Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Cell-to-cell fusion

J.M. White and C.P. Blobel

Departments of Pharmacology and Biochemistry and Biophysics, University of California, San Francisco, California, USA

Current Opinion in Cell Biology 1989, 1:934–939

Introduction

A highly specialized type of cell–cell interaction is fusion between plasma membranes. Cell–cell fusion occurs between cells of the same type (homotypic reactions) as well as between cells of different types (heterotypic reactions). Homotypic cell fusion reactions include fusion of myoblasts to form myotubes, fusion of monocytes to form osteoclasts, and fusion of cytotrophoblasts to form the placental syncytiotrophoblast. The products of homotypic cell fusion reactions are multinucleated cells that perform specialized functions. Heterotypic cell fusion reactions occur between gametes. In all cases, cell–cell fusion reactions lead to profound physiological and developmental changes.

Cell–cell fusion reactions are highly regulated. It is imperative that cells fuse only when the environment is correct for their further differentiation and function. It is equally important that cell fusion events be highly specific (i.e. that the fusing partners be of the correct cell type).

Although some of the proteins that regulate and dictate the target specificity of cell fusion reactions have been identified, the actual molecular basis of fusion remains elusive. Since enveloped viruses use specific proteins to mediate their essential membrane fusion reactions, we will first review what is known about viral membrane fusion proteins. We will then propose and discuss a working hypothesis regarding the possible role of proteins in cell–cell fusion reactions. For more comprehensive recent reviews on this topic see those by Stegmann *et al.* [1] and White (*Annu Rev Physiol*, in press).

The viral paradigm

Enveloped viruses infect cells by fusing with cellular membranes. Viral fusion events share an important feature in common with cell–cell fusion reactions. Both processes are 'exoplasmic' (Fig. 1); the exoplasmic (outer) leaflets of the fusing bilayers make initial contact. This is in contrast to 'endoplasmic' fusion events such as the fusion of transport vesicles carrying material between intracellular organelles. During the latter reactions, the endoplasmic (cytoplasmic) leaflets make initial contact. For a recent review focused on endoplasmic fusion reactions, see that by Wilschut (*Curr Opin Cell Biol* 1989, 1:639–647). Given the differences in the exoplasmic (extracellular) and endoplasmic (cytoplasmic) environments, the mechanisms and proteins involved in exo- and endoplasmic fusion events may differ substantially. Conversely, since cell–cell and virus–cell fusion reactions are exoplasmic, they may share principles in common.

Viral membrane fusion proteins

Two virally encoded activities are necessary for an enveloped virus to enter its host cell. The first is a specific binding interaction between a viral protein and a host cell receptor; the second is the fusion event itself. For paramyxoviruses (e.g. Sendai), these two functions are contained within separate spike glycoproteins; the 'HN' (hemagglutin/neuraminidase) glycoprotein mediates binding while the 'F' (fusion) glycoprotein mediates fusion. For other viruses (e.g. influenza), the binding and fusion functions reside in the same glycoprotein; however, in these bifunctional proteins, the binding and fusion domains are physically distinct (Table 1 and Fig. 2a,b). We believe that the important segregation of binding and fusion functions observed in enveloped viruses will be reiterated in cellular fusion reactions.

The membrane fusion proteins from members of over 25 different virus genera have now been identified, cloned and sequenced. All that have been analyzed further are oligomeric class I integral membrane proteins in which most of the amino acids (>85%) are external to the virus membrane. Most contain N-linked carbohydrates and many are fatty-acylated. They are present at high density in the viral membranes (e.g. $\sim 3 \times 10^4/\mu m^2$ on influenza). Despite these general similarities, the fusion proteins differ in many important respects (Table 1).

Viral fusion proteins can be classified into one of two major categories according to whether they do or do not require exposure to low pH in order to function.

Abbreviations

HA—hemagglutinin; **NCAM**—neural cell adhesion molecule; **PE**—phosphatidylethanolamine; **PI**—phosphatidylinositol; **PS**—phosphatidylserine.

