Chapter 1

Virus-Induced Cell Fusion

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1. INTRODUCTION

1.1. Purpose

Attention is focused in this review on viral proteins that are known to mediate or influence virus-induced cell fusion. Because these proteins also mediate or influence entry of virus into cells, the virion–cell fusion required for this entry is discussed as well. The emphasis is on new information that has emerged since publication of an earlier review on these subjects (White *et al.*, 1983). It was not the intention to provide comprehensive treatment of these subjects.

1.2. Viruses That Induce Cell Fusion

Representatives of many families of animal viruses have been shown to induce cell fusion under appropriate conditions (Table I). These viruses have both similarities and differences that are relevant to an analysis of virus-induced cell fusion. The similarities include the following: All animal viruses capable of inducing cell fusion have one structural compo-

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Table I. Some Fusion-Inducing Viruses and Selected Properties

Virus family	Representative member	Genome		Nucleocapsid		Site of envelopment	Reference[b]
		Nucleic acid[a]	Size (kb or kbp)	Symmetry	Site of assembly		
Rhabdovirus	Vesicular stomatitis (VSV)	ssRNA(−)	12	Helical	Cytoplasm	Plasma membrane	Wagner (1975)
Paramyxovirus	Sendai	ssRNA(−)	16–18	Helical	Cytoplasm	Plasma membrane	Choppin and Compans (1975)
Orthomyxovirus	Influenza	ssRNA(−)	13.5–14	Helical	Cytoplasm	Plasma membrane	Lamb and Choppin (1983)
Bunyavirus	LaCross	ssRNA(−)	14–17	Helical	Cytoplasm	Golgi complex	Bishop and Shope (1979)
Togavirus	Semliki Forest (SFV)	ssRNA(+)	12	Icosahedral	Cytoplasm	Plasma membrane	Garoff *et al.* (1982)
Coronavirus	Infectious bronchitis (IBV)	ssRNA(+)	18	Helical	Cytoplasm	Endoplasmic reticulum	Siddell *et al.* (1983)
Retrovirus	Mouse mammary tumor (MMTV)	ssRNA(+)	8–10[c]	Icosahedral	Cytoplasm	Plasma membrane	Varmus and Swanstrom (1984)
Herpesvirus	Herpes simplex (HSV)	dsDNA	140–250	Icosahedral	Nucleus	Inner nuclear membrane	Spear and Roizman (1980)
Poxvirus	Vaccinia	dsDNA	200–330	Complex	Cytoplasm	Golgi complex[d]	Moss (1974)

[a] ss, single-stranded; ds, double-stranded; (+) or (−) denotes whether genomic RNA has the same polarity as mRNA (+) or opposite polarity (−).
[b] These reviews summarize properties characteristic of the virus families. Replication strategies of most of the RNA viruses listed are also described and compared in Strauss and Strauss (1983). Some of the information is documented in papers cited in the text.
[c] Two identical RNAs of this size are present in each virion.
[d] Poxviruses are assembled in the cytoplasm to form a particle of complex architecture that can be infectious in cell culture without acquiring an envelope derived from cell membrane. The form of the virus that is infectious in nature probably has an envelope, however, and this envelope is derived from membrane of the Golgi complex (Payne and Kristenson, 1979; Hiller and Weber, 1985).

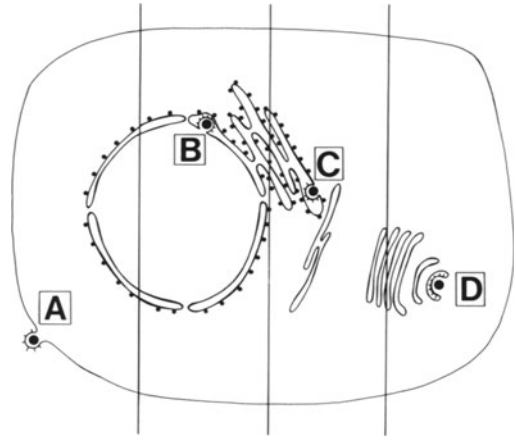

Figure 1. Envelopment of nucleocapsids by modified patches of cell membrane during final stages of virion morphogenesis. Members of different virus families characteristically acquire their envelopes by budding through the plasma membrane (A), the inner nuclear membrane (B), the membrane of the endoplasmic reticulum (C), or the Golgi apparatus (D). Table I lists the sites of envelopment for different virus families.

nent in common—namely, a lipid-containing membrane (envelope) that is the outer coat of the virus particle (virion) containing viral glycoproteins essential for infectivity (ability of the virus to attach to and invade a cell so as to initiate viral gene expression). In all cases this envelope is derived, during virion morphogenesis in the virus-producing cell, from a patch of cell membrane that has been modified by the incorporation of viral-specified glycoproteins and by the exclusion of most or all cell proteins. The viral nucleocapsid (genome plus proteins arranged in a regular helical or icosahedral structure) buds through this modified patch of cell membrane, and the neck of the bud seals up to yield the virion with its intact envelope (Fig. 1). Once released from the virus-producing cell, the virion may infect another cell. Infectivity depends on ability of the viral envelope glycoprotein(s) to mediate attachment of the virion to a cell surface and to induce fusion of the virion envelope with a cell membrane (Fig. 2). This fusion introduces the nucleocapsid into the cytoplasm of the cell so that viral gene expression can be initiated.

Differences among the viruses to be discussed include differences in genetic complexity, in the intracellular site at which envelopment of nucleocapsids characteristically occurs during virion morphogenesis (Table I and Fig. 1) and in the site at which membrane fusion occurs to initiate a new infectious cycle (Fig. 2). To infect a cell and initiate a new infectious cycle, some enveloped viruses probably fuse with the cell at the plasma membrane, some may fuse with the membrane of an endosome following receptor-mediated endocytosis, and some may do either depending on circumstances.

Virus-induced cell fusion can be mediated either by the virion itself or by viral proteins incorporated into the plasma membrane of the infec-

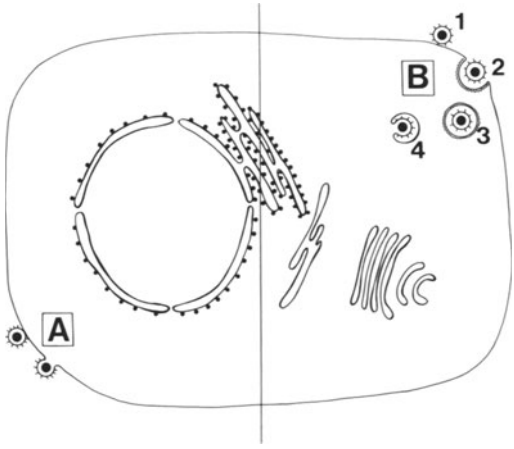

Figure 2. Pathways of viral entry during initiation of infection. The virion–cell fusion required to release the nucleocapsid into the cytoplasm may occur at the plasma membrane (A) or with the membrane of an endosome (B). Stages of the endocytic pathway (B) are thought to be similar to those defined for receptor-mediated endocytosis of other ligands (Goldstein *et al.*, 1985). They include attachment of virus to the cell surface, lateral movement to a clathrin-coated pit, ingestion by the cell in a coated vesicle, transition of the vesicle from coated to uncoated (endosome), and fusion of the virion with the membrane of the endosome.

ted cell. These operationally distinct types of virus-induced cell fusion have been called fusion from without (FFWO) in the first instance and fusion from within (FFWI) in the second (Kohn, 1965; Bratt and Gallaher, 1969). For FFWO the cells must be exposed to large numbers of virions, but the virions need not necessarily be capable of initiating or sustaining viral gene expression. FFWI can be induced by virus at low multiplicities of infection and requires viral gene expression. A number of reviews have been published on the subject of FFWI and FFWO (Roizman, 1962; Poste, 1972; Hosaku and Shimuzu, 1977; Knutton, 1978; Poste and Pasternak, 1978). In this review the emphasis is on FFWI, except where noted otherwise.

