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Cell fusion induced by herpes simplex virus is promoted and suppressed by different viral glycoproteins*

(membranes)

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ABSTRACT **Some of the factors that regulate membrane fusion resulting in polykaryocyte formation have been investigated, using the model system of human cells infected with mutants of herpes simplex virus (HSV). One of the mutant viruses used in this study (MP) failed to produce the viral glycoprotein designated C_2—a nonlethal defect that has previously been correlated with the polykaryocyte-inducing phenotype of this and other mutant strains (wild-type strains of HSV usually induce the aggregation of infected cells rather than their fusion). The other mutant virus (tsB5), a temperature-sensitive conditional-lethal mutant, failed to produce glycoprotein B_2 at nonpermissive temperature, whereas the synthesis of all other viral products appeared to be normal. We produced and isolated seven recombinants of MP and tsB5 that expressed both of the parental alterations in glycoprotein synthesis. All of the recombinant viruses induced the fusion of infected cells at 34° (correlated with the absence of C_2 expression) but were unable to cause cell fusion at 39° (correlated with the absence of C_2 and of B_2 expression), even after infection at multiplicities high enough to ensure that all cells in the cultures synthesized viral macromolecules. These results and studies on the dominance or recessiveness of the fusion-inducing phenotype in mixed infections provide evidence that glycoprotein B_2 plays a critical role in the promotion of cell fusion and that glycoprotein C_2 can act to suppress fusion.**

The fusion of cellular membranes is an important component of several essential biological processes, including both endocytosis and exocytosis and also syncytium formation such as occurs during muscle development. Membrane glycoproteins may play a critical role in promoting membrane fusion, as indicated by studies done with certain enveloped viruses that are capable of inducing cell fusion (reviewed in refs. 1 and 2). Sendai virus, for example, specifies the synthesis of a membrane glycoprotein that is responsible for the ability of this virus to induce cell fusion and is also required for viral infectivity (3, 4), which probably depends in part upon fusion of the virion envelope with the plasma membrane of the host cell (3–5).

Membrane fusion, whether virus-induced or directed by cellular products, must be regulated in some way so that the fusion occurs only between specialized regions of particular membranes in a single cell or between the surface membranes of specific differentiated cell types. An understanding of the process of membrane fusion, therefore, requires identification of membrane constituents or other products that can prevent or limit the fusion of adjacent membranes, in addition to characterization of the membrane constituents that promote fusion. This paper presents evidence that, in human cells infected with herpes simplex virus (HSV), the promotion and suppression of cell fusion are two separate viral functions associated with two different viral glycoproteins.

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Cells infected with HSV exhibit modifications in their social behavior, the nature of which depends upon properties of the particular virus strain used for infection (6–8). Most fresh isolates of HSV cause cell aggregation in which varying degrees of adhesiveness can be evident. Some strains of HSV induce cell fusion, however, and in certain instances it has been shown that the polykaryocyte-inducing phenotype results from a nonlethal mutation of cyto-aggregating virus (6, 9–13). It should be noted that expression of the polykaryocyte-inducing phenotype may depend in part on properties of the host cell because some HSV mutants (and laboratory strains) promote the fusion of certain cell lines or strains but not of others (6,‖).

Previous studies suggested that the kind of altered cellular interactions observed after HSV infection was determined at least in part by the spectrum and properties of viral glycoproteins present in plasma membranes of infected cells (14–16) and, in fact, the polykaryocyte-inducing phenotype of some HSV type 1 (HSV-1) mutants can be correlated with a specific defect in glycoprotein synthesis. Comparisons of the glycoproteins made by different HSV-1 strains revealed that cyto-aggregating isolates specify the synthesis of at least four membrane glycoproteins (13, 15, 17), whereas some fusion-inducing mutants are clearly defective in the synthesis or processing of the glycoprotein designated C_2 (13, 15, 18). It has been observed that the wild-type phenotype (cell aggregation and normal production of all glycoproteins, including C_2) is usually dominant over the fusion-inducing phenotype in mixed infections (6, 19, 20). These findings suggested to us that the glycoprotein whose synthesis is defective in infections with the polykaryocyte-forming mutants may normally act to suppress the fusion-inducing activity of another HSV-1 product. Previous studies have indicated that this fusion-promoting viral product may also be a membrane glycoprotein (21–24).

