Towards a molecular pathway for myoblast fusion in *Drosophila*

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Intercellular fusion among myoblasts is required for the generation of multinucleated muscle fibers during skeletal muscle development. Recent studies in *Drosophila* have shed light on the molecular mechanisms that underlie this process, and a signaling pathway that relays fusion signals from the cell membrane to the cytoskeleton has emerged. In this article, we review these recent advances and discuss how *Drosophila* offers a powerful model system to study myoblast fusion *in vivo*.

Membrane fusion is one of the most fundamental processes in life. Cell–cell fusion is the most poorly understood of the three types of membrane-fusion events (intracellular fusion of organelles; virus–cell fusion and cell–cell fusion). Cell–cell fusion is crucial for the development of multicellular organisms and is required for processes as diverse as fertilization, the formation of bone and placenta, and myogenesis [1,2]. Despite the diversity of the cell types that undergo fusion, the cellular events that are involved in this process – cell recognition, adhesion and membrane merger – are common to all of these cell types, which suggests that shared molecular mechanisms might be used.

Myoblast fusion, by which mononucleated myoblasts fuse to form multinucleated muscle fibers, is an essential early step during skeletal muscle differentiation. Most studies of myoblast fusion during the past three decades have been carried out in mammalian cell-culture systems in which myoblast fusion can be synchronized [3,4]. These *in vitro* studies have implicated several classes of protein in myoblast fusion, including cell-adhesion molecules, metalloproteases, calmodulin, protein kinases and phospholipases [4,5]. However, it remains to be determined whether these proteins are involved in myoblast fusion *in vivo* (for a review of recent advances regarding the genes that regulate mammalian myoblast fusion, see Ref. [6]).

Considering the limitations of *in vitro* studies, an *in vivo* system is desirable for investigating the molecular mechanisms that underlie myoblast fusion. The fruit fly *Drosophila* provides an ideal paradigm for such a purpose. The somatic musculature (or larval body-wall muscle) of *Drosophila* is functionally equivalent to vertebrate skeletal muscle. As in vertebrates, myoblast fusion is an indispensable step during *Drosophila* myogenesis. Furthermore, the distinctive cellular changes during the fusion process, including myoblast recognition, adhesion, alignment and membrane coalescence, are morphologically similar between *Drosophila* and vertebrates [3,4,7]. Thus, it is conceivable that the genes that are involved in myoblast fusion in *Drosophila*, or a portion of them at least, have evolutionarily conserved roles in vertebrate myogenesis. Despite the similarities between fly and vertebrates, the *Drosophila* musculature is much less complex (at most, 30 myoblasts per fiber, compared with thousands of myoblasts per fiber in vertebrates) and its development takes less time (hours, compared with days and weeks in vertebrates) [8]. These features, together with the powerful molecular and genetic tools that are available, make *Drosophila* a tractable system to unravel the molecular mechanisms that control myoblast fusion *in vivo*. In this article, we discuss the basic developmental and cell biology of myoblast fusion in *Drosophila* and highlight recent advances in the molecular and genetic investigations of this process.

The developmental biology of myoblast fusion

*Primary and secondary myotubes in vertebrates*

Vertebrate skeletal muscles originate from the embryonic mesoderm. Skeletal muscle cells, or myoblasts, are derived from epithelial somites and are specified by the sequential actions of the paired-box transcription factor Pax-3 and the myogenic basic helix–loop–helix (bHLH) transcription factors MyoD and Myf5 [9]. The withdrawal of proliferating myoblasts from the cell cycle in response to extracellular cues is accompanied by the fusion of myoblasts to form multinucleated myotubes. The early wave of myoblast fusion produces primary myotubes that function as scaffolds for the later waves of fusion that lead to the formation of secondary and tertiary myotubes.