Events that have been monitored as indicative of virus-induced membrane fusion include hemolysis as well as FFWO and FFWI. Fusion of virions with red cells can result in hemolysis, either as a concomitant of the fusion reaction or due to incorporation of damaged viral envelopes into the red cell membrane.

The viral glycoproteins responsible for mediating virion-cell fusion to initiate infection also mediate cell fusion. A virus may be able to fuse with a cell to initiate infection, however, and yet apparently not induce cell fusion, either FFWO or FFWI. Whether fusion is observed in cultured cells depends on culture conditions and on both viral and cell determinants. For example, different strains of the same virus may differ in their ability to induce cell fusion. Moreover, a single virus strain may fuse some cells but not others.

In general, failure to detect cell fusion after infection with a virus capable of inducing membrane fusion could be due to one of several cir-

cumstances, including the following: (1) failure of the viral fusion protein(s) to be incorporated at sufficiently high concentration into the plasma membrane (this could be compatible with viral replication especially if envelopment occurs at another membrane); (2) inappropriate conditions at the cell surface for activation of the viral fusion protein(s); (3) the presence of viral or cell components in the plasma membrane that inhibit or modulate activity of the viral fusion protein(s); (4) the absence of appropriate receptors in the plasma membrane of uninfected cells—receptors that could be required, not for attachment of virus, but for the membrane fusion process (this could be compatible with viral infectivity provided the virus can attach to the cell surface and then fuse with the membrane of an endosome); or (5) failure to recognize that membrane fusion has occurred because two cells that are perhaps joined by cytoplasmic bridges retain the shape of two adherent spheres (or discoids) instead of assuming the shape of a single sphere.

1.3. Significance of Virus-Induced Cell Fusion

Cell fusion is not an invariant consequence of infection with viruses that have the potential to induce membrane fusion. In fact, for some virus families (orthomyxoviruses, bunyaviruses, togaviruses, retroviruses), infection rarely if ever results in cell fusion under physiological conditions. For other virus families, the extent of cell fusion may be highly strain dependent. Matters seem to have been arranged so that expression of overt membrane-fusing activity is often limited to the virion in its interactions with uninfected cells but is sometimes also observed to occur at infected cell surfaces and to result in cell fusion.

Virus-induced cell fusion can be of conseqence in pathogenicity, as fusion affords a means of spreading infection that does not depend on production or survival of infectious virus. For example, antibodies to the receptor-binding glycoprotein of a paramyxovirus can neutralize viral infectivity but do not prevent spread of infection in cell culture by cell fusion, whereas antibodies to the fusion protein prevent the fusion (Merz *et al.,* 1980). In addition, inactivated vaccines for several paramyxoviruses have proved deficient in inducing antibodies to the fusion protein, but not the receptor-binding protein, and have also proved ineffective (Norrby *et al.,* 1975; Norrby and Penttinen, 1978).

For defining mechanisms by which fusion of biological membranes can be induced and regulated, virus-infected cells provide an attractive experimental system. At least some of the components that either induce or regulate the fusion are viral proteins, and the genes for these proteins are readily altered to provide the mutants that can aid in defining structure-

function relationships. Moreover, there are a variety of ways in which membrane fusion can be assayed in virus-infected cells.

2. INFECTIVITY OF ENVELOPED ANIMAL VIRUSES

2.1. Pathway of Entry into Cells

Defining the site(s) at which a virion can fuse with a cell membrane to initiate infection is relevant to defining the conditions required for cell–cell fusion. For example, if a virion cannot fuse with the plasma membrane but only with the membrane of an endosome, the viral protein(s) responsible for inducing membrane fusion may not be active at the cell surface and therefore may not induce cell fusion, either FFWO or FFWI. As the following illustrates, it can be difficult to establish, however, which cell membranes are targets for virion fusion and which are not.

Viruses such as Semliki Forest virus (SFV, a togavirus), influenza virus (an orthomyxovirus), and vesicular stomatitis virus (VSV, a rhabdovirus) can initiate infection via an endocytic pathway of entry (Marsh, 1984). It has been suggested that the low pH of endosomes may be required to trigger fusion activity of these viruses based principally on the following observations: (1) initiation of infection by these viruses is blocked by agents (ammonium ions, chloroquine, amantadine) that raise the pH of endosomes, and (2) these viruses do not induce FFWO at neutral pH but can do so if the infected cells are exposed briefly to medium of low pH (reviewed by White *et al.,* 1983; Marsh, 1984).

Although these viruses clearly can infect by an endocytic pathway (and this may be the major pathway), the possibility that they can also fuse with the plasma membrane has not been ruled out, despite the apparent requirement for low pH to trigger fusion. There are several relevant arguments. First, both influenza virus and VSV can exhibit membrane-fusing activity at neutral pH, as will be seen.

Second, the findings that FFWO could be induced by SFV, VSV, and influenza virus at low pH but not at neutral pH (White *et al.,* 1981) cannot be taken as evidence that these viruses are unable to fuse at the cell surface at neutral pH. Fusion of virus with the plasma membrane may not necessarily lead to FFWO, particularly if conditions are not appropriate for a virion to fuse with two cells simultaneously, thereby forming a cytoplasmic bridge, or if the bridge cannot enlarge so that fusion can be recognized to have occurred.

Third, if a virus can infect either by fusion at the cell surface or after endocytosis, certain conditions may favor use of one pathway over another. In general, experiments providing evidence for an endocytic

pathway have been done under conditions that may favor this pathway over alternatives. Specifically, cells have been exposed to virus in the cold, washed, and then warmed to 37°C. This kind of temperature shift has been shown to stimulate a burst of endocytosis (Anderson *et al.,* 1977; Marsh and Helenius, 1980).

The possibility exists, therefore, that pH dependence of fusion activity will not provide the full explanation for inability of SFV, VSV, and influenza virus to induce FFWO at neutral pH.