In this paper we present evidence that HSV-1-induced cell fusion is governed by the activities of at least two viral glycoproteins, one of which (glycoprotein B_2) promotes fusion whereas the other (glycoprotein C_2) suppresses it. This conclusion is based on experiments done with HSV-1 mutants, including a polykaryocyte-forming mutant that fails to synthesize glycoprotein C_2, a temperature-sensitive (ts) mutant that is unable to produce glycoprotein B_2 at nonpermissive tempera-

Abbreviations: HSV, herpes simplex virus; HSV-1, HSV type 1; ts, temperature-sensitive; HEp-2, human epidermoid carcinoma no. 2; 199-V, medium 199 supplemented with 1% fetal calf serum; MOI, multiplicity of infection; PFU, plaque-forming units.

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‖ R. Manservigi and P. G. Spear, unpublished data.

Table 1. Numbers and morphologies of plaques formed by the parental mutant viruses and the double-mutant recombinant viruses at permissive and nonpermissive temperatures

Virus	Number of plaques, PFU/ml 34°	39°	Ratio 34°/39°	Morphology of plaques on HEp-2 cells* 34°	39°
		Parental viruses			
MP	6.0×10^8	1.1×10^9	0.5	pk.	pk.
tsB5	1.7×10^9	2.5×10^4	6.8×10^4	cyto-agg.†	—
		Double-mutant recombinants			
tsB5MP-1	3.4×10^8	6.5×10^3	5.2×10^4	pk.	—
tsB5MP-2	3.0×10^8	3.0×10^3	1.0×10^5	pk.	—
tsB5MP-3	2.2×10^9	7.0×10^4	3.2×10^4	pk.	—
tsB5MP-4	2.1×10^8	5.0×10^3	4.2×10^4	pk.	—
tsB5MP-5	5.0×10^8	$<10^4$	$>5.0 \times 10^4$	pk.	—
tsB5MP-6	1.5×10^8	2.0×10^3	7.5×10^4	pk.	—
tsB5MP-7	5.0×10^8	3.6×10^4	1.4×10^4	pk.	—

* Polykaryocytes are indicated by pk. A dash indicates that few plaques were detected; these had the same morphology as those detected at permissive temperature.
† Foci of aggregated or adherent cells.

ture, and seven double-mutant recombinant viruses that are unable to produce C_2 at permissive temperature or B_2 and C_2 at nonpermissive temperature.

MATERIALS AND METHODS

Cells and Media. HEp-2 cells (human epidermoid carcinoma no. 2 cells) were grown in Dulbecco's modification of Eagle's minimal essential medium supplemented with 10% heat-inactivated fetal calf serum and were maintained after infection in medium 199 supplemented with 1% fetal calf serum (199-V).

Viruses and Virus Titrations. The isolation (9) and properties (6, 7, 15, 18, 20, 24) of MP, a spontaneously derived polykaryocyte-forming mutant obtained from the cyto-aggregating strain HSV-1(mP), have been described. The ts mutant tsB5 was isolated by one of us (A.B.) from HSV-1(HFEM) after mutagenesis with 5-bromodeoxyuridine. At both permissive (34°) and nonpermissive (39°) temperatures, tsB5 is capable of carrying out viral DNA synthesis, viral polypeptide synthesis, and nucleocapsid assembly (A. Buchan *et al.*, unpublished data). Virus titrations were performed on HEp-2 cell monolayers using a liquid overlay of medium 199-V containing 0.1% pooled human gamma globulin, as previously described (25).