Table 1. Fusion proteins of enveloped viruses.

Virus	Binding protein	Fusion protein	Fusion pH	Fusion peptide	Precursor processing
Influenza	HA	HA	Low	N-terminal	Yes
Semliki forest	E123	E123	Low	Internal	No
Vesicular stomatitis	G	G	Low	Not obvious	No
Human immunodeficiency virus	*env*	*env*	Neutral	N-terminal	Yes
Rous sarcoma	*env*	*env*	Neutral	Internal	Yes
Coronavirus	E2	E2	Neutral	Not obvious	Yes
Sendai	HN	F	Neutral	N-terminal	Yes

For sources of information in this table and in the text about viral membrane fusion proteins, see citations within reference [1] and/or in White (*Annu Rev Physiol*, in press). 'Yes' or 'no' under the heading 'Precursor processing' refers to whether or not processing of the fusion protein from a larger precursor is required for its fusion function. One of the subunits of the Semliki Forest virus fusion protein is made as a larger subunit; however, in this case, processing does not appear to be required for fusion activity. F, fusion; HA, hemagglutinin; HN, hemagglutinin/neuraminidase.

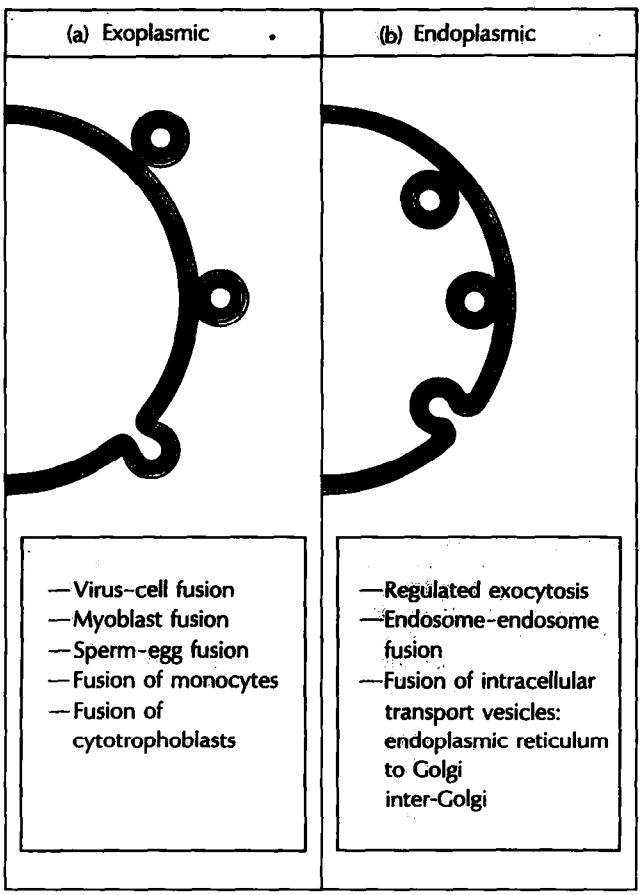

Fig. 1. Exoplasmic versus endoplasmic fusion reactions. (a) Depiction of virus–cell fusion as an example of an exoplasmic fusion reaction. (b) Depiction of regulated exocytosis as an example of an endoplasmic fusion reaction. The shaded areas represent exoplasmic leaflets and the black areas represent endoplasmic leaflets of the fusing bilayers. In exoplasmic reactions, the exoplasmic leaflets make initial contact. In endoplasmic reactions, the endoplasmic leaflets make initial contact. Other examples of exoplasmic and endoplasmic fusion events are listed.

Viruses bearing low pH-activated fusion proteins, such as influenza, are taken into cells by receptor-mediated endocytosis and fuse when they reach an endosome of appropriately low pH (pH 5–6.5, depending on the particular virus). Conversely, viruses whose fusion proteins function at neutral pH, such as the human immunodeficiency virus, are believed to fuse directly with the plasma membrane. Within these two broad groups, the viral fusion proteins can be further subdivided according to whether or not they possess an identifiable 'fusion peptide'.

