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REVIEW ARTICLE

MOLECULAR CORRELATES OF SYNCYTIALIZATION IN MUSCLE AND PLACENTA

MANISHA KAR, DEBABRATA GHOSH AND JAYASREE SENGUPTA*

Department of Physiology,
All India Institute of Medical Sciences,
New Delhi– 110 029

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Abstract : Syncytialization is one of the most fundamental processes in life. It is observed during development of muscle and osteoclast, and syncytiotrophoblast formation in placental villi. Syncytialization involves recognition, migration, adhesion and finally cell fusion between two interacting cells. It is an energy-dependent process which is essentially restricted to a small portion of interacting cellular membranes. Such regions of membranes may differ from other regions of cell surface in terms of physico-chemistry and expression of specific protein biomolecules resulting in restriction of this process to cells of specific competence. Despite the fact that membrane biologists have given significant quanta of efforts to understand the basic principle underlying this fundamental process of life, further large scale initiatives have to be undertaken to dissect the underlying molecular correlates central to this event.

Key words : cell membrane fusogenic proteins syncytialization myoblast cytotrophoblast syncytiotrophoblast

In the narration of ‘Bound Man’ by the Austrian author Ilse Aichinger, the circus master spotted a bound person, who remaining entirely within the limits set by rope, can leap and jump gracefully with purpose. Biological cell membrane exhibits its function similarly: remaining within structural confinement it exercises immense purposeful activities that are fundamental to processes of life. ‘Cell fusion’ is one such process that encompasses diverse fields of life. ‘Fusion’ implies physical fusion of two cells in creating a single new cellular compartment by the mixing of their cell contents. The fusion between archaeon and bacterium is believed to have resulted in the genesis of primordial eukaryote (1). Cell membrane fusion is also implicated in virus-host interaction that allows for the viral nucleocapsid to enter into cell cytoplasm (2). Cell-cell fusion is also involved in development of muscle and placental villous syncytiotrophoblast. In the present review, we shall make an attempt to highlight the present state of knowledge about molecular correlates of processes involved in development of muscle and placental villous syncytiotrophoblast.

*Corresponding Author : Email : jsen47@gmail.com

Definition of Syncytialization

The term ‘syncytium’ was coined more than 100 years back and is defined as a mass of multinucleate protoplasm which is established by the fusion of initially separate cells (3). *Syncytialization* is essentially the process by which ‘syncytium’ is formed as observed during formation of muscle, osteoclast and syncytiotrophoblast. Syncytiotrophoblast is the outermost epithelial layer of placental villi. The ‘syncytium’ is a completely different entity than ‘plasmodium’ which is designated as a mass of multinucleate protoplasm that arises as the result of repeated nuclear division without consequential partitioning of the associated cytoplasm (4). Syncytialization is an energy-dependent process which is essentially restricted to a small portion of the interacting membranes. Such regions of membranes may differ from other regions of cell surface in terms of physico-chemistry and expression of specific protein biomolecules necessary for tissue-specific cell recognition, migration, adhesion and rearrangement of the cell membranes together with the selective restriction of this process to cells of same lineage (5, 6).

Physico-chemistry of membrane fusion

The biological membranes consisting of phospholipid bilayers with embedded and bound membrane proteins is mechanically stable. Because of the electrostatic repulsion between the bilayers and steric interaction of membrane proteins, the initial distances between biological membranes are much larger (7). However, proximity of two opposing membranes is a prerequisite for fusion. This is possible either by overcoming the repulsive hydration force that discourages fusion or by promoting the hydrophobic attractive force that favors fusion (8). Hence, fusion requires energy, which either comes from the thermal fluctuations exerted by the membrane or is delivered to the membrane by specialized proteins (7). Merger of the interacting membrane surfaces requires the formation of transient, intermediate membrane configuration which demands high input of energy (9). Theoretical, biochemical and structural studies with isolated membranes indicate that this energetically costly event can be overcome by the formation of ‘fusion stalk’ – a neck-like structure- connecting only the outer leaflets of biological membranes (10). However, more detailed theoretical analysis has revealed that energy requirement for this stalk formation is too high to allow its formation within a biologically reasonable span of time (see *Box 1*) (11).

Box 1: Criterion for energy requirement for stalk formation.

$F_{bar} = F_{int} - F_0$, where F_{bar} is maximal relative energy overcome by the bilayer membrane; F_0 is energy of the initial membranes; F_{int} is energy of a fusion intermediate.

If F_{int} is too large compared to F_0, formation of fusion intermediate becomes improbable.

When the bilayer membrane is subjected to electrical breakdown, maximal relative energy (F_{bar}) overcome by the membrane within 1 s (characteristic time scale of biological fusion) is estimated to be ≈ 40 k_BT , where k_BT (≈ 4. 10^{-21} J or ~ 10^{-21} cal) is the product of the Boltzmann constant, k_B and absolute temperature, T.