Isolation of Double-Mutant Recombinant Viruses. Monolayer cultures of HEp-2 cells were mixedly infected with the MP and tsB5 mutants at a combined multiplicity of infection (MOI) of 5 plaque-forming units (PFU) per cell (2.5 PFU of each virus per cell) and the cultures were incubated at 34° for 18 hr. Viral progeny obtained from lysates of these cultures were plated on HEp-2 cells at 34° under an agarose overlay for the recovery of virus from isolated plaques that exhibited the polykaryocytic morphology. Virus obtained from each plaque was tested for its ability to form plaques at permissive and nonpermissive temperatures. Seven out of 100 polykaryocyte-forming isolates were found to be ts and each of these was plaque-purified through two additional cycles of replating on HEp-2 cells.

Preparation and Analysis of Radioactively Labeled Cell Extracts. Monolayer cultures of HEp-2 cells were infected by the addition of virus at an MOI of 20 PFU per cell and were incubated from 5 to 19 hr after infection with medium 199-V containing D-[*1*-^{14}C]glucosamine hydrochloride (New England Nuclear, Boston, MA) at a concentration of 1 μCi/ml of medium. The cells were harvested by scraping, collected by centrifugation, and solubilized in a buffer containing sodium dodecyl sulfate for electrophoresis on 8.5% polyacrylamide gel slabs crosslinked with *N-N'*-diallyltartardiamide as previously described (18). After electrophoresis the gels were fixed and stained with Coomassie brilliant blue, dried on filter paper, and placed in contact with x-ray film for autoradiography.

RESULTS

Plating Efficiencies of the Parental Mutant Viruses and Double-Mutant Recombinant Viruses and Morphologies of Plaques Formed. Table 1 lists some pertinent properties of MP and tsB5 and of the double-mutant recombinant viruses that were isolated. Whereas MP formed plaques on HEp-2 cells with equal efficiency at 34° and at 39°, the plating efficiency of tsB5 and the seven recombinant isolates was 10^4- to 10^5-fold higher at 34° than at 39°. On the other hand, MP and all the recombinants produced polykaryocytic plaques on HEp-2 cells, whereas the plaques formed by tsB5 were foci of rounded adherent cells. Thus, each of the seven recombinants expressed the conditional-lethal ts phenotype of tsB5 and the fusion-inducing phenotype of MP; the recombinants were isolated from six different mixedly infected cultures and therefore represent the products of at least six recombinational events.

Glycoproteins Synthesized by the Parental Mutant Viruses and by the Double-Mutant Recombinant Viruses. Fig. 1 shows the electrophoretic separations on sodium dodecyl sulfate/acrylamide gels of [^{14}C]glucosamine-labeled proteins from HEp-2 cells infected either at 34° or at 39° with the parental mutant viruses or with a recombinant. The electrophoretic analysis of only one recombinant virus (tsB5MP-3) is presented because the results of such analyses were identical for all the recombinants. It should be noted that, at permissive temperature, tsB5 specified the synthesis of four major glycoproteins whose electrophoretic mobilities on sodium dodecyl sulfate/acrylamide gels were similar to those of the major glycoproteins (designated A, B_2, C_2, and D_2) made by wild-type HSV-1 (17). [Partially glycosylated forms of three of these proteins (designated B_1, C_1, and D_1 in ref. 17) account for most of the poorly labeled species detected in the autoradiogram of Fig. 1.]

Two points should be made from the data presented in Fig. 1. First, the ts mutation of tsB5 and the recombinants is correlated with a specific alteration in glycoprotein synthesis inasmuch as the fully glycosylated form of polypeptide B (glycoprotein B_2) failed to be produced or failed to accumulate at nonpermissive temperature. The slight difference in electrophoretic mobility between the B_2 of MP and B_2 made by the ts viruses at permissive temperature is typical of variability that has been observed in comparisons of different wild-type HSV-1 isolates (unpublished data) and depends in part on whether the [^{14}C]glucosamine is incorporated primarily into glycoproteins that were processed relatively early or late during the infectious cycle (17). Second, MP and all the recombinants failed to synthesize glycoprotein C_2 at either 34° or 39°. As mentioned above, defects in C_2 production have previously been reported for MP and for two other polykaryocyte-forming mutants (13, 15, 18).