Factors that determine efficiency and pathways of viral entry include the following: Adsorption of an enveloped virus to a cell surface will be followed by elution, fusion, or endocytosis of the virus. If a virion fails to fuse with the plasma membrane and also fails to fuse after endocytosis, it will probably be delivered to a lysosome for destruction. Lysosomal destruction of the virus and differences in the fraction of input virus destroyed by different cells undoubtedly accounts in part for the relatively high particle : plaque-forming unit ratio in most preparations of animal viruses and for differences in this ratio when titrations of plaque-forming units are done on different cell types. The probability that virus attached to a cell surface will fuse with the plasma membrane depends on at least two factors: rate of endocytosis and rate of fusion under conditions that prevail at the cell surface. Endocytosis of different viruses by the same cell type clearly occurs at different rates (Marsh, 1984). Adequate methods for directly measuring the rate of viral fusion at the cell surface have not yet been described. The probability that ingested virus can fuse with the membrane of an endosome probably depends on transit time in prelysosomal vesicles and on the rate of fusion under the conditions prevailing in endocytic vesicles. Too little is known about the range of conditions and factors that influence fusion to permit conclusions that a particular virus can fuse only with either the plasma membrane or the membrane of an endosome.

2.2. Roles of the Viral Glycoproteins

Table II lists the envelope glycoproteins of selected viruses and briefly describes their organization in virions and proposed roles in infectivity. Two activities known to be required for infectivity and expected to be mediated by one or another of the envelope glycoproteins are (1) binding to a cell surface receptor, and (2) induction of fusion between the virion envelope and a cell membrane.

For many of these viruses, the envelope glycoproteins form uniform structures or spikes (on virions but not necessarily in cell membranes) that protrude from the envelope; they can be recognized by electron microscopy to have a characteristic size and shape. In several instances only one

Table II. Envelope Glycoproteins of Selected Viruses

Virus	Glycoprotein: Precursor[a]	Glycoprotein: Mature forms	Glycoprotein: Approx. M_r ($\times 10^{-3}$)	Spike: Subunit associations	Spike: Membrane-spanning domains	Spike: Activities	References[b]
Vesicular stomatitis (VSV)	—	G	62	?	Near C-terminus	Receptor binding; fusion	Rose and Gallione (1981)
Sendai	—	HN	67	?	Near N-terminus	Receptor binding; neuraminidase	Blumberg *et al.* (1985*b*)
	F_0	$F_2 + F_1$	12; 53	$[F_2 + F_1]_?$	Near C-terminus of F_1	Fusion	Blumberg *et al.* (1985*a*)
Influenza	—	NA	60	$[NA]_4$	Near N-terminus	Neuraminidase	Varghese *et al.* (1983)
	HA	HA1 + HA2	44; 30	$[HA1 + HA2]_3$	Near C-terminus of HA2	Receptor binding; fusion	Wilson *et al.* (1981)
Semliki Forest (SFV)	—	E1	51	$[E1 + E2 + E3]_1$	Near C-termini of E1 and E2	Receptor binding; fusion	Garoff *et al.* (1980)
	p62	E3 + E2	11; 52				
Herpes simplex (HSV)	—	gB	120	$[gB]_2$	Near C-terminus	?	See legend to Fig. 5
	—	gC	130/75[c]	?	Near C-terminus	C3b binding	
	—	gD	60	?	Near C-terminus	Receptor binding?	
	—	gE	75	?	Near C-terminus	Fc binding	
	—	gG	?/125[c]	?	?	?	
	—	gH	110	?	?	?	

[a] Only relatively stable precursors detectable in infected cells are listed here.
[b] Most of the publications listed describe primary sequence or three-dimensional structure of the indicated glycoproteins and contain citations to publications providing other information about the proteins.
[c] The number before the slash is for HSV type 1 and the number after the slash for HSV type 2.

kind of spike is present. This spike may be composed of a single or multiple species of glycopolypeptide and has all activities required for infectivity.

In other instances at least two different kinds of spikes are present, and they have different activities. For example, spikes containing the cleaved form of hemagglutinin (HA) (HA1 + HA2) in influenza virus mediate adsorption of virus to sialic acid moieties on cell surfaces as well as fusion of the viral envelope with a cell membrane. The other glycoprotein of influenza virus, neuraminidase (NA), forms separate spikes. For the evolutionary related Sendai virus (a paramyxovirus), these three activities are differently distributed between two kinds of spikes. That is, spikes composed of hemagglutinin-neuraminidase (HN) mediate adsorption to sialic acid moieties and display neuraminidase activity, whereas spikes composed of the cleaved form of the fusion protein F_0 (F_2 + F_1) induce fusion between the virion envelope and a cell membrane. Neuraminidase activity is not found in all enveloped viruses. Although the role of neuraminidase in orthomyxo- and paramyxovirus replication is not fully understood, its expression and presence in the virion envelope is undoubtedly related to the fact that the cell receptor for these viruses is sialic acid. It has been suggested that the binding of virus to sialic acid on certain cell surface components may not lead to fusion and that one role of neuraminidase could be to release virus from these dead-end receptors in order to enable subsequent binding to productive receptors (Haywood, 1974).

In contrast to the RNA viruses, herpesvirus specify the synthesis of more than six different envelope glycoproteins. Their organization in virions and precise roles in infectivity are not yet well defined. Two of the herpes simplex virus (HSV) glycoproteins have unexpected activities (Fc-binding and C3b-binding) that may be irrelevant to infectivity (Spear, 1984).

3. VIRUS-INDUCED CELL FUSION

3.1. Stages in the Process of Cell Fusion

In discussing virus-induced cell fusion, Knutton (1978) proposed that three stages may be required (Fig. 3):

Stage 1 Adhesion between two cells

Stage 2 Membrane fusion to create a cytoplasmic bridge

Stage 3 Enlargement of the bridge to yield what would be recognized as a polykaryocyte—multiple nuclei occupying a single cytoplasm.

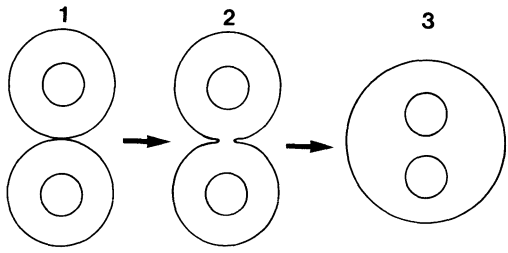

Figure 3. Stages in the process of virus-induced cell fusion as proposed by Knutton (1978). Stage 1 is adhesion between two cells, stage 2 is membrane fusion to form a cytoplasmic bridge, and stage 3 is enlargement of the cytoplasmic bridge with rearrangement and merger of intracellular contents.

Because fusion of cells is usually monitored visually by light microscope, only after the third stage would it be evident that fusion has occurred even though fusion of membranes actually occurs in the second stage.

By analogy with virus attachment and fusion to initiate infection, the first stage of cell fusion could be mediated by the viral receptor-binding glycoprotein and the second stage by the viral fusion glycoprotein. The third stage of cell fusion may not have a counterpart in infectivity unless some specific condition is required to enlarge the initial virion–cytoplasmic bridge so as to permit entry of the nucleocapsid into the cytoplasm.

A change in permeability leading to cell swelling appears to be one aspect of stage 3 (Knutton, 1978; Patel and Pasternak, 1985). For example, preparations of Sendai virus that are most efficient at inducing FFWO have damaged envelopes and apparently, on fusion with cells, introduce lesions into the cell surface that result in increased permeability. Cell lysis rather than fusion can occur unless the medium contains factors, such as calcium, required for repair of the lesions (Okada and Murayama, 1966). Another aspect of stage 3 may be changes in the cell cytoskeleton that could facilitate rearrangement of cell contents.