Fusion peptides are currently defined as stretches of apolar amino acids (in addition to the transmembrane domain) that are conserved within but not between virus families. A feature we believe to be functionally important is that all fusion peptides are located in a polypeptide chain which is anchored in the viral membrane (Fig. 2a). Most fusion peptides are found at the amino terminus of the membrane-anchoring chain, whereas several are found internal to the amino terminus. Although prevalent, fusion peptides have not been identified in all viral membrane fusion proteins (Table 1). Most of the known fusion proteins are made as larger precursors and then cleaved, late in their biosynthetic pathway, into two polypeptide chains that remain associated through disulfide bonds and/or non-covalent interactions. For most of the cleaved fusion proteins, including those with amino-terminal [e.g. the influenza hemagglutinin (HA)], internal (e.g. the *env* glycoprotein of Rous sarcoma virus), and no obvious fusion peptide (e.g. the coronavirus E2 protein), processing appears to be essential for fusion function (Table 1).

Our understanding of how a viral protein promotes fusion is most detailed in the case of the influenza HA, largely because the crystal structure of this molecule is known. The collective findings suggest the following sequence of events: Upon exposure to mildly acidic pH, the trimeric HA spike undergoes a conformational change. The three globular heads dissociate from one another

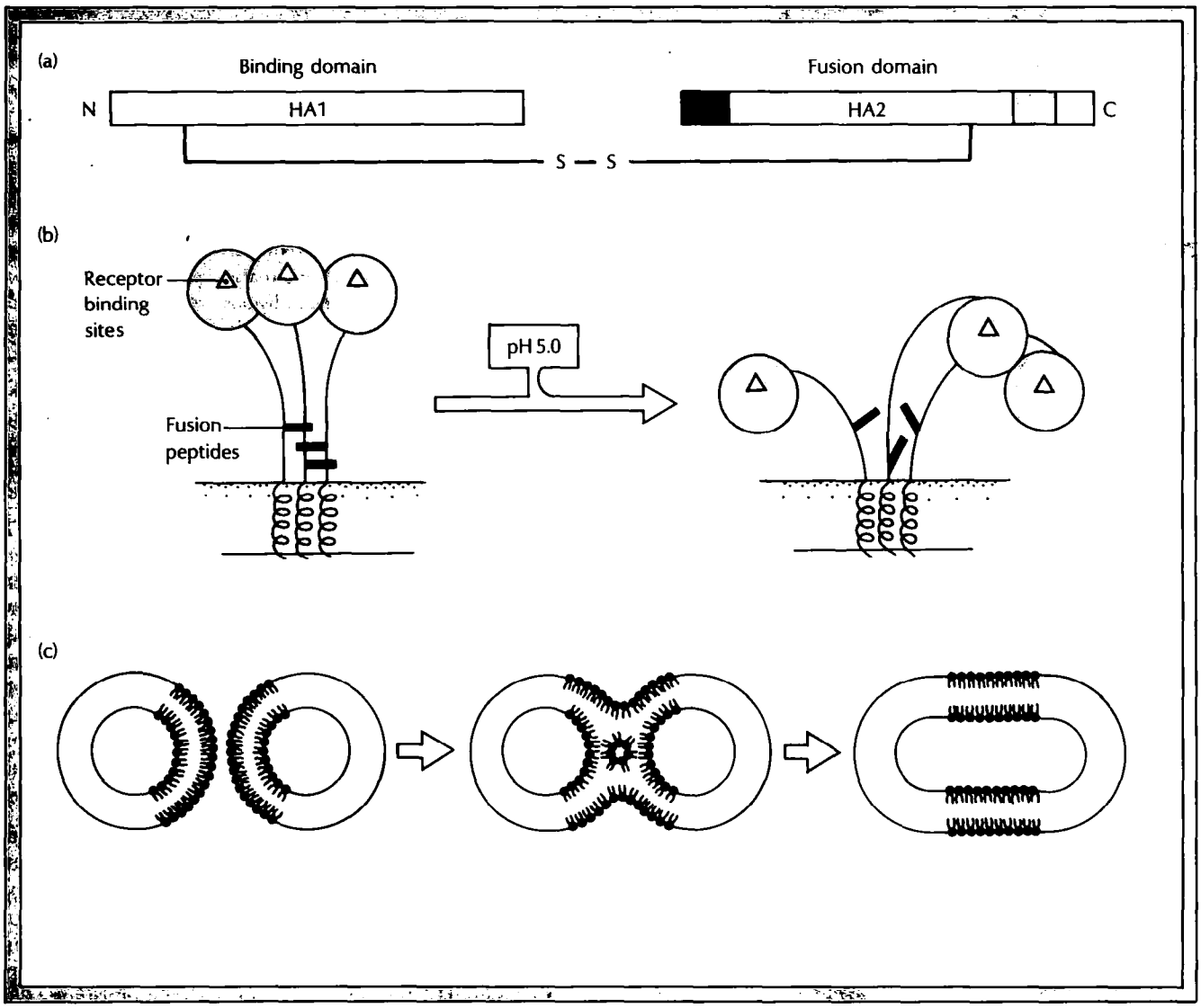

Fig. 2. Proposed fusion mechanism of the influenza hemagglutinin (HA). (a) The influenza HA is a trimer composed of three identical subunits, HA1-S–S-HA2. The receptor binding site is located in the HA1 polypeptide while the fusion peptide (■) is located in the HA1 polypeptide, the chain that anchors the protein into the virus membrane. Transmembrane domain, □. (b) The trimer projects as a spike from the virus membrane. The HA1 polypeptide which houses the receptor binding site forms a globular domain that rests upon a fibrous stalk which houses the fusion peptide. In the neutral pH conformation, the first 10 residues of the fusion peptide are buried inside the