Effect of the ts Mutation on Cell Fusion Induced by the Recombinants. In order to determine whether the recombinant

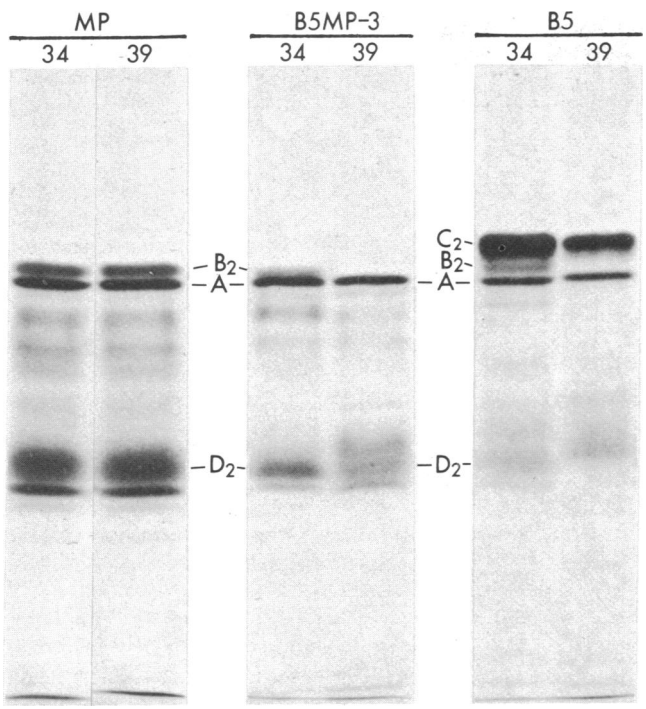

FIG. 1. Autoradiogram of a sodium dodecyl sulfate/acrylamide gel slab showing the electrophoretic separation of [^{14}C]glucosamine-labeled glycoproteins made by MP, tsB5MP-3, and tsB5 at permissive and nonpermissive temperatures. Molecular weights for glycoproteins cannot be reliably estimated from electrophoretic mobilities in sodium dodecyl sulfate gels but, for purposes of comparison, we have previously noted that the HSV-1 glycoproteins comigrate with proteins whose apparent molecular weights are as follows: C_2, 133,000; B_2, 125,000; A, 119,000; and D_2, 63,000. The labeled band near the bottom of each lane contains small molecular weight material that migrates with the bromphenol blue marker.

viruses could induce fusion at 39° as well as at 34°, HEp-2 cells were infected at MOI values of 1, 5, and 10 PFU per cell and incubated either at permissive or at nonpermissive temperature. The results shown in Table 2 and Fig. 2 demonstrate that, whereas MP induced cell fusion at both temperatures and tsB5 did not cause fusion at either temperature, the recombinants induced fusion at 34° but not at 39°. The data shown in Table 2 for two of the recombinants are representative of the results obtained with all seven recombinants; none of them were capable of inducing cell fusion at 39°.

The data presented in Figs. 1 and 2 and Table 2 show that, *in the absence of C_2 expression*, there is a correlation between the production of B_2 and the induction of cell fusion. This correlation was also evident from the results of temperature-shift experiments and from analysis of a revertant. In cells infected with tsB5MP-3, a shift in temperature from 39° to 34° at 8 hr after infection resulted in production of B_2 and in cell fusion. Moreover, a revertant of tsB5MP-3, which had reverted only with respect to the ts mutation, was found to have acquired the ability to synthesize B_2 and to induce cell fusion at 39° along with the capacity to replicate at 39°. Finally, as is demonstrated by data presented in the next section, the inability of the recombinants to induce cell fusion at 39° was due to the absence of fusion-inducing activity rather than to its suppression. This absence of fusion-inducing activity at 39° correlates well with the temperature-dependent alteration in glycoprotein B_2 production inasmuch as the ts mutation did not alter the synthesis of the other HSV-1 glycoproteins (Fig. 1) and did not prevent incorporation of these glycoproteins into the cell surface, as indicated by analyses of radioiodinated cell surface components (data not shown).