3.2. Viral Proteins That Induce or Influence Cell Fusion

For the viruses listed in Table II, attention is focused here on the conditions required for cell fusion, the identities of the viral glycoproteins for inducing membrane fusion, structural features of these glycoproteins thought to be important for fusion activity, and the identities and properties of other viral proteins that may influence fusion activity.

The most compelling evidence that a viral glycoprotein has fusion-inducing activity has come from demonstration that the product of a single molecularly cloned viral gene can induce cell fusion. Findings that antibodies of a given specificity can block virus-induced cell fusion do not necessarily identify the fusion protein, despite published claims. For ex-

ample, antibodies specific for either HN (Miura *et al.,* 1982) or F (Merz *et al.,* 1980) of a paramyxovirus can block fusion, and yet the cleaved form of F alone is sufficient to induce membrane fusion, as discussed in section 3.2.2a. Experiments using liposomes containing purified viral glycoproteins (virosomes) to assess fusion activity were summarized by White *et al.* (1983) and have given results consistent for the most part with the conclusions presented below.

Attempts to relate structure and function of viral fusion proteins are complicated by lack of knowledge as to general features of the mechanism by which these proteins induce membrane fusion. Attention has focused on hydrophobic domains (other than the membrane-spanning regions) of the fusion proteins in part because these domains seem to be more highly conserved than others among related viruses and because, in some instances, these hydrophobic domains are at the new N-termini generated by cleavages required for fusion activity.

Barriers to the close approach of two lipid bilayers required for membrane fusion include the physical bulk of membrane proteins and charge repulsion. There are several ideas about the role of viral fusion proteins in overcoming these barriers. A hydrophobic region of the fusion protein may interact with lipids or proteins in the target membrane, either to draw the target membrane and viral membrane into closer proximity or to destabilize the lipid bilayer of the target membrane for initiation of fusion. Alternatively, the fusion protein may promote aggregation or patching of the viral (and perhaps cell) membrane proteins so that lipid bilayers devoid of protein can approach closely enough to fuse (White *et al.,* 1983). Enhanced curvature of either the viral or target membrane may also be necessary to overcome charge repulsion (Haywood and Boyer, 1981).

If fusion activity requires insertion of a hydrophobic N-terminus of the fusion protein into the lipid bilayer of the target membrane, then one might expect hydrophobicity, but not necessarily amino acid sequence, to be conserved at the N-terminus, as is the case for signal sequences and membrane-spanning domains. The conservation of sequence, within a virus family, at the relevant N-termini of fusion proteins (Fig. 4) suggests that there are requirements for interaction of these domains with cell or viral components other than lipid bilayers, in addition to any other requirements. If members of the different virus families listed in Figure 4 cause fusion by similar mechanisms, common features of the fusion process must not depend on relatedness of amino acid sequence at the N-termini of the proteins shown.

Although it can be shown that individual viral glycoproteins are both necessary and sufficient to induce cell fusion under a particular set of conditions, there is increasing evidence that other viral proteins influence fusion-inducing activity. These other viral proteins may be required for

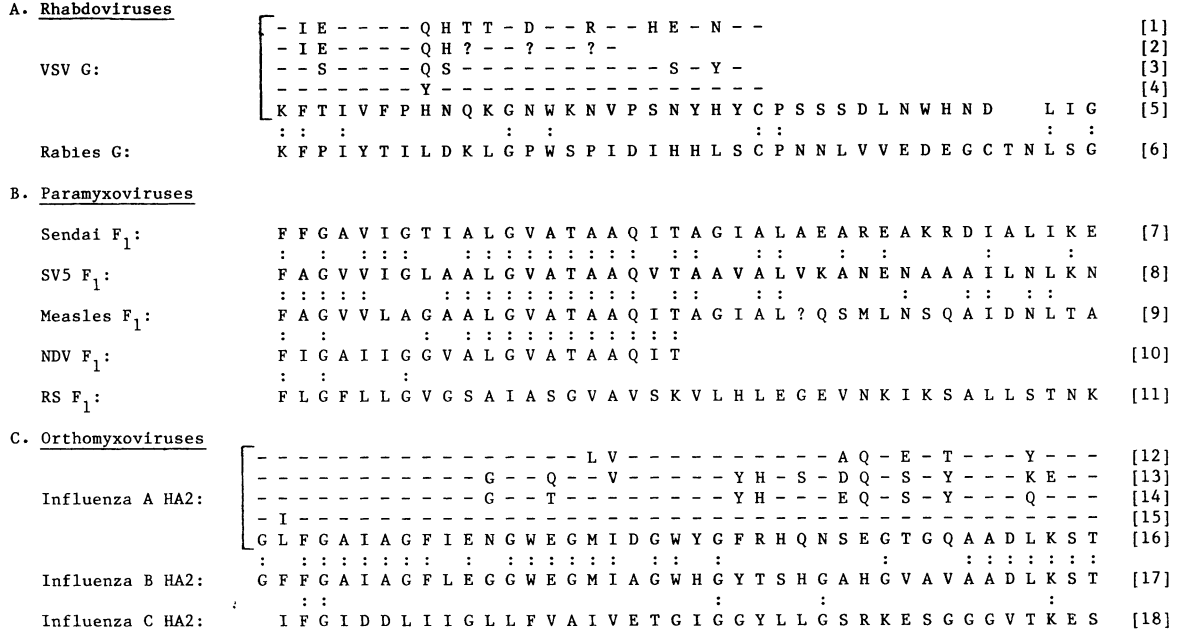

Figure 4. Comparisons of N-terminal amino acid sequences for the fusion proteins or fusion protein subunits indicated. For different strains of the same virus, the dashes indicate identity with the reference sequence. For different viruses within a single family the double dots indicate identity between sequences. The viruses and strains for which data are presented and citations are as follows. *Rhabdoviruses.* VSV G: strains [1] New Jersey Ogden, [2] New Jersey Concan, [3] Cocal, [4] Indiana Toronto (Kotwal *et al.,* 1983), [5] Indiana San Juan (Rose and Gallione, 1981), [6] rabies G (Rose *et al.,* 1982). *Paramyxoviruses.* Primary data for measles F_1 and citations of papers providing information for the other viruses are in Varsanyi *et al.* (1985). *Orthomyxoviruses.* Influenza A HA2: strains [12] fowl 1979, [13] A/Japan/305/57, [14] A/WSN/33 and A/PR/8/34, [15] A/Victoria/3/75, A/England/321/77 and A/Bangkok/1/79, [16] A/NT/60/68/29C, A/Aichi/2/68, A/Mem/102/72 and A/duck/Ukraine/63 (from the protein sequence database of the National Biomedical Research Foundation, Georgetown University Medical Center); influenza B HA2: strains [17] B/Lee/40, B/HK/8/73 and B/Md/59 (Krystal *et al.,* 1983, 1984); [18] influenza C HA2 (Pfeifer and Compans, 1984).

cell fusion under certain conditions. Alternatively, they may modulate or inhibit cell-fusing activity.

