Myoblast fusion: lessons from flies and mice
Susan M. Abmayr1,2,* and Grace K. Pavlath3

Summary
The fusion of myoblasts into multinucleate syncytia plays a fundamental role in muscle function, as it supports the formation of extended sarcomeric arrays, or myofibrils, within a large volume of cytoplasm. Principles learned from the study of myoblast fusion not only enhance our understanding of myogenesis, but also contribute to our perspectives on membrane fusion and cell-cell fusion in a wide array of model organisms and experimental systems. Recent studies have advanced our views of the cell biological processes and crucial proteins that drive myoblast fusion. Here, we provide an overview of myoblast fusion in three model systems that have contributed much to our understanding of these events: the Drosophila embryo; developing and regenerating mouse muscle; and cultured rodent muscle cells.

Key words: Founder cell, Fusion-competent myoblast, Myoblast, Myocyte, Myotube, Myofiber, Satellite cell, Regeneration, Migration, Adhesion, Myogenesis

Introduction
The process of fusing two adjacent membranes accomplishes many goals that are crucial to the development and maintenance of living organisms. Broadly, membrane fusion can occur intracellularly, as with synaptic vesicles, or between cells, as for sperm-egg fusion. Cell fusion occurs in a broad range of organisms, including Saccharomyces cerevisiae and Caenorhabditis elegans, and between several cell types, such as macrophages, placental trophoblasts and myoblasts. Experimental analysis of fusion in these systems has revealed the involvement of a diverse array of specialized molecules.

Myoblast fusion, a fundamental step in the differentiation of muscle in most organisms, can involve tens of thousands of myoblasts. Given the complexity of the musculature, fusion must be a regulated process in which the appropriate number of cells fuse at the appropriate time and place. Often these cells migrate long distances prior to fusion, and fusion can involve multiple cell types, necessitating cell recognition and adhesion, which are crucial to accurate and efficient fusion. In addition to the early fusion events that occur during embryogenesis, vertebrate muscle tissue is able to regenerate in response to damage and disease. This regeneration involves proliferation of muscle satellite cells (see Glossary, Box 1) and their subsequent fusion to repair the damaged muscles. The focus of this review is the process of myoblast fusion, which involves cell migration, adhesion and signaling transduction pathways leading up to the actual fusion event. We review the crucial role of the actin cytoskeleton and actin polymerizing proteins, and recently revealed membrane protrusions that may drive fusion itself. We describe three powerful systems for this analysis: Drosophila embryo; mouse embryogenesis and regeneration; and rodent tissue culture. We highlight the genes and morphological events involved in myoblast fusion, as revealed by each system. With the emergence of Danio rerio as another model for myoblast fusion, we also list relevant homologs in this system (Tables 1, 2).

Experimental systems for the analysis of myoblast fusion
The ability to isolate and propagate mammalian myoblasts that could differentiate and fuse in vitro launched the analysis of myoblast fusion several decades ago. This experimental system was the first to implicate specific molecules in myoblast fusion.

Box. 1 Glossary
Embryonic myoblast. A proliferative muscle progenitor cell found in early embryonic mouse development that differentiates and fuses to form primary myofibers. In Drosophila it also refers to either the founder cell or fusion-competent myoblasts prior to fusion.
Fetal myoblast. A proliferative muscle progenitor cell found in later embryonic mouse development that differentiates and fuses to form secondary myofibers.
Founder cell. A mono-nucleate cell in Drosophila that determines the properties of a myofiber (size, shape and attachment to the epidermis). It arises by asymmetric division of a muscle progenitor and does not proliferate further. There is one founder cell for each embryonic/larval somatic muscle.
Fusion-competent myoblast. A mono-nucleate cell in Drosophila that is committed to a muscle-specific program of differentiation but not yet fused. It undergoes very limited additional proliferation. Many fusion-competent myoblasts will fuse with a single founder cell, taking on the identity of that founder cell.
Myofiber (also referred to as a muscle fiber). A single syncytia in Drosophila embryos that results from the fusion of one founder cell and multiple fusion-competent myoblasts. In mice, it refers to a multinucleated muscle cell in vivo formed by the fusion of multiple myocytes.
 Mature myotube. A large mouse muscle cell in vitro that contains many nuclei. These cells are not as developed in size, myonuclear number or internal structure as myofibers in vivo.
Myocyte. A differentiated mononucleated muscle progenitor cell in mice.
Nascent myotube. A newly formed multinucleated mouse muscle cell in vitro that results from the fusion of a few myocytes.
Primary myofiber. A multinucleated muscle cell present early in embryonic mouse development.
Satellite cell. A muscle stem cell in mice that lies in close apposition to a myofiber underneath the basal lamina surrounding the myofiber. These cells are normally quiescent in uninjured muscle but begin to proliferate upon muscle injury to give rise to myoblasts and also self-renew to form new satellite cells.
Secondary myofiber. A multinucleated muscle cell present later in embryonic mouse development that develops in close apposition to primary myofibers.