Table 2. Fusion-inducing ability of the parental mutant viruses and double-mutant recombinant viruses at permissive and nonpermissive temperatures

Virus	MOI, PFU/cell	Fusion of HEp-2 cells* 34°	39°
	Parental viruses		
MP	1	+++	+++
	5	+++	+++
	10	++	++
tsB5	1	r	r
	5	r	r
	10	r	r
	Double-mutant recombinants		
tsB5MP-3	1	++++	r
	5	++++	r
	10	++++	r
tsB5MP-7	1	+++	r
	5	++	r
	10	+	r

* The plus symbols (+) indicate that a significant number of cells in the culture fused with neighboring cells; the variable number of plus symbols denotes the extent of fusion or the approximate percentage of nuclei that were in polykaryocytes (+, 25%; ++, 50%; +++, 75%; ++++, 100%). The letter *r* indicates that the cells rounded up and exhibited cytopathology characteristic of cells infected with wild-type HSV but that cell fusion did not occur (fewer than 5% of nuclei in polykaryocytes).

We have observed that the tsB5MP recombinants differ from one another in one respect—namely, in the extent to which cell fusion is partially inhibited by high MOI at permissive temperature. As has previously been reported for MP (6, 26), we found that MP and most of the recombinants induced maximal levels of fusion at MOI values between 0.1 and 1 PFU per cell, whereas fusion was somewhat inhibited at higher MOI (Table 2). The recombinants exhibited this property to various degrees, however, or not at all, inasmuch as one recombinant (tsB5MP-3) induced complete fusion of HEp-2 cell monolayers at MOI values from 1 to 50. As yet we have no explanation of the basis for this partial inhibition of fusion at high MOI.

Dominance or Recessiveness of the Fusion-Inducing Phenotype in Mixed Infections. Our results suggest that the failure of HSV-1 to induce cell fusion can be due either to the presence of functional glycoprotein C_2 (putative fusion-suppressing function), or, in the absence of C_2, to a defect in glycoprotein B_2 (putative fusion-promoting function). This hypothesis leads to certain predictions as to the dominance or recessiveness of the fusion-inducing phenotype in cells mixedly infected with particular viral mutants. Specifically, the fusion-inducing phenotype should be recessive in cells infected with both a polykaryocyte-forming mutant and a virus that expresses a functional C_2; on the other hand, the fusion-inducing phenotype should be dominant in cells infected with a fusion-inducing mutant and with a virus that does not cause fusion because it fails to make both B_2 and C_2. In fact, these predictions were borne out by results of experiments summarized in Table 3. The mutant tsB5 was able to suppress the fusion-inducing capacity of MP at both 34° and 39°, and several considerations, including the data in Fig. 1, suggest that the expression of C_2 at both temperatures was responsible for this suppression and also for the inability of tsB5 to induce the fusion of HEp-2 cells at permissive temperature. In contrast, the tsB5MP recombi-