trimer interface. After exposure to low pH, the globular heads dissociate from one another and the fusion peptides are released from the interface. The released fusion peptides are then capable of interacting with lipid components of the target membrane. For details see reference [1], White (*Annu Rev Physiol*, in press), and citations within. (c) Steps in a membrane fusion reaction. Left: the bilayers approach each other closely. Center: a non-bilayer structure is formed at the contact site. Right: the unstable non-bilayer intermediate breaks in the plane perpendicular to the original bilayers so as to form one united bilayer. How the partially unfolded HA causes the formation of a non-bilayer structure is under active investigation.

and the three fusion peptides are liberated from the trimer interface (Fig. 2b).The fusion peptides then interact with lipids of the target membrane. In this manner, the fusion protein is thought to associate simultaneously and hydrophobically with both the viral and target membranes, leading to the production of a non-bilayer structure at the contact site (Fig. 2c), a necessary intermediate in the fusion process. Although other viral fusion proteins appear to undergo conformational changes and express hydrophobic moieties under fusion-inducing conditions, we do not yet know whether the fusion mechanism of the HA can be generalized to other viral proteins. We anticipate that the various viral membrane fusion proteins will exhibit interesting variations on the HA theme, and possibly unexpected mechanisms.

Hypothesis: specific proteins mediate cell-to-cell fusion reactions

Fusion is an inherently unfavorable process because of a large energy barrier which prevents membranes from approaching closer than ~2.0 nm (Rand, *Annu Rev Biophys Bioeng* 1981, 10:277–314). Because of both this energy barrier and the viral precedent, we propose that in addition to proteins that regulate and dictate the specificity of cell–cell fusion events, specific proteins facilitate the final bilayer perturbation necessary for cellular union. Variations of this hypothesis have been proposed [1–3] (Strittmatter *et al.*, *In* Cell Fusion edited by Sowers. Plenum Press 1987, pp 99–121).