From the theoretical analysis, the value of the energy for stalk formation (assuming membranous structures are axially symmetric) is estimated to be 220 k_BT.

Therefore energy requirement for this stalk formation is too high to allow its formation within a biologically reasonable span of time.

Further modification of this model system includes tilting of hydrocarbon chains of lipid molecules of the membranes which essentially decreases estimated energy requirement for the stalk formation (9). Moreover, stalk formation requires fusion proteins that bend the membrane in order to overcome this 'energy crisis' (12).

It is also suggested that local perturbations of hydrophilic-hydrophobic boundary or of phospholipid packing results in fusion (13). Phospholipids are normally maintained in an asymmetrical distribution between extracellular and intracellular domains of plasma membrane of eukaryotic cells. The cholinephospholipids (about 80% of the sphingomyelin and 75% of the phosphatidylcholine) are located in the outer leaflet, while the aminophospholipids (80% of the phosphatidylethanolamine [PE] and almost 100% of the phosphatidylserine [PS]) are located in the inner leaflet of the plasma membrane (14). The asymmetric distribution of aminophospholipids is actively maintained through the action of a Mg^{++}/ATP-dependent aminophospholipid translocase that moves errant phosphatidylserine and phosphatidylethanolamine from the outer leaflet to the inner leaflet of the plasma membrane (15, 16). Phospholipid asymmetry is disrupted when phosphatidylserine is preferentially externalized which is known as 'phosphatidylserine (PS) flip' (Fig. 1). PS flip occurs during apoptosis (17) and intercellular fusion, as observed in the process of myogenesis and morphogenesis of syncytiotrophoblast (18, 19).

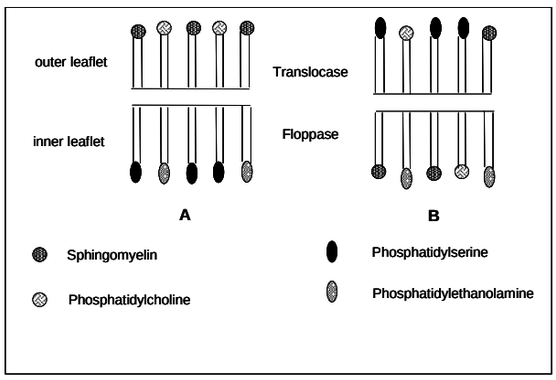

Fig. 1: Schematic illustration of phosphatidylserine flip. Phospholipids are normally maintained in an asymmetrical distribution between outer and inner leaflets of plasma membrane of eukaryotic cells by the action of aminophospholipid translocase. Phospholipid asymmetry is disrupted when phosphatidylserine is preferentially externalized (phosphatidylserine flip) by the action of activated floppase and simultaneous inactivation of translocase. A. before phosphatidylserine flip, B. after phosphatidylserine flip.

Fusogenic proteins

Fusogenic proteins are a group of specific protein biomolecules which are involved in membrane fusion by enhancing membrane curvature and thereby decreasing 'energy crisis' during stalk formation. Table I shows representative fusogenic proteins that have been implicated in eukaryotic syncytialization.

Viral fusogenic proteins

Research on viral fusion proteins paved our way towards understanding the process of membrane fusion. During viral infection, virus binds to receptor present on host cell membrane and both membranes fuse so that viral nucleocapsid could enter the cytoplasm of host cell (2, 12). There are two classes of viral fusogenic proteins which are implicated in membrane fusion, namely class I and class II proteins. The basic unit of most viral fusion proteins contains one or two sets of

TABLE I: Fusogenic proteins involved in eukaryotic syncytialization.

Name of fusogenic protein	*Localization*	*References*
Syncytin 1	Placenta and trophoblast-derived cell lines	Blond et al. (20); Mi et al. (23)
Syncytin 2	Placental tissue	Potgens et al. (6)
ADAM (a disintegrin and a metallo-proteinase domain)	Sperm head, myoblast, myogenic cell line, placental tissue	Cho et al. (28); Gilpin et al. (27)
Gap junctional protein Connexin 43	Muscle tissue, placental tissue	Lowenstein (33); Cronier et al. (35); Frendo et al. (36)
Amino acid transporter CD98	Osteoclast, placental tissue	Kudo et al. (39)
Apoptotic protein Caspase 8	Placental tissue	Black et al. (42)