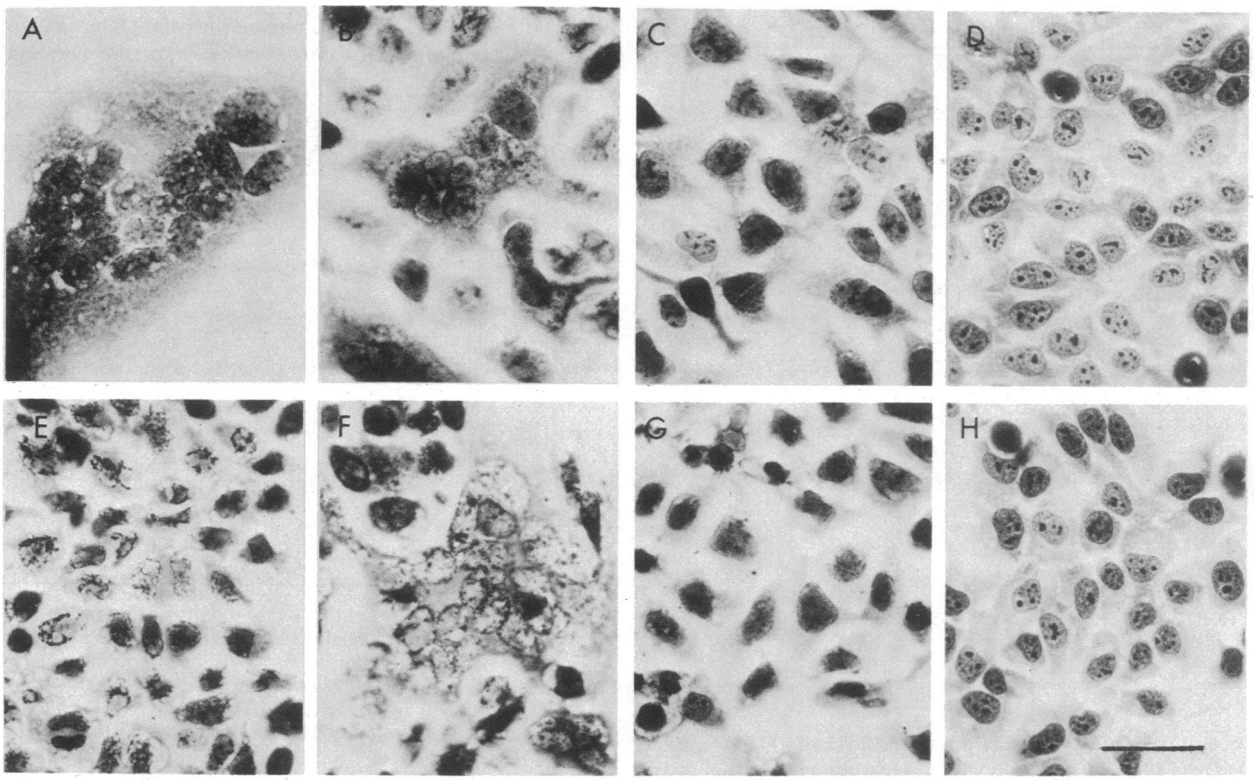

FIG. 2. Photomicrographs of HEp-2 cells infected with the recombinant tsB5MP-3 (*A* and *E*), with MP (*B* and *F*), with tsB5 (*C* and *G*) or of mock-infected cells (*D* and *H*), 24 hr after infection with incubation at 34° (*A* through *D*) or at 39° (*E* through *H*). In all the infected cultures, alterations in nuclear morphology, which are characteristically induced by HSV, were evident and were somewhat more pronounced at 39° than at 34°. The bar represents 50 μm.

nants did not suppress the fusion-inducing capacity of MP at 39°, although the extent of fusion was somewhat less than at 34°, possibly because less functional B_2 would have been made in the mixedly-infected cells at 39°. These results provide additional evidence that the inability of the recombinants to induce fusion at 39° was due to absence of the fusion-promoting function rather than to suppression of this function.

DISCUSSION

The activities of at least two HSV-1 glycoproteins appear to determine whether infected cells will form polykaryocytes or not. Our results indicate that one glycoprotein promotes cell fusion whereas another suppresses it. This discussion will focus on the evidence that glycoproteins B_2 and C_2, respectively, are responsible for these activities and also on the possible roles of these glycoproteins in HSV-1 replication.

Suppression of Fusion. The existence of an HSV-1 product that suppresses HSV-induced cell fusion could have been inferred from earlier observations that (*i*) wild-type HSV-1 isolates usually do not cause cell fusion (6, 9–15), (*ii*) a mutation can "unmask" the fusion-inducing capacity of HSV-1 (6, 8–11, 13, 18), and (*iii*) the fusion-inducing phenotype is recessive to the wild-type phenotype (6, 19, 20). The previous results also suggested that the fusion-suppressing activity might be associated with glycoprotein C_2 expression because, in separate studies, three fusion-inducing mutants were shown to be defective in the production of this glycoprotein (13, 15, 18). The results presented in this paper provide the most compelling evidence to date that glycoprotein C_2 can suppress or modulate the activity of a viral fusion-inducing factor. Specifically, all the polykaryocyte-forming ts recombinants that we isolated (at least six of which were from independent recombinational events) failed to synthesize C_2 (Fig. 1), and results obtained in mixed infections (Table 3) provided evidence that the expression of C_2, even by a ts virus at nonpermissive temperature, correlated with the suppression of fusion in HEp-2 cells.