It should be kept in mind that, in most of the studies done to date, attempts have not been made to differentiate between the postulated stages 2 and 3 of the cell fusion process (Fig. 3). Usually cell fusion is judged to have occurred only if the final product, the polykaryocyte or greatly enlarged red cell, is observed. The possibility exists, however, that viral proteins may induce cytoplasmic bridges as depicted for stage 2 without inducing the progression to stage 3. Special techniques would be required to detect these bridges. The possibility also exists that formation of the cytoplasmic bridges is reversible if transition to stage 3 does not occur. The transition from stage 1 to stage 2 is topologically equivalent to fusion of a virion with the cell at the cell surface. The reverse reaction (stage 2 to stage 1) is topologically equivalent to the purse-string closure and annular fusion that occur to separate a budding virion from the cell surface.

3.2.1. Rhabdoviruses

FFWO has not been reported for VSV except after exposure of infected cells to low pH (White *et al.,* 1983). Certain strains of VSV induce the FFWI of certain cell types at neutral pH, whereas other VSV strains do not (Takehara, 1975; Nishiyama *et al.,* 1976; Chany-Fournier *et al.,* 1977). The genetic differences responsible for the phenotypic differences are for the most part unknown. Strains of VSV that do not ordinarily cause FFWI can be induced to do so under the following conditions: (1) exposure to low pH of cells infected with VSV or cells expressing only the VSV G protein (Florkiewicz and Rose, 1984; Riedel *et al.,* 1984); (2) infection of cells at nonpermissive temperature with the temperature-sensitive mutant *ts*G31, which has a lesion in the gene for M protein (Hughes *et al.,* 1979*b;* Handa *et al.,* 1982; Storey and Kang, 1985); and (3) inhibition of protein synthesis, especially relatively late in the infectious cycle, in cells infected with wild-type virus (Storey and Kang, 1985). Under conditions (2) and (3), the fusion occurs at neutral pH and may occur only in certain cell types and not others.

3.2.1a. VSV G Protein. Expression of G protein alone (of the five VSV proteins) is sufficient to induce cell fusion provided the cells are exposed to low pH (Florkiewicz and Rose, 1984; Riedel *et al.,* 1984). Cells that expressed a truncated form of G that lacked the membrane-spanning domain and was secreted into the medium failed to fuse, indicating that fusion activity probably depended on anchorage of G to the cell surface (Florkiewicz and Rose, 1984).

In contrast to some other viruses, there is no evidence that proteolytic cleavage of G protein (other than that which removes the signal sequence) is required to activate fusion activity. The N-terminus of G (Fig. 4) may be important for fusion activity, based on findings that a synthetic peptide (24 amino acids) similar in sequence to the N-terminus has hemolytic activity at pH 5.0 (Schlegel and Wade, 1984; see Chapter 2, this volume). VSV virions and purified G protein in liposomes also have hemolytic activity at pH 5.0 (Bailey *et al.*, 1981, 1984). Reducing the size of the synthetic peptide from 25 amino acids to 6 retained hemolytic activity and abolished the low pH dependence of this activity. The basic amino acid at the N-terminus of the peptide (Lys-Phe-Thr-Ile-Val-Phe) may be essential for activity. Activity was retained when Lys was substituted with Arg but not with Glu (Schlegel and Wade, 1985).

In native G protein at neutral pH, the N-terminus appears not to be accessible to antibodies (Schlegel and Wade, 1984) or to protease (Capone *et al.*, 1982). If indeed fusion activity of G depends on interaction of the N-terminus with the target membrane, activation of fusion may require a change in conformation of G. Exposure to low pH may be one way to induce the postulated change in conformation, but presumably it is not the only way.

3.2.1b. VSV M Protein and Nucleocapsids. Although low pH appears to be a requirement for induction of cell fusion in cells expressing only G protein, cell fusion (FFWI) can occur at neutral pH in infected cells expressing other viral proteins. Mutation in the M protein or partial inhibition of viral protein synthesis, as well as low pH, can cause a nonfusing VSV strain to induce cell fusion.

Why should mutation in M protein (as in *ts*G31) permit cell fusion to occur at neutral pH? M protein lines the undersurface of the virion envelope. Interactions of M protein with both the nucleocapsid and G protein are required for virion morphogenesis (Knipe *et al.*, 1977). Apparently the mutation in *ts*G31 prevents budding of nucleocapsids (Hughes *et al.*, 1979*a*) but may not prevent all interactions of M (possibly aberrant) with either G protein or the nucleocapsid. The effects of protein synthesis inhibitors could be to create an imbalance of G protein, M protein, and nucleocapsid available for interaction.

A hypothesis consistent with the results summarized above proposes that interactions of M and G without nucleocapsid trigger fusion activity at neutral pH, whereas adherence of nucleocapsids to the complex blocks fusion activity and initiates budding for virion morphogenesis. A prediction of this hypothesis is that cells expressing only M and G should fuse at neutral pH.

3.2.2. Paramyxoviruses

Paramyxoviruses can induce both FFWO and FFWI at neutral pH (Choppin and Compans, 1975; Hosaka and Shimizu, 1977). No enhancement of fusion activity by exposure to low pH has been reported. On the contrary, it was shown that transient exposure of Sendai virus to pH 9.0 enhanced fusion activity, as assessed by hemolysis and FFWO, and caused an irreversible change in the conformation of F protein (Hsu *et al.*, 1982). Cell fusion is not an invariant consequence of infection with paramyxoviruses, for reasons that are not understood.

3.2.2a. F Protein. A large body of evidence indicates that the F protein of paramyxoviruses displays fusion activity (Choppin and Compans, 1975; White *et al.*, 1983). In contrast to two other well-characterized fusion proteins (VSV G and influenza virus HA), which exhibit receptor-binding as well as fusion activity, F protein apparently has only fusion activity. It has been reported (Peterhans *et al.*, 1983), however, that F can bind to cells independently of HN, the recognized receptor-binding protein. Cells infected with a simian virus (SV40) vector carrying and expressing the F gene only of SV5 (a paramyxovirus) were shown to fuse at neutral pH. The active cleaved form of F was produced by the cells (Paterson *et al.*, 1985).

Fusion activity of the F protein depends on a specific proteolytic cleavage yielding the subunits F_2 and F_1, which remain covalently joined by disulfide linkage (White *et al.*, 1983). The F_1 subunit contains the membrane-spanning domain of F (C-terminus). The N-terminus of F_1, generated by the cleavage required for fusion activity, is hydrophobic and more conserved among different paramyxoviruses (Fig. 4) than are other domains. The sequences shown in Figure 4 are for viruses from four animal species including human.

The possibility that the N-terminus of F_1 may interact directly with the target membrane was suggested by results obtained with synthetic peptides. It was found that peptides similar in sequence to the N-terminus could inhibit viral infectivity, FFWI, and hemolysis without blocking virus adsorption and that these peptides interacted with the cell, not the virus (Richardson *et al.*, 1980; Richardson and Choppin, 1983). There are, however, aspects of the results that are difficult to reconcile with the straightforward interpretation that the peptides act by competing with the F_1 polypeptide for specific sites on the target cell membrane. First, the most effective inhibition was obtained with the peptide Z-D-Phe-L-Phe-Gly (where Z is a carbobenzoxy group), and optimal inhibitory activity depended on presence of both the Z group and the D form of Phe in position 1. Second, much greater inhibition was obtained with measles virus

than with Sendai virus, even though the sequence of the inhibitory peptide matches better that of Sendai virus at the N-terminus of F_1 (Fig. 4).