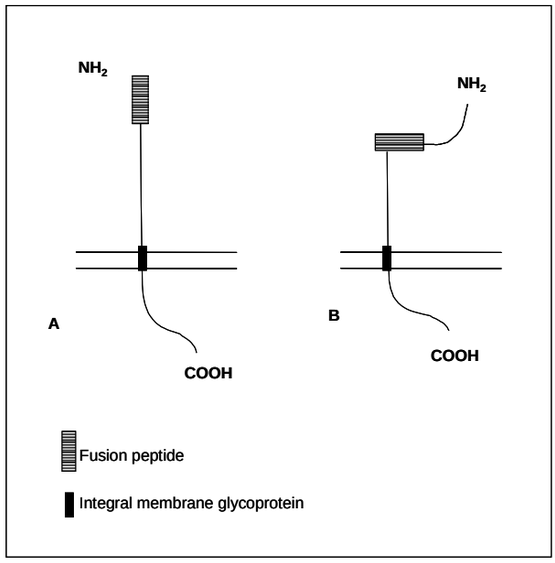

Fig. 2: Schematic illustration of class I viral fusion protein (A) and class II viral fusion protein (B). See White (8) for details.

type 1 integral membrane glycoproteins which project from viral envelope (Fig. 2). Class I viral fusion proteins (exemplified by HA and HIV gp120/gp41) are oriented perpendicularly to the envelope surface and feature α-helical coiled-coil domains (Fig. 2A). A highly conserved sequence located at or near the N-terminal of the fusion protein appears critical for 'fusion peptide' (20). Class II viral fusion proteins (exemplified by E1 protein of alphaviruses and E protein of flaviviruses) lie tangential to the virus membrane and have an 'internal' rather than 'terminal' fusion peptide (Fig. 2B). Class II proteins contain predominantly β-strand secondary structures and are not predicted to form coiled-coils (21). Fusion peptides are short (16 to 26 amino acids) and relatively hydrophobic (8). On activation of viral fusion protein by a shift in pH (mild acidic) or binding of fusion protein to its host cell receptor, integral membrane glycoprotein of viral fusion protein gets proteolytically cleaved and hidden 'fusion peptide' is exposed which triggers fusion (8).

A new human endogenous retrovirus (HERV-W) family has been discovered (22). HERV-W is not detectable as a complete provirus in the human genome, but its envelope is expressed in the placenta and in trophoblast-derived cell lines. It is further characterized as being encoded on chromosome 7 and expressed as two major transcripts with a length of 4 and 8 kb (22, 23). The mature envelope protein consists of 518 amino acids and is presumed to be a membrane protein (22, 23). It was reported that on transfection into COS cells, it induces the formation of multinuclear syncytia. Hence, the protein has been designated as syncytin (23). Syncytin

interacts with a specific receptor, known by the acronym ASCT2. It functions as a retrovirus receptor and as a sodium-dependent neutral amino acid transporter (24). A second fusogenic envelope protein derived from envelope of endogenous retrovirus HERV-FRD has also been described (25). This protein is named as syncytin 2. Syncytin 2 is appeared to utilize a receptor different than that of syncytin. The mRNA encoding syncytin 2 has been specifically found in placenta tissue (6).

A disintegrin and a metalloproteinase domain (ADAM)

ADAMs (for a disintegrin and a metalloproteinase domain) are a family of transmembrane glycoproteins encoded by at least 30 genes identified in *C. elegans*, *Drosophila*, *Xenopus* and various mammalian species (26). They are about 800 amino acids long and have a unique domain organization, containing a prometalloprotease domain, a metalloprotease domain, a disintegrin domain, a cysteine-rich domain, an epidermal growth factor (EGF)-like domain, a transmembrane domain and a cytoplasmic tail (Fig. 3) (26). 18 different members of ADAMs family have been proposed as candidates for modulating proteolysis, cell adhesion, cell fusion and signaling (27). ADAM 1 and 2 (fertilin α and β) have been shown to be crucial for sperm-egg fusion in the mouse and disintegrin domain of fertilins (localized to the posterior head domain of the sperm) is responsible for sperm-egg fusion (28). On the assumption that myoblast fusion may be similar to sperm-egg fusion, homologues of ADAMs 1 and 2 were searched in a mouse myogenic cell line and ADAM 12 (meltrin α) has been identified (29). ADAM 12 shows strong expression in neonatal skeletal muscle and bone. In mouse C2 myoblast cultures, the expression of ADAM 12 becomes apparent on muscle cell differentiation. Evidence for a role in muscle cell fusion has been provided by studies showing that transfection of mouse C2 cells with a minigene of *adam 12* lacking the pro- and metalloprotease domains accelerated cell fusion, whereas antisense constructs blocked myoblast fusion (27). ADAM 12 has also been found to be involved in the formation of