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Drosophila then emerged as a model organism for the study of embryonic myoblast (see Glossary, Box 1) fusion owing to its easily manipulated genetics and the ability to screen for mutations that impact fusion (Fig. 1). One can readily examine the developing musculature in both fixed and live embryos (see Box 2). Indeed, the identification of crucial genes in Drosophila has provided a valuable entry into the study of myoblast fusion in higher organisms. Nevertheless, insect muscles differ in fundamental ways (see Box 2) from vertebrate muscles. With the emergence of gene knockout and siRNA strategies, and the ability to manipulate primary myoblasts and myoblast cell lines, rodent systems have become powerful models for the analysis of myoblast fusion that more accurately recapitulate that occurring in humans (see Box 2). The details of each of these systems are described below.

The Drosophila embryo

The muscles used by the Drosophila larva develop in the embryo over a period of 10–12 hours. These 30 segmentally repeated abdominal muscles differ from each other in size, shape, location, pattern of innervation and site of attachment to the epidermis (Bate, 1990) (Fig. 1A). In contrast to vertebrate muscles, which are composed of large bundles of myofibers (see Glossary, Box 1), each muscle is a single myofiber that arises by fusion of one founder cell (see Glossary, Box 1) with several fusion competent myoblasts (FCMs; see Glossary, Box 1). The founder cells and FCMs arise from the same population within the somatic mesoderm. Founder cells are initially selected in a complex process that involves overlapping signal transduction pathways, lateral inhibition mediated by Notch and asymmetric cell division (Tixier et al., 2010). This heterogeneous population of cells controls the muscle pattern, with a single founder cell for each muscle fiber (Bate, 1993). The identity and subsequent behavior of each muscle founder cell is then controlled through combinatorial expression of one or more muscle identity genes (Frasch, 1999; Enriquez et al., 2010). This heterogeneous population as the founder cells, but appear to be specified as a homogenous population by the Gli-family transcription factor Lame duck (Lmd) (Duan et al., 2001; Ruiz-Gomez et al., 2002). Following the initial fusion event between a founder cell and an FCM, additional rounds of fusion continue between the developing myotube and FCMs until the final muscle size is achieved. Thus, diversification of the muscle fibers is accomplished in a cell-autonomous manner, within the muscle cells themselves, rather than through the influence of external cues. Overall, the smallest muscles of the embryo will be formed by fusion of as few as two or three cells, whereas larger muscles can include more than a dozen cells (Bate, 1990; Richardson et al., 2007) (Fig. 1). Neither of these cell populations is highly proliferative. Progenitors of the

### Table 1. Proteins that function in migration, adhesion and recognition events prior to myoblast fusion

<table>
<thead>
<tr>
<th>Role</th>
<th>Drosophila</th>
<th>Zebrafish</th>
<th>Mouse</th>
<th>Protein class</th>
<th>References</th>
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<tbody>
<tr>
<td>Cell migration</td>
<td>Cd164</td>
<td>Sialomucin</td>
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<td>(Bae et al., 2008)</td>
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<td></td>
<td>Cxcr4</td>
<td>Chemokine receptor</td>
<td>Cytokine</td>
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<td>(Griffin et al., 2010)</td>
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<td></td>
<td>Interleukin 4</td>
<td>Endocytic C type lectin</td>
<td>G protein-coupled receptor</td>
<td>Prostaglandin</td>
<td>(Jansen and Pavlath, 2006)</td>
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<td></td>
<td>(Griffin et al., 2009)</td>
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<tr>
<td>Cell recognition and adhesion</td>
<td>Duf/Kirre (CG3653)</td>
<td>Kerrel</td>
<td>Rst/Irre C (CG4126)</td>
<td>Immunoglobulin superfamily</td>
<td>(Bondesen et al., 2007)</td>
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<td>(Moore et al., 2007; Ruiz-Gomez et al., 2000; Strunkenberg et al., 2001; Lafreniere et al., 2006; Sohn et al., 2009)</td>
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<td>(Strunkenberg et al., 2001; Srivastava et al., 2007)</td>
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<td>(Bour et al., 2000; Sohn et al., 2009)</td>
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<td>(Artero et al., 2001; Dworak et al., 2001; Shelton et al., 2009)</td>
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<td>(Chen and Olson, 2001; Menon and Chia, 2001; Rau et al., 2001)</td>
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<td>(Brzoska et al., 2006)</td>
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<td>α3 integrin</td>
<td>Integrin</td>
<td>Integrin</td>
<td>Integrin</td>
<td>Protein tyrosine kinase</td>
<td>(Bour et al., 2000; Sohn et al., 2009)</td>
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<td>β3 integrin</td>
<td></td>
<td>Integrin</td>
<td>Integrin</td>
<td></td>
<td>(Quach et al., 2009)</td>
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<td>β1 integrin</td>
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<td>Integrin</td>
<td>Integrin</td>
<td></td>
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<tr>
<td>Focal adhesion kinase</td>
<td></td>
<td>Protein tyrosine kinase</td>
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<tr>
<td>Kindlin 2</td>
<td>Integrin-associated cytoplasmic adaptor</td>
<td>(Dowling et al., 2008)</td>
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<td>M-cadherin</td>
<td>Cadherin</td>
<td>(Zeschner et al., 1995; Charrasse et al., 2006; Charrasse et al., 2007)</td>
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<td>N-cadherin</td>
<td>Cadherin</td>
<td>(Abramovic and Gee, 2007)</td>
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<td>Phosphatidylycerine</td>
<td>Cell surface phospholipid</td>
<td></td>
<td>Metalloprotease</td>
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<td>(van den Eijnde et al., 2001)</td>
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<tr>
<td>Adam12</td>
<td>MARVEL domain protein</td>
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<td></td>
<td>(Lafuste et al., 2005)</td>
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<tr>
<td>Sing (CG13011)</td>
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<td>(Estrada et al., 2007)</td>
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founder cells often undergo only a single cell division subsequent to fusion (Tixier et al., 2010). Some FCMs also exhibit very limited cell division prior to fusion (Beckett and Baylies, 2007).