During the final wave of embryonic myogenesis, a pool of ‘muscle satellite cells’ is formed. Some satellite cells remain quiescent for a period of time, after which they proliferate, differentiate and fuse with existing muscle fibers during exercise and injury, and in degenerative muscle diseases [10,11].
Muscle founder cells and fusion-competent cells in Drosophila

Based on their different behaviors during fusion, two myoblast cell types have been revealed by studies of Drosophila myogenesis: muscle founder cells and fusion-competent cells. Muscle founder cells function as ‘attractors’ for the surrounding fusion-competent cells and they prefigure many properties of future muscle fibers, including position, orientation, size, attachment sites and patterns of nerve innervation [8]. Muscle founder cells are further divided into different subsets by the expression of different ‘selector’ transcription factors such as Nautilus, Krüppel, S59, Apterous, Vestigial, Even skipped and Ladybird [12,13]. The neighboring fusion-competent cells fuse with founder cells and, thereafter, adopt the same selector-gene expression profile. Initially, a founder cell fuses with one or two competent cells to form binucleated or trinucleated muscle precursors [14]. Additional rounds of fusion between these precursors and fusion-competent cells result in the formation of multinucleated myotubes [14]. Thus, myoblast fusion in Drosophila occurs in two step-wise phases. Recent in vitro studies of mammalian myoblast fusion have also revealed two phases of fusion: first, the fusion between a subset of myoblasts to form nascent myotubes and, second, additional rounds of fusion between myoblasts and nascent myotubes [6]. However, it is not clear whether the two-phase fusion process occurs in vivo and whether a founder-cell population exists during the first phase of mammalian myoblast fusion.

Muscle founder cells and fusion-competent cells are specified by a hierarchy of transcription factors during Drosophila myogenesis [5,12,13,15] (Figure 1). During early embryogenesis, the bHLH transcription factor Twist (Twi) is required to specify the embryonic mesoderm. After gastrulation, the mesoderm is subdivided into regions of alternating high and low Twi expression. The domains with high levels of Twi expression contain clusters of cells that express another gene, lethal of scute, that encodes a bHLH transcription factor. These clusters of cells form the so-called myogenic equivalence groups. One muscle progenitor cell from each myogenic equivalence group is then specified by a Notch- and Delta-mediated lateral inhibition process. This single cell undergoes one round of asymmetric cell division to generate either two muscle founder cells or one founder cell and one adult muscle precursor. The remaining cells of the myogenic equivalence group differentiate as fusion-competent cells. This later stage of myogenic differentiation also seems to be controlled by additional transcription factors. For example, lame duck (lmd) [also called myoblast incompetent (minc) and gleeful (glee)] encodes a Gli family transcription factor that is required for the differentiation of fusion-competent cells [16–18]. In lmd/minc-mutant embryos, there is an absence of fusion-competent cells, whereas founder cells are properly specified. Interestingly, one of the downstream target genes of lmd/minc/glee is Dmef2, which encodes a MADS-box transcription factor that is required for the differentiation of all the somatic, cardiac and visceral muscle lineages. At present, it is not clear whether other transcription factors are required for the differentiation of all muscle founder cells, as Lmd/Minc/Glee is in fusion-competent cells.

Cellular aspects of myoblast fusion

Like other types of cell–cell fusion events, myoblast fusion is a multistep process. The initial steps of cell recognition and adhesion can be observed readily at the light-microscopy level. In Drosophila, for example, fusion-competent cells are seen to extend membrane protrusions (filopodia) towards founder cells and the tips of the filopodia are observed to be attached to the founder-cell membrane [19]. The electron microscopy (EM) studies of Drosophila myoblast fusion that were carried out by Doberstein et al. are particularly informative with respect to the subcellular changes that follow the initial recognition and adhesion of myoblasts [7] (Figure 2). The authors observed paired vesicles (called fusion complexes) that had electron-dense margins at the sites of cell–cell contact. These vesicles line up with each other across the apposed membranes of two adhering myoblasts. The prefusion complex then resolves into electron-dense plaques between apposed myoblasts while the two cells become elongated and align themselves along their long axes. Subsequently, cytoplasmic continuity forms through multiple small zones (fusion pores) between the apposed plasma membranes, followed by vesiculation of the residual membranes. Eventually, these events lead to the formation of multinucleated myotubes.