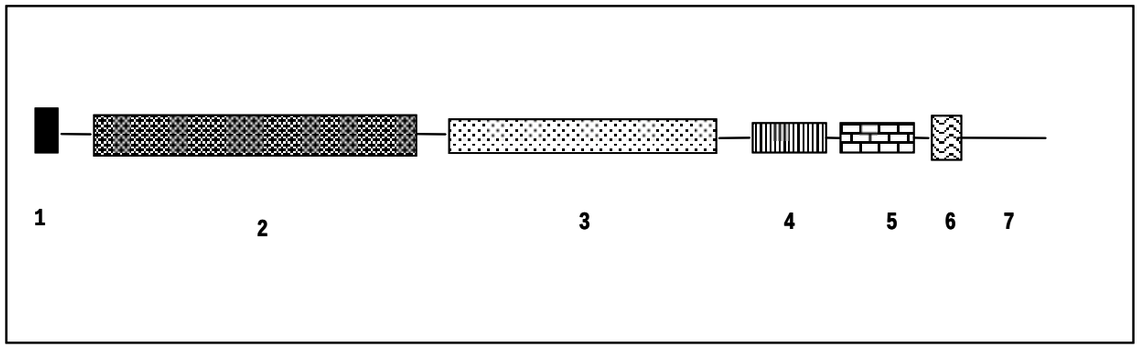

Fig. 3: Schematic diagram of ADAM (a disintegrin and a metalloproteinase domain) protein. 1. a prometalloprotease domain, 2. metalloprotease domain, 3. disintegrin domain, 4. cysteine-rich domain, 5. epidermal growth factor (EGF)-like domain, 6. transmembrane domain, and 7. cytoplasmic tail. For details see Gilpin et al. (27).

osteoclasts (30). ADAM 12 (meltrin-α) mRNA has been detected in human placenta, but it is not clear whether trophoblast or other cell type (blood mononuclear cells) is responsible for this expression (27).

Gap junctional protein

Gap junctions are clusters of transmembrane channels composed of connexin (Cx) hexamers. Connexins represent a family of closely related membrane proteins, which are encoded by a multigene family that contains at least 20 members in humans. In general, effects of connexin expression have been attributed to gap junctional intercellular communication (GJIC) and sharing a common pool of intracellular messengers and metabolites (31). In addition, connexin expression varies during differentiation, proliferation and transformation processes, and following treatment with growth factors and hormones (32). The exchange of molecules through gap junctions is thought to be involved in the control of cell proliferation, in the control of cell and tissue differentiation, in metabolic cooperation and in spatial compartmentalization during embryonic development (33). Gap junctional communication is essential in myogenesis in the rat and the chick models (34). Connexin 43 (C×43) mRNA and protein have been found to be present between cytotrophoblastic cells and syncytiotrophoblast of placental villi and has been shown to be involved in trophoblast differentiation and fusion (35, 36).

Amino acid transporter

CD98 is a multifunctional protein and like syncytin receptor, it is involved in amino acid transport. CD98 is an integral membrane protein which is expressed on the cytotrophoblast and membrane of syncytiotrophoblast of placental villi (37). This molecule is found to be similar to fusion regulatory protein-1 (FRP-1) and its expression is necessary for virus-induced cell fusion, for osteoclast formation (38) and for syncytialization in placenta (39).

Pro-apoptotic protein

One of the mechanisms to initiate apoptosis is binding of a death-inducing ligand to its cognate receptor. This ligand-receptor interaction results in formation of a death-inducing signaling complex (DISC) which initiates distinct pathways to start caspase-dependent cascade of apoptosis. Caspases are present as inactive proforms in most cells and only the activation of caspases leads to apoptotic death of cell. The initiator caspases such as caspase 8 are directly activated by DISC and are active during early and reversible stages of the apoptotic cascade (40, 41). Caspases normally have key functions in apoptosis and are also involved in differentiation processes as shown in case of differentiation of cytotrophoblast cells and its fusion to form syncytiotrophoblast (42). This finding emphasizes the crucial role played by apoptosis related biomolecules in syncytial fusion.

Molecular correlates of cell fusion: myoblast model

The multinucleated muscle fiber develops during myogenesis by fusion of mononucleated myoblasts (43). Several ultrastructural studies have provided the

basis for the current appreciation of this fusion process (44, 45). Fusion events in vertebrate embryos are asynchronous across muscles and depending on the species, can take days, weeks or months. In contrast, myoblast fusion in the *Drosophila* embryo occurs over a period of several hours. The combination of short time frame for fusion, simple anatomy of the somatic musculature and genetic amenability makes *Drosophila* an ideal model organism for analyzing the genetic and molecular basis of myoblast fusion (46).