Identification of proteins involved in cell-to-cell fusion

For simple viruses such as influenza and Sendai, the fusion function resides in a single oligomeric spike glycoprotein. For these viruses, assignment of the fusion function has been relatively straightforward, involving demonstration of fusion activity following either expression of the cloned gene encoding the fusion protein or reconstitution of the purified fusion protein into artificial vesicles. Identification of the fusion proteins of more complex viruses has proved more difficult. For herpes simplex virus, which expresses seven distinct glycoproteins, three of them, gB, gD and gH, may be required for optimal fusion. Given the enhanced complexity of cellular membranes, it is therefore fully expected that identification of proteins involved in cell–cell fusion will be a challenging task.

Of the known cell–cell fusion reactions, the two that have been investigated most intensely are myoblast fusion and gamete fusion. Therefore, in the ensuing discussion we will focus on these two cell–cell fusion processes. After discussing proteins that determine the specificity and regulation of these events, we will discuss proteins that have been implicated in reactions more proximal to the final membrane joining.

Proteins involved in myoblast fusion

Knudson and Horwitz (*Dev Biol* 1977, 58:328–338; *Dev Biol* 1978, 66:294–307) proposed that myoblast fusion be considered a sequence of events: cell–cell recognition and adhesion, and then membrane fusion. As in the viral systems, the binding and fusion steps are biochemically separable events.

Specific cell surface molecules are certainly required to provide for the close adhesion and cell type specificity required for myoblast fusion. Two types of cell adhesion systems have been described for both avian (Gibralter and Turner, *Dev Biol* 1985, 112:292–307; Knudson, *J Cell Biol* 1985, 101:891–897) and mammalian [4] myoblasts: a calcium-dependent system and a calcium-independent system. Both classes of interactions involve glycoproteins and both may employ multiple components. Likely participants in calcium-independent myoblast adhesion are one or more variants of the neural cell adhesion molecule (NCAM). This prediction derives from the observation of both qualitative and quantitative changes in the expression of various NCAM isoforms during myogenesis. Interestingly, the levels of phosphatidylinositol (PI)-linked NCAM(s) increase during myogenesis *in vitro* (Moore *et al.*, *J Cell Biol* 1987, 105:1377–1386) and treatment of myoblasts with PI-specific phospholipase C interferes with myoblast adhesion (Knudson *et al.*, *J Cell Biol*, in press). In terms of calcium-dependent myoblast adhesion, one or more members of the cadherin family (see Kemler *et al.*, this issue, pp 892–897) are likely to be involved.

Many factors that appear to be inhibitors of myoblast fusion are actually inhibitors of differentiation. For example, a monoclonal antibody against chicken integrin, the extracellular matrix receptor, prevents myoblast fusion by preventing differentiation (Menko and Boettiger, *Cell* 1987, 51:51–57). This observation, which in its own right is significant and intriguing, highlights an important experimental point. In assessing the effects of inhibitors of myoblast fusion, it is important to determine whether differentiation or a process more directly involved in fusion has been blocked. An additional complication is that myoblast differentiation consists of at least two separable stages, commitment,which is reversible, and terminal differentiation which is not reversible and which, apparently, begins with the onset of fusion [5]. Therefore it would be expected that a fusion inhibitor would block terminal differentiation without blocking commitment.

Several factors have been implicated as regulators of myoblast fusion. Calcium figures prominently in this context. Calcium influx is known to precede membrane fusion (David *et al.*, *Dev Biol* 1981, 82:297–307), and low concentrations of calcium prevent fusion without blocking myoblast differentiation. Calcium channels, such as the embryonic acetylcholine receptor [6], or a recently described stretch-activated Ca^{2+}-channel that is most active when myoblasts are fusing (Franco and Lansman, *Biophys J* 1989, 55:491a), are thought to mediate the Ca^{2+} influx. The mechanism by which Ca^{2+} influx eventually leads to membrane fusion is unclear. Given that prostaglandins [6], PI bisphosphate [7], protein kinase C and other factors associated with the PI second messenger pathway have also been implicated [2], Ca^{2+} could well be part of a second messenger pathway that ultimately triggers myoblast fusion.