3.2.2b. HN Protein. Although expression of F alone (from at least one paramyxovirus) can induce cell fusion, HN influences cell fusion in ways that are not completely understood. The effect of HN on cell fusion appears to differ for different paramyxoviruses.

Several lines of evidence suggest that HN of Sendai virus may be required for fusion activity under certain circumstances and that this requirement is not solely for the receptor-binding or neuraminidase activity of HN. First, an anti-HN monoclonal antibody that did not inactivate hemagglutination or neuraminidase activity was shown to block Sendai virus-induced cell fusion and hemolysis as well as fusion with cells of virosomes (assessed by delivery of toxin from the virosomes to the cells) (Miura *et al.,* 1982). Second, using this latter assay, fusion activity was found to depend sharply on the ratio of F to HN in the virosomes (optimal being 2), suggesting that HN was not merely serving a receptor-binding function (Nakanishi *et al.,* 1982). Third, under conditions such that the requirement for the receptor-binding activity of HN was bypassed, fusion activity depended on the presence of HN nonetheless. Specifically, Sendai virus induced the fusion and hemolysis of neuraminidase-treated red cells provided that antibodies reactive with the virus were coupled to the red cells. Virosomes could also induce hemolysis of red cells treated in this way, but only if both F and HN were present (Nussbaum *et al.,* 1984). (In an earlier study, virosomes containing only F were shown to lyse red cells provided that wheat germ agglutinin was used to mediate attachment of the virosomes to the red cells (Hsu *et al.,* 1979). The reasons for the apparent discrepancy in these results are unknown.)

Although all aspects of the role of HN in Sendai virus-induced cell fusion are not understood, there are some suggestive observations. For example, it was recently reported that virosomes fused in a nonleaky fashion with liposomes composed of phosphatidylcholine and cholesterol but lysed liposomes also containing sialoglycolipids and sialoglycoproteins (Citovsky and Loyter, 1985). Presence of both F and HN in the virosomes was required for both activities (fusion and lysis). In this same study it was also reported that virosomes could fuse with neuraminidase- and pronase-treated red cell vesicles, provided that the medium was hypotonic. Citovsky and Loyter (1985) suggested that normal biological membranes containing the usual complement of proteins and glycolipids may not have areas of protein-free bilayer accessible to the virus for fusion. Interactions of the virus with sialic acid-bearing receptors could somehow induce changes in membrane permeability, leading to cell swelling and

membrane stretching, which could be a prerequisite to fusion of the virus with a biological membrane.

Another role of HN in the fusion induced by Sendai virus has been suggested. Haywood and Boyer (1981) examined by electron microscopy the interaction of virus with liposomes containing sialoglycolipids. Within 1 min. at 37°C, many virions appeared to be partially or almost entirely engulfed by a liposome. Fusion occurred at the leading edge of the developing pit, resulting in a small bridge between interiors of the virion and liposome. Because at this stage most of the virion surface remained in close contact with the surface of the pit in the liposome, probably due to the receptor-binding activity of HN, it was proposed that destruction of the receptors by neuraminidase would be required to enable enlargement of the bridge and complete merging of virion and liposome contents.

If HN is required for fusion activity, as these results suggest, then what is the explanation for cell fusion induced by F alone? At this stage it seems necessary to conclude either that other interpretations will be found for the results discussed above or that certain cell surface components can substitute for HN under some conditions. It should be noted that most of the experiments suggesting a role for HN were done with liposomes or red cells, whereas the cells fused after expression of the cloned F gene were monkey fibroblasts.

With mumps virus it appears that HN in the cell surface may inhibit cell fusion, at least in part because of its neuraminidase activity. Strains of mumps virus differ in their ability to induce cell fusion (FFWI), and there is an inverse correlation between cell fusing activity and level of neuraminidase activity associated with HN (Merz and Wolinsky, 1981). Moreover, treatment of infected cells with chymotrypsin cleaves HN, but not F, and induces fusion of cells infected with nonfusing virus strains, whereas exogenous neuraminidase inhibits the fusion of cells infected with fusing strains (Merz and Wolinsky, 1983). Similar but not identical results have been reported for bovine parainfluenza 3 (Shibuta *et al.*, 1983).

The apparently opposite effects of HN on cell fusion induced by different paramyxoviruses have not yet been explained.

3.2.3. Orthomyxoviruses

Influenza viruses induce neither FFWO or FFWI unless the infected cells are exposed to low pH (White *et al.*, 1983).

3.2.3a. Influenza HA Protein. Transformed cells expressing HA of influenza virus fused after addition of trypsin to the medium and exposure of the cells to medium of low pH (White *et al.*, 1982). The addition of tryp-

sin was required to convert the HA made by the cells to the active cleaved form. This study also showed that a secreted form of HA could not induce cell fusion.

HA of influenza virus is the best characterized of the viral fusion proteins in terms of primary and tertiary structure. The use of bromelain to separate the HA spikes (consisting of the cleaved form of HA) from their membrane-spanning domains permitted crystallization and determination of structure at 3-Å-resolution (Wilson *et al.,* 1981). Each monomer of the trimer has been described as a long fibrous stem topped by a globular domain. The globular domain is composed entirely of HA1 and forms the receptor-binding site for sialic acid located about 135 Å from the viral membrane. The fibrous stem is composed of both HA1 and HA2, with the N-terminus of HA2 located about 35 Å from the membrane and probably buried in the interior of the trimeric stem. The C-terminus of HA2 (in the intact spike) has the membrane-spanning domain for anchorage to the viral envelope or cell membrane.

Cleavage to yield HA1 plus HA2 is essential for fusion activity (Lamb and Choppin, 1983). The N-terminus of HA2 is hydrophobic and is more highly conserved than other parts of HA, even between strains of influenza A and B (Fig. 4). Influenza C seems to have diverged considerably from A and B. Consistent with similiarities between orthomyxoviruses and paramyxoviruses that suggest a common evolutionary origin, similarities between amino acid sequences at the N-terminus of HA2 and the N-terminus of F_1 have been noted (Blumberg *et al.,* 1985*a*).

Opinions differ as to whether exposure of HA1–HA2 to low pH is required for fusion activity. There is general agreement that influenza virus does not induce FFWO, FFWI, or hemolysis at neutral pH but that it can be induced to do so at low pH (<6.0) (White *et al.,* 1983). Although it has been concluded from such results that influenza virus has no fusion activity at neutral pH, it can be argued that FFWO, FFWI, and hemolysis do not necessarily follow virion–cell fusion. Therefore, failure to observe these phenomena may not imply failure of virion–cell fusion to occur.

A more telling argument is the evidence obtained, using different kinds of assays, that influenza virus can exhibit fusion activity at neutral pH. Electron microscopic examination showed that influenza virus fused at neutral pH with liposomes containing sialic acid-bearing glycolipids (Haywood and Boyer, 1985) and with red cell membranes (Fidgen and Tisdale, 1981) and that liposomes containing influenza virus glycoproteins fused with cells (Huang *et al.,* 1980). Also, hemolysis and fusion of red cells could be induced by influenza virus at neutral pH provided the red cells were first pretreated with a solution of low pH (Huang *et al.,* 1985). The latter result indicates that fusion occurs because the target membrane, not the virus, is altered by low pH. On the other hand, when virus and red

cells both are exposed to low pH, the pH optimum for hemolysis depends on proteins of the virus. Different strains of influenza virus exhibit different pH optima (Huang *et al.,* 1981) and, in particular, amantadine-resistant mutants of influenza virus induce hemolysis at higher pH than do the parental strains (Daniels *et al.,* 1985).