These detailed cell biology studies of myoblast fusion have raised many questions regarding the mechanisms that underlie this process. How do fusion-competent cells sense the signal from founder cells for fusion? What mediates the attraction and adhesion between the two cell populations? How are fusion signals transduced to the cytoskeleton to affect its rearrangement, which is a prerequisite for cell alignment and fusion? What are the components of the prefusion complex? What mediates the breakdown of the plasma membrane and how do fusion pores form? A genetic approach to address these fundamental questions is to isolate mutations that cause specific defects in myoblast fusion. The identification and the functional characterization of the corresponding genes are beginning to reveal a signaling cascade that transduces the fusion signal from the cell surface to changes in the cytoskeleton during Drosophila myoblast fusion. These recent advances are discussed later.

The molecular biology of myoblast fusion

Myoblast recognition and adhesion: the transmembrane receptors

The first step during myoblast fusion is the recognition between muscle founder cells and fusion-competent cells. This seems to be mediated by cell-type-specific transmembrane receptors (Figure 3 and Table 1). In founder cells, two immunoglobulin (Ig)-domain-containing cell-adhesion molecules – Dumbfounded (Duf) [also called Kin of Irregular chiasm C (Kirre)] and Roughest (Rst) [also called Irregular chiasm C (IrreC)] – function redundantly to attract fusion-competent cells [20,21]. The deletion of both duf and rst causes a complete block of fusion, whereas the overexpression of either gene can attract fusion-competent
myoblasts to the ectopic sites of expression. In fusion-competent cells, Sticks and stones (Sns), which is also an Ig-domain-containing cell-adhesion molecule, is required for fusion because the loss of \( \text{sns} \) results in a lack of fusion [22]. Another fusion-competent cell-specific cell-adhesion molecule is the paralog of Sns Hibris (Hbs) [23,24]. Hbs is not essential for myoblast fusion but it seems to inhibit Sns function. The overexpression of \( \text{hbs} \) blocks myoblast fusion, whereas the loss of \( \text{hbs} \) causes only minor fusion defects.

The careful examination of the cellular behavior of fusion-competent cells in \( \text{duf} \) \( \text{rst} \) double-mutant or \( \text{sns} \) single-mutant embryos revealed that these myoblasts do extend filopodia, albeit with random orientations [5,20]. The failure of these filopodia to attach to founder cells is consistent with the hypothesis that Duf, Rst and Sns are required for the initial recognition and adhesion between the two cell populations. In addition, there is evidence that Duf and Sns might interact directly with each other to mediate cell adhesion because cultured \text{Drosophila} cells (S2 cells) that express Duf can aggregate with Sns-expressing cells [15,23].

It remains to be determined how fusion-competent cells are attracted to the founder cells initially. One possibility is that fusion-competent cells randomly extend filopodia to locate the founder cells. Alternatively, fusion-competent cells might sense a kind of concentration gradient from the founder cells and extend filopodia specifically in that direction. It is also unclear how the sites of fusion are selected. For example, the transmembrane protein Duf might be localized to predetermined sites in founder cells by intrinsic cues. Alternatively, extrinsic contacts made by the filopodia from fusion-competent cells could have a role in determining Duf localization in founder cells. Detailed studies of receptor localization during the fusion process will provide clues to the answers to these questions.

**Signal transduction: from membrane to cytoskeleton**

Two events occur after a fusion-competent cell makes contact with a founder cell. First, the fusion-competent cell moves towards the founder cell. Second, the fusion-competent cell aligns with the founder cell, thus juxtaposing the two cell membranes. These cellular events require changes in the actin cytoskeleton. Thus, rearrangement of the actin cytoskeleton in both founder cells and fusion-competent cells is a prerequisite for myoblast fusion. How is the fusion signal transduced to the cytoskeleton to effect the rearrangement of the cytoskeleton? The recent
identification of Antisocial (Ants) [also called Rolling pebbles (Rols7)], which is an adaptor protein that links the fusion receptor to components of the cytoskeleton, and Loner, which is a regulator of the ADP-ribosylation factor (ARF)6 small GTPase, has provided insights into the signaling mechanisms that relay the fusion signal from the fusion receptor to the cytoskeleton in founder cells (Figure 3 and Table 1).