And what of protein involved in the final fusion of the myoblast membranes? At present we can only list several candidates. These include a variety of proteins that are developmentally expressed or modified (Kaufman *et al.*, *J Cell Biol* 1985, 100:1977–1987); Rosenberg *et al.*, *Proc Natl Acad Sci USA* 1985, 82:8409–8413; Lognonne and Wahrmann, *Cell Diff* 1988, 22:245–258). Although a soluble metalloendoprotease has been implicated in myoblast fusion (Strittmatter *et al.*, 1987), neither the precise

step(s) at which it acts (Baldwin and Kayaler, *Proc Natl Acad Sci USA* 1986, 83:8029–8033) nor its substrates have been identified.

Proteins that maintain lipid asymmetry may be important determinants of the fusogenic capacity of membrane surfaces. The exoplasmic leaflet of chick (but not rat) myoblast plasma membranes contains two to three times more phosphatidylethanolamine (PE) and phosphatidylserine (PS) than the exoplasmic leaflets of fibroblast or erythrocyte plasma membranes. Although this enrichment cannot account in total for the fusogenic property of the myoblast surface, it may play an indirect role (Sessions and Horwitz, *Biochim Biophys Acta* 1983, 728:103–111). In this context it is interesting that an ATPase has recently been described which maintains a relatively high amount of PS in the endoplasmic leaflet of chromaffin granules [8]. It will be interesting to see whether chick myoblasts use related mechanisms to maintain relatively high amounts of PS and PE in their fusing surfaces.

Proteins involved in gamete fusion

Gamete fusion is clearly an important cell–cell fusion reaction. It is under study in both lower and higher eukaryotes using a combination of genetic, immunological and biochemical approaches. Clues to some of the proteins involved in gamete binding and fusion are beginning to emerge.

In the baker's yeast *Saccharomyces cerevisiae* two genes, FUS1 and FUS2, have been identified whose products are involved in gamete fusion (McCaffrey *et al.*, *Mol Cell Biol* 1987, 7:2680–2690; Trueheart *et al.*, *Mol Cell Biol* 1987, 2316–2328). Fus1 is an 80 kD integral membrane protein which localizes to the gamete attachment site. It has a relatively small extracellular domain with multiple O-linked carbohydrates, a single transmembrane domain, and a relatively large cytoplasmic domain. Characterization of FUS2 is in progress. Future studies are necessary to determine whether either or both of these proteins are involved in breakdown of the cell wall, in binding the plasma membranes of cells of opposite mating types or in the final membrane fusion reaction. A polyclonal antibody blocking approach has been used in preliminary studies of mating in *Dictyostelium discoideum*. Based on adsorption of the fusion-inhibitory activity of a polyclonal antiserum, a protein in the 70 kD range has been implicated in a post-aggregation step required for gamete union [9].

In sperm–egg fusion in higher eukaryotes the initial binding and the fusion reaction are clearly separate events. Initial binding occurs between acrosome-intact sperm and the extracellular glycoprotein coat of the egg, the zona pellucida. Sperm and egg proteins involved in this critical binding interaction have recently been identified and characterized (Shur, this issue, pp 905–912). Following binding to the zona pellucida, sperm undergo the acrosome reaction and migrate toward the egg plasma membrane. Once the sperm and egg plasma membranes meet, the fusion reaction occurs.