What are the effects of low pH on both the target membrane and the virus or viral proteins? Even transient exposure of the target membrane to low pH may induce irreversible changes in organization and conformation of membrane components or alter permeability. Possibly changes in membrane permeability before or during exposure to virus can permit cell-virion-cell fusion (stage 2) to progress to visually evident cell fusion (stage 3).

Influenza virions aggregate when exposed to low pH in the absence of cells and are rapidly inactivated with respect to infectivity and fusion. If attachment of virions to cells is permitted prior to exposure to low pH, virion-cell fusion (and FFWO) can occur, but initiation of infection does not result (Matlin *et al.,* 1981). Exposure of purified HA or virions to low pH causes a change in conformation of HA as assessed by altered sensitivity to protease and altered reactivity with monoclonal antibodies (Skehel *et al.,* 1982; Daniels *et al.,* 1983*a*). Moreover, in mutants that induced hemolysis at higher pH in comparison with parental strains, the altered conformation of HA detectable by susceptibility to proteolytic cleavage also occurred at higher pH (Daniels *et al.,* 1985). Altered conformation of purified HA (bromelain-released spike) after exposure to low pH is also evident from its enhanced binding of nonionic detergent and ability to bind to liposomes lacking sialic acid receptors (Skehel *et al.,* 1982); the hydrophobic domain exposed as a result of exposure to low pH appears to be the N-terminus of HA2 (Daniels *et al.,* 1983*b*). Virions exposed to low pH acquire the ability to bind irreversibly at 0°C to liposomes bearing sialic acid receptors, such that elution cannot be achieved with competitive inhibitors for the viral receptor-binding site (Haywood and Boyer, 1985).

Several points emerge from a consideration of the above observations:

1. Fusion of the virion with a liposome or red cell apparently does not require exposure of either to low pH.
2. Exposure of virion-cell complexes to low pH probably enhances the adherence of virion to cell and may accelerate the rate of virion-cell fusion. This statement is based in part on findings that both virions and HA (bromelain-released spike), after exposure to low pH, adhere tightly to membranes even if sialic acid-bearing receptors are absent.

3. If the low pH-induced change in conformation of HA is obligatory for fusion activity, this change should be inducible by conditions other than low pH and should be demonstrable when fusion is induced at neutral pH.
4. The induction of cell–cell fusion (FFWO or FFWI), in contrast to virion–cell fusion, seems to be dependent on exposure of the cells to low pH. It remains to be determined exactly how the effects of low pH on the target membrane and on HA contribute to the induction of cell fusion.

3.2.3b. NA, M_1, and M_2 Proteins. The suggestion that influenza NA has a role in fusion activity comes from several observations. First, virosomes composed of cleaved HA and NA were shown by electron microscopy to fuse with cells at neutral pH. If HA was not cleaved, NA was omitted, or the virosomes treated with anti-NA antibodies, this fusion did not occur. Soluble neuraminidase of influenza virus or *Vibrio cholerae* could substitute for virosome-bound NA, permitting virosomes containing only cleaved HA to fuse (Huang *et al.,* 1980). Second, both fusion and lysis of red cells induced by influenza virus at pH 5.5 could be blocked by an anti-neuraminidase antiserum and restored by addition of soluble *V. cholerae* neuraminidase (Huang *et al.,* 1985).

Whereas HN of paramyxoviruses has both receptor-binding and neuraminidase activities, and possibly other activities that influence fusion, only neuraminidase activity has been associated with influenza NA. It is this activity that appears to be required for the kinds of fusion measured by the assays described above. Why was there no apparent requirement for neuraminidase activity in fusion of the cells induced by expression of the cloned HA gene? There is not yet an answer to this question, although it has been suggested that a cell neuraminidase could have substituted for viral NA (Huang *et al.,* 1985).

Results implicating M_1 or M_2 in fusion activity were obtained in investigations of amantadine-resistant viral mutants. M_1 and M_2 are encoded by overlapping reading frames on the same genome segment (here designated M), with M_2 being translated from a spliced messenger RNA (mRNA). M_1 of influenza virus, similarly to M of rhabdoviruses, lines the inner surface of the virion envelope and probably interacts both with the nucleocapsid and internal domains of the viral glycoproteins (Lamb and Choppin, 1983). M_2 is an integral membrane protein expressed on the surface of infected cells but not present in virions (Lamb *et al.,* 1985). Amantadine is a drug that interferes with influenza virus replication and is known to increase the pH of intracellular vesicles. Amantadine-resistant mutants have been shown to induce hemolysis with pH optima about 0.1–0.6 higher than that for the parental viruses (Daniels *et al.,* 1985). Naturally

occurring strains of influenza differ in their sensitivity to amantadine (Appleyard, 1977) and in their pH optima for hemolysis (Huang *et al.,* 1981), but whether these two types of genetic variability are always correlated is unknown.

What viral gene or genes determine amantadine sensitivity? Because the eight genome segments (each containing one or two genes) of influenza virus vary in size among different strains, it is often possible to correlate segregation of defined phenotypes among recombinant viruses with segregation of particular genome segments. There are limitations to genetic analysis of this kind, in part because two different strains of influenza virus may have diverged sufficiently such that recombinants formed between the two may exhibit altered phenotypes not expressed by either parental virus.

Nevertheless, depending on assays used and on the particular parental strains of influenza virus used to produce recombinants, it has been shown that sensitivity or resistance to amantadine segregated with the HA genome segment (Scholtissek and Faulkner, 1979), with the M genome segment (Lubeck *et al.,* 1978; Hay *et al.,* 1979), or was not assignable to a single genome segment (Appleyard, 1977; Lubeck *et al.,* 1978; Scholtissek and Faulkner, 1979).

More recently, a number of amantadine-resistant mutants were compared with their respective amantadine-sensitive parental strains with respect to pH optima for hemolysis, pH threshold for changes in conformation of HA as assessed by sensitivity to protease, and nucleotide sequences of the HA gene (Daniels *et al.,* 1985). Although all the mutants discussed had amino acid substitutions in HA, genetic tests were not performed to determine whether these substitutions were responsible for all the mutant phenotypes. Undoubtedly, the amino acid substitutions detected account for the altered pH dependence of conformational changes in HA, as these tests were done with bromelain-released soluble HA spikes. Daniels and co-workers suggested that some of the amino acid substitutions could destabilize either the pH 7.0 conformation that holds the N-terminus of HA2 in a hydrophobic crevice or the subunit interactions necessary for trimer stability. The other mutant phenotypes (amantadine resistance, altered pH optima for hemolysis), however, could depend on mutations other than, or in addition to, those detected in the HA gene.

