than purified restriction fragments, of
Fusion-inducing mutants of HSV-1

HSV-1 DNA because of the possible cross-contamination with restriction fragments of similar sizes.

Two mutations have been identified in \textit{ts-B5} that are separable by genetic recombination; one leads to the \textit{syn} phenotype and the other to the temperature-sensitive production of \textit{gB} (Honess \textit{et al.}, 1980). The authors did not distinguish between the mutations being in the same genes or in different genes. Marker-rescue and marker-transfer co-transfection experiments have shown that the two mutations have an average separation of 2.55 kilobase pairs (N. A. DeLuca \textit{et al.}, unpublished results). Since a minimum of 2.7 kilobase pairs is required to specify the peptide backbone of \textit{gB} (about $95 \times 10^3$ mol. wt.), the mutations could be in the same gene. If both mutations are in the same gene, then the expression of the \textit{syn} phenotype as well as \textit{gB} production would be temperature-sensitive. If the mutations are in separate genes, the \textit{syn} mutation would be temperature-independent and the temperature sensitivity of the \textit{syn} phenotype would only reflect a requirement for \textit{gB} production for fusion. However, if the mutations are in separate genes, then fusion should not be temperature-sensitive either in mixed infections with non-syncytial (Fig. 3) or with syncytial (Fig. 5) viruses. We conclude that the two mutations in \textit{ts-B5} are in the same gene. This conclusion would be invalidated if the \textit{syn} mutation was also temperature-sensitive, but \textit{syn} segregants from \textit{ts-B5} are not temperature-sensitive for fusion in a line of Vero cells (N. A. DeLuca \textit{et al.}, unpublished results).

Recombination and complementation data between different \textit{syn} mutants are of limited use in genetic localization. For recombination data to be reliable, closely linked markers are required. Complementation is ambiguous, possibly because of the co-dominance of some mutants in mixed infections with parental viruses for HEL cells. Nonetheless, we have tentatively assigned six of the nine mutants to the \textit{ts-B5} or \textit{syn-20} complementation group and excluded two of the remaining three from the \textit{ts-B5} complementation group. We emphasize the limitations stated in Results in making these assignments and note that final assignments will require the use of different experiments. It is surprising that two out of three mutants isolated from HFEM were assigned to the \textit{ts-B5} group and zero out of six isolated from KOS were assigned to this group. The latter finding is consistent with the earlier report that eight out of eight mutants isolated from KOS were in the \textit{syn-20} gene (Read \textit{et al.}, 1980). We do not understand this finding, but note that we have recently isolated six \textit{syn} mutants by \textit{in vitro} mutagenesis of KOS-derived, cloned restriction fragments that encode the \textit{ts-B5} complementation group region (N. A. DeLuca, unpublished results).

The SDS-gel electrophoresis peptides and glycopeptides of the mutants are of some interest. Although most mutants gave patterns that were indistinguishable from or very similar to those observed for the parental viruses, some exceptions were noted. \textit{syn-101} produced markedly less \textit{gC} at 38 °C relative to 34 °C and, in contrast to MP, which completely lacks \textit{gC}, produced less fusion at 38 °C. This finding is in agreement with the results of Honess \textit{et al.} (1980) who showed, using recombination analysis, that the absence of \textit{gC} is not sufficient to induce fusion, and the presence of \textit{gC} is not sufficient to inhibit fusion. However, as mentioned earlier, the ability to unambiguously establish the role of a missing glycoprotein may be complicated by the finding that mutants such as MP also show a decreased processing of other glycoproteins (S. Person \textit{et al.}, unpublished results). Both the amounts and possibly the mobilities of some glycopeptides are altered in \textit{syn-102}-infected cells. \textit{syn-106} does not cause the synthesis of a non-glycosylated, infected-cell protein, probably ICP6. The lack of this protein may be coincidental to the fusion process.

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REFERENCES


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