The zona pellucida of the egg can be removed such that binding and fusion between (fully differentiated) sperm and egg plasma membranes can be investigated *in vitro*. Various types of perturbants have been used to begin to identify proteins involved in these events. Protease digestion experiments suggest that proteins on the mouse egg plasma membrane may be required for sperm binding [10]. Monoclonal antibodies have been used to probe the molecular basis of sperm–egg fusion [11] (Saling *et al.*, *Biol Reprod* 33:515–526). Based on these studies, we feel that a good candidate to play a role in sperm–egg fusion is PH-30, a complex of two proteins located in the posterior head region of guinea pig sperm, a region where fusion with the egg occurs. Of two monoclonal antibodies that react with PH-30, one inhibits fusion whereas the other does not. Neither antibody prevents binding between acrosome-reacted sperm and eggs whose zonae pellucidae have been removed (Primakoff *et al.*, *J Cell Biol* 1987, 104:141–149). Interestingly, inhibitors of metalloendoproteases have been shown to block fusion of sea urchin sperm [11] and human sperm [12] with eggs. As is the case for myoblasts, the substrates of the implicated metalloendoproteases are not yet known.

Perspectives

Given the energy barrier to fusion we believe that all cellular fusion reactions are, at some level, protein-mediated. More specifically, we speculate that cellular fusion reactions are mediated by membrane fusion proteins. Until proven otherwise we adhere to a rather strict definition of a membrane fusion protein as a protein (or protein assembly) that interacts with lipid components of two apposed bilayers so as to bring about their unification.

To date several proteins have been implicated in cell–cell fusion reactions. However, the precise role of any of these candidates in the overall fusion process remains to be determined. Since cell–cell fusion reactions are exoplasmic, it is tantalizing to speculate that proteins involved in cell–cell fusion events will resemble viral membrane fusion proteins. However, lacking any evidence, at this point in time we must consider alternative possibilities. For example, it has been suggested that lysin, a soluble protein from the acrosome granule of abalone sperm, may play a direct role in fusion (Hong and Vacquier, *Biochemistry* 1986, 25:543–549). It is also conceivable that phospholipid modifying-enzymes may play an active role in fusion.

In conclusion, we feel that the most pressing questions in the field of cell–cell fusion are the following: (1) Do specific proteins mediate cell–cell fusion reactions? (2) If so, do cell–cell fusion proteins share structural features in common with viral membrane fusion proteins? (3) If specific proteins mediate cell–cell fusion reactions, do they function like viral membrane fusion proteins, or

(4) do they employ novel mechanisms to promote the final bilayer destabilization required for membrane fusion? In pursuing these questions it will be important to keep abreast of concurrent progress in understanding viral membrane fusion proteins as well as proteins involved in endoplasmic fusion reactions.

Annotated references and recommended reading

- • Of interest
- •• Of outstanding interest

1. •• STEGMANN T, DOMS RW, HELENIUS A: **Protein-mediated membrane fusion.** *Annu Rev Biophys Biophys Chem* 1989, 18:187–211.

An excellent review of the role of proteins in biological membrane fusion events. The review focuses on the influenza virus HA, but good discussions of fusion by other viral proteins and by amphiphilic peptides are also presented. Several proteins implicated in cellular fusion events are also described.

2. •• WAKELAM MJ,: **Myoblast fusion.A mechanistic approach.** *Curr Top Membr Transp* 1989, 32:87–112.

A well balanced review discussing factors involved in myoblast fusion and membrane-associated changes accompanying myoblast fusion. Several possible mechanisms of myoblast fusion are discussed.

3. •• YANAGIMACHI Y: **Sperm egg fusion.** *Curr Top Membr Transp* 1988, 32:3–43.

A comprehensive review of sperm–egg fusion in a variety of systems. Factors regulating fusion as well as the effects of numerous perturbants are summarized. A specific hypothesis regarding the role of fusogenic proteins is proposed.

4. • PIZZEY JA, JONES GE, WALSH FS: **Requirements for the Ca^{2+}-independent component in the initial intercellular adhesion of C2 myoblasts.** *J Cell Biol* 1988, 107:2307–2318.