It should be noted that some polykaryocyte-forming HSV strains have no readily detectable alterations in glycoprotein synthesis (22,‖). We predict, however, that either (*i*) the C_2 made by these mutants will be found to differ structurally and functionally from wild-type C_2 or (*ii*) the fusion-promoting glycoprotein will be altered so that its activity is no longer suppressible by wild-type C_2. An interesting property of these mutants is that they express the fusion-inducing phenotype in some cells but not in others, whereas the strains that fail to make C_2 are much less host-dependent in expression of the mutant phenotype.‖ In fact, tsB5 at permissive temperature and its parental strain HFEM can induce cell fusion in certain cell lines, but not in HEp-2 cells. The possibility exists that certain cell lines such as HEp-2 may process some mutated forms of poly-

Table 3. Dominance or recessiveness of the fusion-inducing phenotype after mixed infections of HEp-2 cells

Viruses*	Fusion of HEp-2 cells† 34°	39°
MP	+++	+++
tsB5	r	r
tsB5MP-3	++++	r
tsB5MP-7	++	r
MP + tsB5	r	r
MP + tsB5MP-3	+++	++
MP + tsB5MP-7	++	+

* The MOI values were 5 PFU/cell total (2.5 PFU of each virus per cell in the cultures that were mixedly infected).
† See footnote in Table 2 for explanation of symbols used.

peptide B or polypeptide C so that the fully glycosylated products have wild-type function, whereas other cell lines yield products with altered function. A necessary corollary of this hypothesis is that the HSV glycoprotein percursors can be processed differently by different cell lines; some preliminary data suggest that this is indeed the case (15,‖).

Promotion of Fusion. Our results indicate that glycoprotein B_2 is the HSV-1 fusion-promoting factor or, at least, is one of the viral products required for the induction of cell fusion. This conclusion is based on our findings that, in all of our independently derived recombinants, the ts mutation selectively interfered with the normal production or accumulation of glycoprotein B_2 at 39° and eliminated the capacity to induce cell fusion at 39°. Fusion-inducing activity was absent rather than suppressed at 39°, as indicated by the inability of the tsB5MP recombinants to prevent MP-induced cell fusion at 39° (Table 3). Finally, in a revertant of tsB5MS-3, the capacities to synthesize glycoprotein B_2 and to induce cell fusion at 39° were restored along with the ability to produce infectious virus at 39°.

Roles of Glycoproteins B_2 and C_2 in HSV-1 Replication. A consideration of the possible roles of glycoproteins B_2 and C_2 in viral replication allows certain predictions to be made regarding the nature of their functional interactions and activities. In the first place, it should be noted that both of these glycoproteins are constituents of the virion envelope (17, 18, 27) as well as of infected cell membranes (13–17). It has been proposed that efficient penetration of the HSV nucleocapsid into a host cell requires the occurrence of fusion between the virion envelope and the cell surface (28, 29). If this hypothesis is correct, then glycoprotein B_2 will probably be found to play an essential role in inducing this fusion. The sequence of events required for initiation of infection might include (*i*) attachment of the virion to a receptor on the cell surface, mediated perhaps by the viral product that is responsible for the increased adhesiveness of HSV-infected cells, and then (*ii*) promotion of membrane fusion by the interaction of B_2 with some cell surface component.

The role of glycoprotein C_2 in viral replication is more difficult to assess. It is necessary, first of all, to determine whether the suppression of fusion-promoting activity requires that C_2 interact physically with B_2. If so, then the functional interaction leading to suppression of fusion could occur between molecules made by the same cell (*cis* interaction) or, alternatively, between B_2 on one cell and C_2 on a neighboring cell (*trans* interaction). Because both B_2 and C_2 are in the virion envelope and because fusion is probably required for infectivity, it seems unlikely that the *cis* configuration would lead to suppression of fusion. On the other hand, suppression of fusion by the *trans* interaction could conceivably have selective advantage and facilitate the dissemination of infection because the appearance of C_2 in the membranes of infected cells could prevent progeny virus from fusing with and being eclipsed by the already infected cell.