Cellular characteristics

During myoblast fusion, founder cells and fusion competent myoblasts make contact, recognize one another and fuse (Fig. 4). 'Prefusion complex' appears following cell-cell recognition and adhesion between fusion competent myoblasts and founder cells (Fig. 4A, B). Prefusion complexes are evident in the cytoplasm adjacent to sites of cell-cell contact and consist of as many as 50 electron-dense granules per contact site. These vesicles line up with one another, forming pairs across the apposing plasma membranes (Fig. 4C). An electron-dense material appears both along the vesicle margins and in the extracellular space between the vesicle pairs. Electron-dense plaques then form in areas where membrane breakdown ultimately occurs, presumably through the fusion of the electron-dense vesicles with plasma membrane (Fig. 4D). After plaque formation, myoblasts elongate and align more extensively with one another. Membrane breakdown then begins and pores form, creating cytoplasmic continuity between fusing cells (Fig. 4E). Finally the remaining excess membrane is removed in clear vesicles, which are most likely recycled (Fig. 4F) (47).

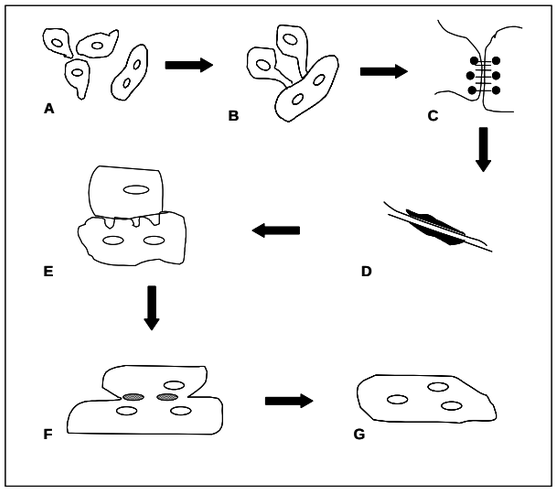

Fig. 4: Schematic representation of subcellular steps of myoblast fusion. Fusion competent myoblast cells (*tear shaped*) (A) recognize and adhere to founder cell (B). Vesicles (prefusion complexes) accumulate at sites of cell-cell contact (C). Electron-dense plaques then form presumably through fusion of electron-dense vesicles with plasma membrane (D). Fusion pores are formed at the site of membrane breakdown (E), and then membrane vesicularization takes place (F). Finally the membrane vesicles disappear and cytoplasmic continuity is established (G). Adapted from Doberstein et al. (47).

Founder cells and fusion-competent myoblasts

The process of myoblast fusion in *Drosophila* is a dynamic relationship between two myoblast cell types: 'founder cells' and 'fusion competent myoblasts' (48). The genetic basis of formation of founder cell and fusion competent myoblast is an area of intense research. Both are known to derive from areas of mesoderm expressing high levels of the transcription factor 'Twist' (49). Clusters of cells within the 'Twist' domain

express *Lethal-of-scute* (L'Sc), a transcription factor (50). Through Notch-mediated lateral inhibition one cell within each L'Sc cluster is singled out to become a progenitor myoblast, while the remaining cells become fusion competent myoblasts (50). Each progenitor myoblast then divides to give rise to a founder cell and one fusion competent cell or two founder cells (50).

Molecular correlates

Utilization of reverse and forward genetic strategies in *Drosophila* has resulted in the identification of molecular components that support myoblast fusion. The findings allow for a preliminary model of molecular interactions that facilitate myoblast fusion *in vivo* (Fig. 5). The extracellular proteins *Sticks and stones* (Sns), *Hibris* (Hbs) and *Irregular-chiasm-C/Roughest* (IrreC/Rst) are found on fusion competent myoblast cells, and *Dumbfounded/Kin of Irregular-chiasm-C* (Duf/Kirre) and *IrreC/Rst* are localized on founder cells. Binding of Sns and Hbs localized on fusion competent myoblast with Duf/Kirre present on founder cell has been detected; IrreC/Rst is known to act homotypically between these two cell types. These extracellular proteins are likely to be involved in cell-cell recognition and adhesion events in both cell types. In the fusion competent myoblasts, Sns and Hbs potentially interact with protein kinase C (PKC), protein kinase A (PKA) and protein kinase G (PKG) through adaptor proteins to mediate intracellular signaling as Sns and Hbs have no intracellular signaling domains. The intracellular domain structure of Duf/kirre and IrreC/Rst suggests that their signaling would also be mediated through adaptors. Indeed the first interaction identified between extracellular proteins and intracellular effectors in founder cell is with an adaptor protein. The interactions of the adaptor protein Rolling Pebbles/Antisocial (Roles7/Ants) with Duf/Kirre and Myoblast city (Mbc), and potentially with irreC/Rst and D-titin has provided an exciting first glimpse of possible links between a cell surface receptor and modulation of the cytoskeleton. D-titin and Mbc (through adaptor protein D-crk and D-Rac [Rho family GTPases]) are thought to organize actin and myosin, thereby remodeling the cytoskeleton (46). Interestingly, for most of the proteins identified in *Drosophila* there already exists at least one homologue in vertebrates (see Table II). The mouse homologues of Rols/Ants are expressed in the developing mesoderm (55), suggesting an immediate suitable genetic entry point for examining analogous function. Comparing and contrasting the functions of such homologues will yield invaluable insights into the molecular nature of myoblast fusion across organisms.