The authors have developed an elegant assay for monitoring the initial stages of adhesion between C2 myoblasts. As observed in the avian system, they find that intercellular adhesion involves both a Ca^{2+}-dependent and a Ca^{2+}-independent system. The Ca^{2+}-independent system is sufficient for initial cell–cell adhesion and appears to be composed, at least in part, of glycoproteins. Somewhat surprisingly the RGDS tetrapeptide stimulates Ca^{2+}-independent adhesion.

5. • FLORINI JR, MAGRI KA: **Effects of growth factors on myogenic differentiation.** *Am J Physiol (Cell Physiol)* 1989, 256:C701–C711.

An up-to-date review discussing the processes of myoblast commitment and terminal differentiation. Emphasis is on the role of growth factors in these events.

6. • ENTWISTLE A, ZALIN RJ, BEVAN S, WARNER AE: **The control of chick myoblast fusion by ion channels operated by prostaglandins and acetylcholine.** *J Cell Biol* 1988, 106:1693–1702.

This and the accompanying paper (Entwistle *et al.*, *J Cell Biol* 1988, 106:1703–1712) suggest that ion channels affected by prostaglandins and acetylcholine are critically involved in controlling myoblast fusion. The authors suggest that depolarization of the myoblast membrane leads to a rise in intracellular calcium which, in turn, initiates myoblast fusion.

7. • SAURO VS, BROWN GA, HAMILTON MR, STRICKLAND CK, STRICKLAND KP: **Changes in phospholipid metabolism dependent on calcium-regulated myoblast fusion.** *Biochem Cell Biol* 1988, 66:1110–1118.

L6 rat myoblasts triggered to fuse by return to calcium-containing medium show a relatively rapid decrease (to 50% in 60 min) in PI 4,5-bisphosphate.

8. • ZACHOWSKI A, HENRY J-P, DEVAUX PF: **Control of transmembrane lipid asymmetry in chromaffin granules by an ATP-dependent protein.** *Nature* 1989, 340:75–76.

Spin-labeled phospholipids were used to demonstrate an ATP-dependent mechanism for maintaining high concentrations of PS in the cytoplasmic (endoplasmic) leaflet of chromaffin granules. Based on its sensitivity to N-ethylmaleimide, the authors propose that the aminophospholipid translocase activity is provided by the granule ATPase II. Aminophospholipid asymmetry may be a determinant of chromaffin granule fusion competence.

9. • URUSHIHARA H, HABATA Y, YANAGISAWA K: **A membrane protein with possible relevance to sexual cell fusion in *Dictyostelium discoideum*.** *Cell Differ Develop* 1988, 25:81–88.

Immunoadsorption studies suggest that a protein in the 70 kD range is involved in a post-aggregation step required for cell fusion in *Dictyostelium discoideum*.

10. • BOLDT J, HOWE AM, PREBLE J: **Enzymatic alteration of the ability of mouse egg plasma membrane to interact with sperm.** *Biol Reprod* 1988, 39:19–27.

Treatment of zona-free mouse eggs with trypsin or chymotrypsin inhibits sperm binding and subsequent fusion.

11. •• ROE JL, FARACH AH, STRITTMATTER WJ, LENNARZ WJ: **Evidence for involvement of metalloendoproteases in a step in sea urchin gamete fusion.** *J Cell Biol* 1988, 107:539–544.

A careful study showing that metalloendoprotease inhibitors prevent fusion between sea urchin sperm and eggs without inhibiting sperm–egg binding. A previous study showed the involvement of metalloendoproteases in the acrosome reaction (Farach *et al.*, *J Biol Chem* 1987, 262:5483–5487).

12. • DIAZ-PEREZ E,THOMAS P, MEIZEL S: **Evidence suggesting a role for sperm metalloendoprotease activity in penetration of zona-free hamster eggs by human sperm.** *J Exp Zool* 1988, 248:213–221.

Metalloendoprotease inhibitors were shown to inhibit (by about 50%) the number of sperm that fuse per egg. The authors suggest that during or as a result of the acrosome reaction, a metalloendoprotease modifies the sperm plasma membrane, somehow increasing its fusion competence.