Regardless of the roles of glycoproteins B_2 and C_2 in the viral infectious cycle, use of HSV-infected cells to study cell fusion offers a genetically manipulatable system in which the structures of the viral membrane glycoproteins can eventually be precisely related to their functions in the processes that govern membrane fusion.

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1. Choppin, P. W. & Compans, R. W. (1975) in *Comprehensive Virology*, eds. Fraenkel-Conrat, H. & Wagner, R. R. (Plenum Press, New York) Vol. 4, pp. 95–178.
2. Spear, P. G. (1977) in *Cell Membranes and Viral Envelopes*, eds. Blough, H. A. & Tiffany, J. M. (Academic Press, Inc., New York), in press.
3. Homma, M. & Ohuchi, M. (1973) *J. Virol.* **12**, 1457–1465.
4. Scheid, A. & Choppin, P. W. (1974) *Virology* **57**, 475–490.
5. Cohen, A. (1963) in *Mechanisms of Virus Infection*, ed. Smith, W. (Academic Press, Inc., New York), pp. 153–199.
6. Roizman, B. (1962) *Cold Spring Harbor Symp. Quant. Biol.* **27**, 327–342.
7. Ejercito, P. M., Kieff, E. D. & Roizman, B. (1968) *J. Gen. Virol.* **2**, 357–364.
8. Gray, A., Tokumaru, T. & Scott, T. F. (1958) *Arch. Gesamte Virusforch.* **8**, 60–76.
9. Hoggan, M. D. & Roizman, B. (1959) *Am. J. Hyg.* **70**, 208–219.
10. Nii, S. & Kamahora, J. (1961) *Biken J.* **4**, 75–96.
11. Brown, S. M., Ritchie, D. A. & Subak-Sharpe, J. (1973) *J. Gen. Virol.* **18**, 329–346.
12. Timbury, M., Theriault, A. & Elton, R. A. (1974) *J. Gen. Virol.* **23**, 219–224.
13. Cassai, E., Manservigi, R., Corallini, A. & Terni, M. (1975/76) *Intervirology* **6**, 212–223.
14. Spear, P. G., Keller, J. M. & Roizman, B. (1970) *J. Virol.* **5**, 123–131.
15. Keller, J. M., Spear, P. G. & Roizman, B. (1970) *Proc. Natl. Acad. Sci. USA* **65**, 865–871.
16. Heine, J. W., Spear, P. G. & Roizman, B. (1972) *J. Virol.* **9**, 431–439.
17. Spear, P. G. (1976) *J. Virol.* **17**, 991–1008.
18. Heine, J. W., Honess, R. W., Cassai, E. & Roizman, B. (1974) *J. Virol.* **14**, 640–651.
19. Roizman, B. (1970) in *Viruses Affecting Man and Animals*, ed. Sanders, M. (Warren Green Publishers, St. Louis, MO), pp. 37–71.
20. Keller, J. M. (1976) *Virology* **69**, 490–499.
21. Gallaher, W. R., Levitan, D. B. & Blough, H. (1973) *Virology* **55**, 193–201.
22. Knowles, R. W. & Person, S. (1976) *J. Virol.* **18**, 644–651.
23. Levitan, D. B. & Blough, H. A. (1976) *J. Virol.* **18**, 1081–1087.
24. Keller, J. M. (1976) *Virology* **72**, 402–409.
25. Roizman, B. & Roane, P. (1961) *Virology* **15**, 75–79.
26. Roizman, B. (1962) *Proc. Natl. Acad. Sci. USA* **48**, 228–234.
27. Spear, P. G. & Roizman, B. (1972) *J. Virol.* **9**, 143–159.
28. Morgan, C., Rose, H. M. & Mednis, B. (1968) *J. Virol.* **2**, 507–516.
29. Hummeler, K., Tomassini, N. & Zajac, B. (1969) *J. Virol.* **4**, 67–74.