Molecular correlates of cell fusion: syncytialization in placental villi

The floating chorionic villi of villous placenta form the feto-maternal interface. The floating villi consist of stromal core surrounded by double-layered epithelial membrane which in turn consists of outer syncytiotrophoblast and inner single layer of cytotrophoblast cells resting on the basement membrane. The daughter cells of cytotrophoblast stem cells (Langhans' cell) differentiate depending on their position into either villous syncytiotrophoblast or invasive extravillous cytotrophoblast (60).

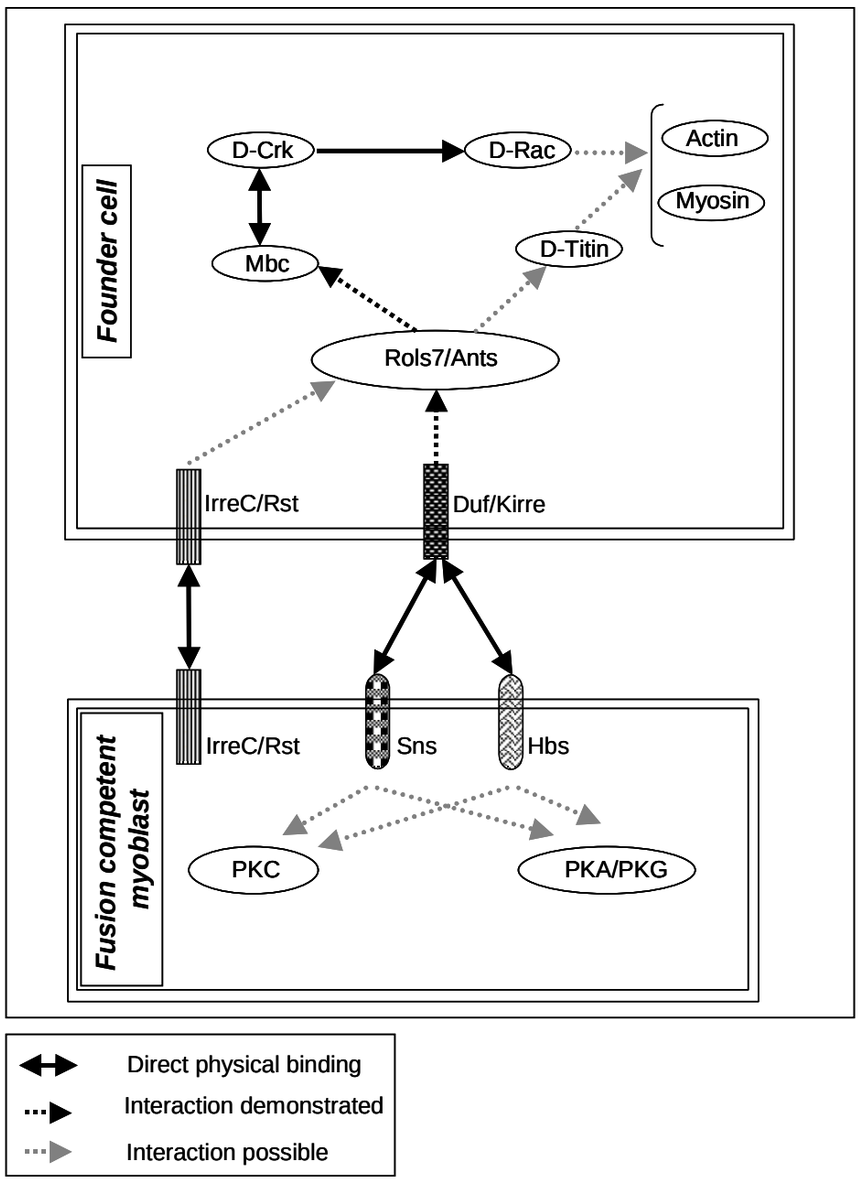

Fig. 5 : Schematic representation of molecular events in myoblast fusion. Extracellular proteins Sns and Hbs present on fusion competent myoblasts can bind with *Duf/Kirre* (extracellular proteins) present on founder cells. *IrrC/Rst* (extracellular proteins) present on both fusion competent myoblasts and founder cells interact with each other homotypically. In the fusion competent myoblasts, Sns and Hbs potentially interact with protein kinase C (PKC), protein kinase A (PKA) and protein kinase G (PKG) through adaptor proteins to mediate intracellular signaling. In founder cells, Duf/kirre interacts with adaptor proteins *Rolling Pebbles/Antisocial* (Roles7/Ants) and *Myoblast city* (Mbc). In turn, Mbc interacts with D-titin (through adaptor protein *D-crk* and *D-Rac* [Rho family GTPases]) which suggestively organizes actin and myosin, thereby remodeling the cytoskeleton. *IrreC/Rst* potentially also interacts with *Roles7/Ants* and *D-titin*. From Dworak et al. (54) after modification.