Despite the complexities of the genetic analyses, they strongly suggest that properties of M_1 or M_2, as well as HA, can in part determine amantadine resistance. If amantadine resistance invariably correlates with altered pH optima for virion-induced hemolysis, these results also suggest that M_1 (but not M_2, as it is apparently not present in virions) influences the fusion activity measured by this assay. Because M_1 probably interacts directly with HA, at least in virions, it seems reasonable that M_1 could in-

fluence conformation and function of HA. The possibility also exists that M_2, the function of which is unknown, could in part determine whether fusion activity is expressed at the surfaces of infected cells.

3.2.4. Togaviruses

Similarly to orthomyxoviruses, togaviruses usually do not induce FFWO or FFWI except after exposure of infected cells to low pH (White *et al.,* 1981).

It is experimentally difficult to assign functions to the individual viral glycoproteins because the capsid protein and three glycoproteins are all derived by proteolytic cleavage of a single translation product. Cells microinjected with a cloned cDNA containing the coding region for this translation product of SFV were able to express all the normal cleavage product, or all but E1, depending on how the cDNA was cloned. The cells expressing E1 + E2 + E3, but not those expressing only E2 + E3, were induced to fuse by exposure to low pH (Kondor-Koch *et al.,* 1983). These results indicate that E1 is required for fusion activity but they do not rule out a requirement for E2 as well. E3 is thought not to be required for fusion because it can be removed from some togaviruses without loss of infectivity and because it is not an integral membrane protein (Garoff *et al.,* 1982). It has been reported that monoclonal antibodies to E1 can block fusion activity, as assessed by hemolysis, whereas antibodies to E2 and E3 were without this effect (Chanas *et al.,* 1982). Near, but not at, the N-terminus of E1 is located a hydrophobic segment of about 17 amino acids. This segment is highly conserved in the proteins of Sindbis virus and SFV and could have a role in fusion activity (White *et al.,* 1983).

3.2.5. Herpesviruses

Most wild-type strains of HSV do not cause the fusion of cultured cells despite the fact that polykaryoctyes are a common histological feature of herpetic lesions. Cell fusion (FFWI or FFWO) cannot be induced by exposure of infected cells to low pH. Mutants of HSV can induce cell fusion, however, as a consquence of nonlethal alterations in any one of at least four different genes (Fig. 5).

It is probable that the products of some half-dozen viral genes influence the cell-fusing activity of HSV (Spear, 1984). These products include the genes encoding the fusion protein or proteins as well as the genes that must be mutated to permit cell fusion to occur. The latter are designated Syn genes and may include the gene or genes for fusion proteins.

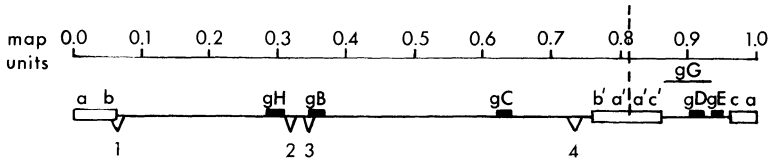

Figure 5. Genome of HSV and locations of genes for glycoproteins (e.g., gB, gC) and of Syn mutations (bracketed regions indicated 1, 2, 3, and 4). The open boxes indicate the positions of inverted repetitive sequences and the filled bars indicate the positions of glycoprotein genes (reviewed in Spear, 1984; see also Buckmaster *et al.*, 1984).

The fusion protein or proteins of HSV have not yet been identified. Temperature-sensitive mutations in the gB gene result in temperature sensitivity of cell fusion (Manservigi *et al.*, 1977), and monoclonal antibodies specific for gD (Noble *et al.*, 1983) or gH (Gompels *et al.*, 1985) can block HSV-induced cell fusion. These three glycoproteins are therefore likely to either induce or influence cell-fusing activity.

Information about the nature of the Syn mutations and the products altered by these mutations is limited. Syn mutations have been mapped on the viral genome by marker transfer or marker rescue experiments. That is, infectious genomic DNA from a wild-type strain is co-transfected into cells with cloned DNA fragments from the mutant, in order to identify which fragments can recombine with the wild-type DNA to yield recombinants with the Syn phenotype (marker transfer). In marker rescue experiments the converse is done.

Most of the Syn mutations mapped appear to be located within the region indicated by the number 4 in Figure 5 (Little and Schaffer, 1981; Bond and Person, 1984; Pogue-Geile *et al.*, 1984). For at least two mutants, nucleotide substitutions have been identified that alter translation in a single open reading frame, the only one likely to be expressed from the region (Debroy *et al.*, 1985; Pogue-Geile and Spear, 1987). Assuming that a protein is translated from this open reading frame, which has not yet been demonstrated, this protein would be very hydrophobic and would probably be membrane-associated and made on membrane-bound ribosomes with a cleavable signal sequence.

Only one Syn mutation has been mapped to region 3 (Fig. 5). This mutation is in a strain designated HSV-1(HFEM)*ts*B5, which also has a ts mutation. Both the ts and Syn mutations, which are segregable by recombination, appear to be in the structural gene for gB. Nucleotide sequence comparisons of *ts*B5 and an unrelated wild-type strain, coupled with the mapping results mentioned above, suggest that the Syn phenotype of

HSV-1(HFEM)*ts*B5 is attributable to an amino acid substitution in the cytoplasmic tail (near the C-terminus) of gB (Bzik *et al.*, 1984).

The Syn mutations of region 2 (Fig. 5) all result from deletions in the thymidine kinase gene (Sanders *et al.*, 1982). Because it seems unlikely that absence of thymidine kinase should result in cell fusion, it is reasonable to speculate that the deletions produced may alter the expression of some other gene, possibly even the adjacent gene for gH.

One mutation, in a strain designated HSV-1(KOS)804, has been mapped to region 1 by marker transfer to the genome of the wild-type parental strain HSV-1(KOS) (Little and Schaffer, 1981). Nothing is known about the nature of the mutation or the product mutated.

In summary, only one Syn mutation (region 3) is in an identified membrane glycoprotein. This glycoprotein, gB, possibly mediates membrane fusion or forms part of an active fusion complex, based on the phenotypes of mutants with ts lesions in this gene. Other Syn mutations (region 4) may be in a previously unrecognized membrane glycoprotein. Syn mutations in regions 1 and 2 must be further characterized to identify the altered products. Identification and characterization of the proteins that must be mutated to permit cell fusion should lead to the formulation of testable hypotheses as to how cell fusion is regulated. It is of interest that the Syn mutation in region 3 appears to alter the cytoplasmic tail of gB. This focuses attention on cytoplasmic and virion proteins that could interact with this domain of gB.

4. CONCLUDING REMARKS

It is evident from the foregoing that the phenomena of virus-induced cell fusion are beginning to be explicable, at least in part, by properties and activities of specific viral proteins. The mechanisms are not yet understood, however. Attention will continue to be focused on the viral fusion proteins. It will be important to define the interactions of these proteins with other viral proteins, cell components, and factors in the medium and to determine how these interactions modulate fusion activity. Better structural definition of the initial, intermediate, and end stages of the virion–cell and cell–cell fusion processes is also needed. Finally, it seems likely that common mechanisms and phenomena may be found in comparing virus-induced cell fusion with other kinds of fusion, such as myoblast fusion. Attempts to define similarities and differences could synergistically advance the studies of both kinds of fusion.

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