**Table 1. Proteins involved in *Drosophila* myoblast fusion**

<table>
<thead>
<tr>
<th>Protein (or Rolling pebbles)</th>
<th>Localization</th>
<th>Structure</th>
<th>Vertebrate homolog</th>
<th>Refs</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Transmembrane receptors</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dumbfounded (or Kin of irregular chiasm C)</td>
<td>FC</td>
<td>Ig domains; TM; APD; PDZ-binding motif</td>
<td>DM-GRASP/BEN/SC1</td>
<td>[20]</td>
</tr>
<tr>
<td>Roughest (or Irregular chiasm C) Sticks and stones</td>
<td>FC, FCC</td>
<td>Ig domains; TM; APD; PDZ binding motif</td>
<td>DM-GRASP/BEN/SC1</td>
<td>[21]</td>
</tr>
<tr>
<td>Hibris</td>
<td>FCC</td>
<td>Ig domains; fibronectin type-III domain; TM; target sites for kinases</td>
<td>nephrin</td>
<td>[22]</td>
</tr>
<tr>
<td><strong>Intracellular proteins</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Antisocial (or Rolling pebbles)</td>
<td>FC</td>
<td>Lipolytic-enzyme signature sequence; ATP- and GTP-binding site; ankyrin repeats; TPR; coiled-coil domain</td>
<td>mants</td>
<td>[25–27]</td>
</tr>
<tr>
<td>Loner</td>
<td>FC</td>
<td>IQ motif; Sec7 domain; PH domain; coiled-coil domain</td>
<td>ARF–GEP&lt;sub&gt;100&lt;/sub&gt;</td>
<td>[30]</td>
</tr>
<tr>
<td>Myoblast city DCrk</td>
<td>FC, FCC</td>
<td>SH3 domain; Crk binding sites; Docker domain</td>
<td>Dock180</td>
<td>[31,32]</td>
</tr>
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<td>Drac1 and Drac2</td>
<td>Mesoderm</td>
<td>GTPase</td>
<td>Rac</td>
<td>[39]</td>
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<td>GTPase</td>
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<td>[30]</td>
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<td>Titin</td>
<td>[28,29]</td>
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<td>Mesoderm</td>
<td>Coiled-coil domains</td>
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<td>Blown fuse</td>
<td>FC, FCC</td>
<td>PH domain</td>
<td>Unknown</td>
<td>[7]</td>
</tr>
</tbody>
</table>

*Abbreviations: APD, autophosphorylation domain; ARF, ADP-ribosylation factor; FC, founder cell; FCC, fusion-competent cell; GEP, guanine-nucleotide-exchange protein; Ig, immunoglobulin; PH, pleckstrin homology; SH, Src homology; TM, transmembrane domain; TPR, tetratricopeptide repeat.*

**Figure 2.** Myoblast fusion is a multistep process. (i) A fusion-competent cell (green) extends filopodia towards a binucleated muscle precursor (brown). (ii) The fusion-competent cell recognizes and attaches to the muscle precursor. (iii) Paired vesicles with electron-dense margins (prefusion complexes) form along the apposed membranes. (iv) An electron-dense plaque forms along the membranes. (v) The cells align along their entire long axes. (vi) The apposed membranes break down, accompanied by the formation of fusion pores. (vii) A multinucleated myotube is formed. The genes illustrated in blue have been shown to function at different stages of myoblast fusion. Modified, with permission, from Ref. [7]. © (1997) Rockefeller University Press.
coiled-coil domain. It also contains a RING finger and a lipolytic-enzyme signature sequence. Mutations in ants/rols7 block the fusion process after the initial step of myoblast recognition and adhesion. Occasionally, fusion proceeds to a binucleated or trinucleated stage, which suggests that ants/rols7 is essential for the second phase of fusion but might have a redundant role or no function during the first phase [26,27]. ants/rols7 is expressed specifically in founder cells at the time of fusion and, strikingly, the protein is localized to discrete subcellular foci [25,26]. These discrete foci correspond to subcellular sites of fusion, as revealed by their colocalization with the structural protein D-Titin that localizes to the sites of myoblast contact [26,28,29]. Interestingly, the specific subcellular localization of Ants/Rols7 depends on the founder-cell-specific transmembrane receptors Duf and Rst. Ants/Rols7 is distributed throughout the cytoplasm in duf/rst double-mutant embryos, whereas it is localized to specific subcellular foci in wild-type embryos [25,26]. Consistent with Duf being required for the subcellular localization of Ants/Rols7, Duf can associate physically with Ants/Rols7 and recruit it from the cytoplasm to the membrane-contact regions between aggregating S2 cells [25,30].