TABLE II: Vertebrate homologues of *Drosophila* fusogenic proteins.

Drosophila protein	*Localization in Drosophila*	*Vertebrate homologue*	*Reference*
Extracellular			
Sticks and stones	Fusion competent myoblast	Nephrin	Bour et al. (51)
Dumfounded/Kin of Irregular-chiasm-C	Founder cell	Dm-Grasp/Ben/SC1	Ruiz-Gomez et al. (52)
Irregualr-chiasm-C/Roughest	Founder cells and fusion competent myoblasts	Dm-Grasp/Ben/SC1	Strunkelnberg et al. (53)
Hibris	Fusion competent myoblasts	Nephrin	Dworak et al. (54)
Intracellular			
Rolling Pebbles/Antisocial	Founder cells	Mants	Chen et al. (55)
D-Titin	Founder cells and fusion competent myoblasts	Titin	Machado et al. (56)
Myoblast City	Founder cells and fusion competent myoblasts	DOCK 180	Nolan et al. (57)
D-Crk	Mesoderm	Crk-II and CrkL	Galletta et al. (58)
Rac1/Rac2	Ubiquitous	Rac	Luo et al. (59)

Cellular characteristics

Based on morphologic studies on early placenta of the rhesus monkey (61) and the human (62), sufficient explanation of the origin of the multinucleate trophoblast or syncytiotrophoblast has not been achieved. This issue has been addressed by studying human placentae in the first three months of gestation, and it has been proposed that syncytiotrophoblast may originate from either (i) cytotrophoblast while it undergoes nuclear division without cellular division, or (ii) adjacent cytotrophoblasts that unite and fuse to form syncytiotrophoblast, or (iii) cytotrophoblast that fuses with overlying syncytiotrophoblast (63).

An electron microscopic study of first trimester human placenta indicates that syncytiotrophoblast expansion involves plasmodial division though interplasmodial gaps have not been found (64). However, evidence of syncytiotrophoblast arising from fusion of cytotrophoblast with syncytiotrophoblast has been provided (65) along with morphological evidence for the presence of intermediate cytotrophoblast cell type (60, 63). The cellular features of intermediate cytotrophoblasts are closely comparable with features described by earlier investigators for transitional stage cytotrophoblasts (66, 67). The evidence of syncytial clefts with attached desmosomes in syncytiotrophoblast also provides evidence in support of the possible cell fusion between cytotrophoblast and syncytiotrophoblast (63).

Two-stage model of syncytialization

On the basis of ultrastructural characteristics of first trimester human villous trophoblast, a ‘two-stage’ model of cell fusion has recently been proposed (68). Figure 6 gives a cartoon depicting the ‘two-stage’ model of syncytialization in human first trimester villous placenta. In the first stage, a ‘pre-fusion complex’ is established

between 'transition' stage cytotrophoblast and its adjoining syncytiotrophoblast, and it is characterized by closely approximating dense membrane plaques between apposed plasma membranes enclosing mutual hiatus. Such gaps often show vesiculating membranous structures and electron-dense, liposome-like structures. In the second stage, a 'fusion complex' forms with punctuate areas of plasma membrane breakdown resulting in focal cytoplasmic continuity. Additional evidence in form of the presence of intracytoplasmic desmosomes and membrane fragments inside adjoining syncytiotrophoblast further substantiates the proposed 'two-stage' model of villous placental syncytialization (Fig. 6).

Molecular correlates

In choriocarcinoma and in primary trophoblast cells phosphatidylserine flip has been shown to be involved in syncytial fusion. Blockage of phosphatidylserine by antibodies hinders syncytium formation (69, 70, 71). Focal presence of phosphatidylserine flip has been documented in syncytiotrophoblast and discrete membrane bound phosphatidylserine are observed in villous cytotrophoblast in explant culture model system of term villous placenta (19). The same group of investigators has shown that inhibition of caspase 8 (initiator caspase) resulted in failure of trophoblast fusion *in vitro* by virtue

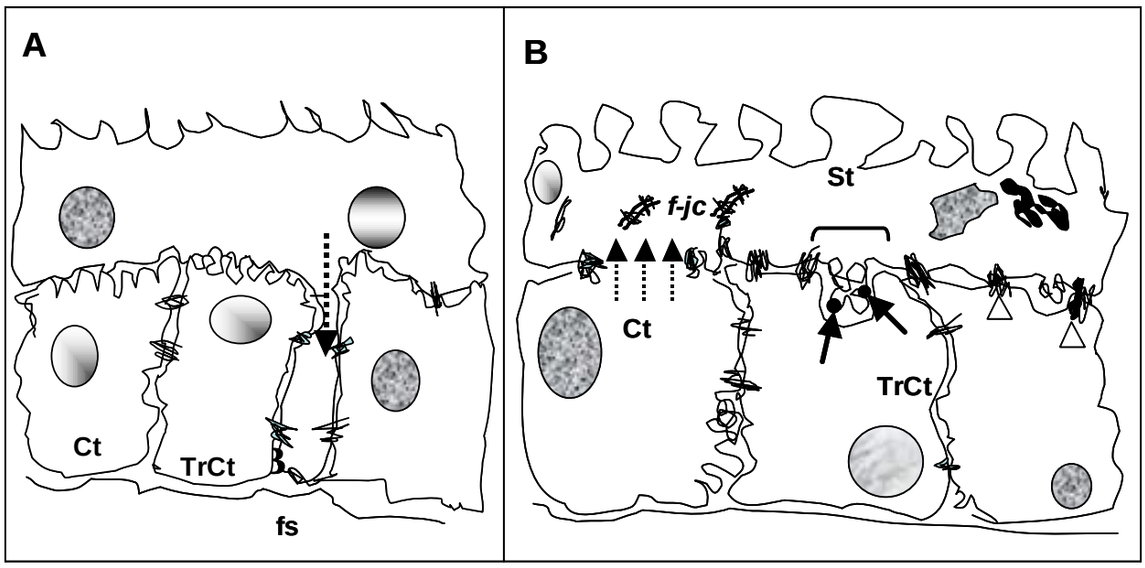

Fig. 6 : Scale-free cartoon representation of 'two-stage' model of syncytialization in human first trimester villous placenta. Transitional cytotrophoblast (TrCt) showing numerous blebbing of apical plasma membrane forms desmosomal junctional complexes with neighbouring cytotrophoblast (Ct) and with invading tongue of syncytial cell (*dotted single arrow*) as it approaches fetal stroma, *fs* (A). Transitional cytotrophoblast (TrCt) with 'pre-fusion zone' of complex membraneous vesiculation (*bracket*) that encloses electron-dense liposome like vesicles (*arrow*) and are bound by dense plaques (*hollow triangle*). The 'fusion zone' is evident from punctate areas of plasma membrane breakdown (*stippled arrows*) resulting in cytoplasmic continuity between cytotrophoblast (Ct) and syncytiotrophoblast, (St), fragments of plasma membrane bearing junctional complexes (*f-jc*) are seen in syncytial cell (B). Adapted from Kar et al. (68).

of causing inhibition of tanslocase enzyme, critical for phosphatidylserine flip (42). This report supports the proposed hypothesis that the molecular machinery of the early apoptosis cascade is utilized for early phase of differentiation of villous trophoblast cells and it precedes syncytial fusion (19, 72). However using BeWo cells *in vitro* (cell line derived from choriocarcinoma that exhibit features of syncytialization on forskolin stimulation), trophoblast fusion has been found to be dependent on phosphatidylserine efflux, but independent of apoptosis (73). Data obtained from an *in vitro* model system using permanent cell lines has genuine caveats that temper their extrapolation to the biological system *in situ*.

In human villous cytotrophoblast cells endogenous retroviral envelope proteins have been reported to decrease cell proliferation, initiate intercellular fusion with an efflux of phosphatidylserine independent of apoptosis (74), and results in hCG production characteristic of syncytialization (75).

The expression of syncytin has been shown to be upregulated after stimulating the fusion of primary cytotrophoblast isolates into syncytia by forskolin, an activator of adenylate cyclase (36). However using a two-color fluorescence assay for quantification of cell-cell fusion in choriocarcinoma cell lines BeWo, JAR and JEG3, fusion index has not been found to be associated with comparable levels of mRNAs of syncytin and its receptor RDR (ASCT2) (67). It is however likely that different set of fusogenic proteins may also be involved in syncytial fusion. CD98, a subunit of a family of amino acid transporter molecules has been shown to play a role in osteoclast formation and in mediating virus-induced cell fusion and trophoblast cell fusion (24, 37). Inhibition of expression of CD98 by antisense strategy, results in failure of fusion in BeWo cells (24). Antisense strategy for connexin 43 reveals that this gap junctional protein is involved in trophoblast fusion in isolated trophoblast cells from term villous placenta (36).

In lieu of conclusion

Syncytialization is not an isolated event during myogenesis, formation of osteoclasts or morphogenesis of syncytiotrophoblast. It is a process that evolves within structural and functional constraints of a given tissue and involves tools of a complex homeodynamic process including proliferation, differentiation and apoptosis. To this effect, it appears imperative that multiparametric large scale studies are to be undertaken in near future towards delineating syncytialization as a procees.

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