Further insights into the function of Ants/Rols7 have come from physical interactions detected between Ants
and Myoblast city (Mbc) [25], which is another essential component of the myoblast-fusion process [31,32].

Drosophila Mbc belongs to the CDM family of proteins that also includes Caenorhabditis elegans Ced-5, and mammalian Dock180 and Dock2 [33]. CDM proteins in C. elegans and mammalian cells are involved in an evolutionarily conserved signaling pathway (Ced-2, Ced-12, Ced-5 and Ced-10 in C. elegans and CrkII, ELMO, Dock180 and Rac in mammals) that modulates the small GTPase Rac, which is a crucial regulator of cytoskeletal dynamics [34–36]. This pathway mediates cytoskeletal rearrangements during the phagocytosis of apoptotic cells and during cell movements [37]. It has been suggested that Dock180 forms an unconventional two-part guanine-nucleotide-exchange factor (GEF) for Rac with the ELMO protein [38]. It is conceivable that Drosophila Mbc also regulates the activity of the small GTPase Drac during myoblast fusion, although the signaling mechanisms of Mbc are understood less well. Consistent with this hypothesis, Drac1 and Drac2 are required for myoblast fusion in Drosophila [39]. The physical interactions between Ants and Mbc and between Ants and Duf suggest that Ants could function as an intermediary protein that relays the fusion signal from the cell-surface receptor Duf to the cytoskeleton through the regulation of Mbc and Drac activity [25]. It remains to be determined whether Ants/Rols7 regulates the GEF activity or the subcellular localization of Mbc. Furthermore, it will be interesting to investigate whether the homologs of CrkII and ELMO are involved in myoblast fusion in Drosophila [40].

Loner: a guanine-nucleotide-exchange factor that regulates the ARF6 small GTPase during myoblast fusion

The recent characterization of the fusion-defective mutant loner has provided a new element to the understanding of the signaling cascade that regulates cytoskeletal rearrangement during Drosophila myoblast fusion (Figure 3 and Table 1). The loner gene encodes a putative GEF that contains a Sec7 domain and an adjacent pleckstrin homology (PH) domain [30]. The Sec7 domain is found in GEFs for the ARF family of small GTPases [41], whereas PH domains have been implicated in binding to phospholipids in the plasma membrane [42]. Rescue experiments have demonstrated that both of these domains are essential for the function of Loner in vivo [30]. Loner is expressed in founder cells, in which it is localized in discrete subcellular foci (as is the case for Ants/Rols7). However, Loner is colocalized only partially with Ants, which suggests that only a portion of the Loner protein is localized to the sites of fusion. The transmembrane receptor Duf is required for the proper subcellular localization of Loner in founder cells, which is also the case for Ants. Furthermore, Duf can recruit Loner from the cytoplasm to the membrane-contact regions between aggregating S2 cells. However, the subcellular localization of Loner is not dependent on that of Ants and vice versa. Thus, it seems that Ants and Loner are recruited independently to sites of fusion by the transmembrane receptor Duf [30].

How does Loner mediate myoblast fusion? The presence of a Sec7 domain suggests that Loner might function as a GEF for the ARF family of small GTPases. In vitro, the purified Sec7 domain of Loner displays specific GEF activity towards Drosophila ARF6 (dARF6), which suggests that dARF6 might be a physiological target of Loner [30]. Consistent with this hypothesis, the overexpression of a dominant negative form of dARF6 in founder cells blocks myoblast fusion [30]. Together, these observations reveal a novel Loner–dARF6-mediated signaling module that has an essential role in myoblast fusion. However, loss-of-function mutations of dARF6 will, ultimately, be required to strengthen this conclusion.

The relationships between the small GTPases dARF6 and Drac1

The identification of dARF6 and Drac1 as essential components of myoblast fusion raises important questions regarding the relationships between these two small GTPases during the fusion process. Studies in cultured mammalian cells have implicated ARF6 in membrane trafficking and actin-cytoskeleton rearrangements, which are two processes that have potential relevance to myoblast fusion [43]. In particular, there is evidence that ARF6 regulates cytoskeletal rearrangement by controlling the subcellular localization of Rac1 [44]. In Drosophila muscle founder cells, the Loner–dARF6 module seems to control the subcellular localization of Drac1. In loner mutant embryos, Drac1 is distributed throughout the cytoplasm rather than being concentrated to the sites of fusion, as is seen in wild-type embryos [30]. Thus, similar to what occurs in mammalian cells, the Loner–dARF6 module could signal to the actin cytoskeleton through the regulation of Drac1 (Figure 3 and Table 1). However, considering the widespread roles for ARF6 in diverse processes, such as its regulation of the enzymes that are responsible for lipid modification and its involvement in regulated secretion events, it remains to be determined whether these other functions of ARF6 also contribute to myoblast fusion.

The downstream effectors of Drac

Considering the pivotal role of Drac in Drosophila myoblast fusion, it would be interesting to determine the downstream effectors of Drac during actin-cytoskeleton rearrangement. The characterization of the structural proteins D-Titin and Paramyosin in muscle development might help to do this. D-Titin and Paramyosin were identified initially as sarcomeric proteins. However, recent studies have revealed unexpected functions for them during myoblast fusion [28,45]. Both proteins are present at myoblast-contact sites during fusion and are important, although not essential, for the fusion process [28,29,45]. In addition, the proper localization of D-Titin is dependent on Ants/Rols7 (the adaptor protein that is associated with the putative Drac1 GEF Mbc) [27]. These studies, together with the interactions between D-Titin and the actin cytoskeleton and between Paramyosin and the actin cytoskeleton, have led to suggestions that the two structural proteins have a role in the organization of the actin-cytoskeleton elements that are required for fusion [28,29,45] and that they might be among the many downstream effectors of Drac (Figure 3 and Table 1).
Questions outstanding

Studies of *Drosophila* myoblast fusion are beginning to reveal a signaling pathway in muscle founder cells that transduces signals from fusion receptors into changes in the cytoskeleton. Meanwhile, these studies raise new questions for future investigations, as highlighted next.

Identification of components of a ‘fusion complex’

The presence of multiple potential protein–protein-interaction motifs in Ants/Rols7, combined with the observation that Duf recruits both Ants/Rols7 and Loner to sites of fusion, suggests that Duf and Ants/Rols7 might function within a scaffold to anchor multiple proteins to the sites of fusion, where a multiprotein ‘fusion complex’ mediates the cellular changes that accompany myoblast fusion. The identification of additional components of this fusion complex, through both genetic and biochemical approaches, is likely to provide important insights into myoblast fusion. It will also be important to examine the subcellular localization of the fusion complex at the EM level to determine how the fusion complex relates to the distinct ultrastructural entities that have been observed during myoblast fusion, such as paired vesicles and plaques.

How do juxtaposed membranes fuse with each other?

Cytoskeletal rearrangement is a prerequisite for the membrane merger of two apposing cells. It is required for the two membranes to align effectively so that their lipid bilayers are closely juxtaposed for fusion to proceed. Little is known about the actual fusion process. For example, it is unclear how the two membranes are destabilized, how fusion pores form and which molecules are involved in these events. During virus–cell fusion, a hydrophobic peptide in the fusogenic viral glycoprotein mediates the juxtaposition and fusion of two membranes [2,46], although no fusogen-like sequences have been identified in the known proteins that are involved in myoblast fusion. However, the founder-cell adaptor protein Ants/Rols7 contains a lipolytic-enzyme signature sequence that is often present in lipases that are involved in the modification of the lipid bilayer [47]. An isoform of Ants/Rols7 that lacks the N-terminal region that includes the lipolytic-enzyme signature sequence can no longer rescue the myoblast-fusion phenotype in *ants/rols7*-mutant embryos [26]. It will be interesting to determine the specific contribution of this lipolytic-enzyme signature sequence to membrane dynamics during myoblast fusion.

Signal transduction in fusion-competent cells

Little is known about how fusion signals are transduced in fusion-competent cells. The cytoplasmic region of the transmembrane receptor Sns, which is specific to fusion-competent cells, contains proline-rich sequences, potential phosphorylation sites for various kinases and stretches of evolutionarily conserved sequences that have unknown physiological functions [22]. Mbc, which regulates the cytoskeleton in founder cells, is also present in fusion-competent cells and might provide a similar function by regulating Drac and cytoskeletal rearrangements during fusion [32]. It will be interesting to determine whether *Drosophila* homologs of CrkII and ELMO, in addition to Mbc and Drac, are required in fusion-competent cells. It will also be interesting to find out whether there is an adaptor protein in fusion-competent cells that is equivalent to Ants/Rols7 in founder cells and that links the Sns receptor to the cytoskeleton. Ongoing genetic screens in *Drosophila* might identify these and other potential components of fusion-competent cells and shed light on the signal-transduction pathway that is employed in this cell type.

*Drosophila* myoblast fusion: relevance to mammalian myogenesis

Given the conserved cellular events that are involved in *Drosophila* myoblast fusion and mammalian myogenesis, it is conceivable that the genes that are required for *Drosophila* myoblast fusion might have conserved roles in mammalian myogenesis. Curiously, the mammalian homologs of the Ig-domain-containing transmembrane receptors Duf, Rst, Sns and Hbs are not expressed in developing mesoderm. In fact, the mouse homolog of *duf* and *rst* (*SC-1*) is expressed predominantly in the nervous system [48]. In addition, the mouse homolog of Sns and Hbs (nephrin) has been implicated in kidney development [49]. Thus, it seems that the initial recognition and adhesion between myoblasts during vertebrate myoblast fusion might use a different set of transmembrane receptors. This might reflect the differences in the molecular events that lead to the specification of myoblasts in flies and vertebrates. However, preliminary studies suggest that the intracellular components of the myoblast-fusion network might be conserved between *Drosophila* and vertebrates after a fusion signal has triggered the recognition and adhesion of myoblasts. One of the mouse orthologs of *ants, mants1*, is expressed in a variety of mesodermal tissues, including somites, limb buds and body-wall muscles [25]. The transient expression of *mants1* coincides with muscle differentiation, which suggests that it might have a role in muscle differentiation and myoblast fusion. The Loner–ARF6 module might also have a role in mammalian myogenesis because a dominant negative form of ARF6 blocks MyoD-induced myotube formation in a cell-culture model [30]. Future experiments involving knockout or transgenic mice should address definitively whether these fusion genes have conserved roles in mammalian myoblast fusion.

Myoblast fusion and muscle disease

Most studies of human muscle disease have focused on genes such as dystrophin that affect the sarcolemma [47]. Because embryonic myogenesis requires myoblast fusion to occur, complete loss-of-function mutations in fusion genes are likely to cause embryonic lethality. However, hypomorphic alleles of these genes might result in congenital or postnatal muscle diseases. In fact, both centronuclear myopathy and myotonic dystrophy are characterized by minute myofibers, which suggests that myoblast fusion might be defective in these muscle diseases [50,51]. In addition to its role during myogenesis, myoblast fusion is also required for muscle growth and repair during exercise and muscle injury. For example,
Our understanding of this fascinating biological process.

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