Muscle development and regeneration in rodents
Mice and rats are used extensively to study muscle formation in vertebrates with similar processes occurring in both species. In contrast to flies, specific subsets of myoblasts that seed the formation of myofibers have not been identified. Furthermore, myoblast fusion during rodent embryogenesis occurs in two stages known as primary and secondary myogenesis (Kelly and Zacks, 1969; Ontell and Kozeka, 1984; Ross et al., 1987), which results in individual muscles that are composed of multiple myofibers.

Embryonic muscle development
During primary myogenesis, embryonic myoblasts fuse to form primary myofibers (see Glossary, Box 1). Several days later, fetal myoblasts (see Glossary, Box 1), which are cells that develop from distinct, but related progenitors (Hutcheson et al., 2009) and differ in proliferation and fusion capacities (Biressi et al., 2007a; Biressi et al., 2007b), become prevalent and fuse with each other to form secondary myofibers (see Glossary, Box 1) in close apposition to primary myofibers (Duxson et al., 1989). As secondary myogenesis progresses, fetal myoblasts preferentially fuse with the ends of both primary and secondary myofibers (Zhang and McLennan, 1995). During secondary myogenesis, myofibers begin to differ in their expression of myosin heavy chain isoforms that distinguish between future fast- and slow-contracting muscles of the adult (Lyons et al., 1990). Late in development, a third type of muscle precursor cell, the adult satellite cell, is present between the basal lamina and the myofiber plasma membrane (Lepper and Fan, 2010). Myoblasts derived from satellite cells are responsible for adult muscle growth and regeneration (Lepper et al., 2011; Murphy et al., 2011; Sambasivan et al., 2011). During muscle growth in the postnatal period, large numbers of myoblasts fuse to the ends of myofibers, resulting in increased myofiber length and girth (Kitityakara and Angevine, 1963; Williams and Goldspink, 1971). Adult myofibers contain one nucleus approximately every 20 μm, depending on age and muscle type (Bruusgaard et al., 2003; Bruusgaard et al., 2006); thus, a 5 mm muscle fiber contains several hundred nuclei.

Regeneration in adult muscle
Myoblast fusion during regeneration in adult muscle is the best-studied model in vertebrates (Fig. 2). Degeneration can be induced by local physical or chemical trauma, resulting in rapid focal necrosis of myofibers with new membrane formation demarcating the ends of the surviving stumps and probably limiting the extent of myofiber necrosis (Carpenter and Karpati, 1989; Papadimitriou et al., 1990). A localized cellular immune response ensues (Snow, Table 2. Proteins that function in signal transduction, actin remodeling and membrane fusion

<table>
<thead>
<tr>
<th>Role</th>
<th>Drosophila</th>
<th>Zebrafish</th>
<th>Mouse</th>
<th>Protein class</th>
<th>References</th>
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<tr>
<td>Membrane fusion: cell signalling</td>
<td>Arf51F/Arf6 (CG8156)</td>
<td>Arf6</td>
<td>GTPase</td>
<td>(Chen et al., 2003)</td>
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<tr>
<td></td>
<td>Blow (CG1363)</td>
<td>Cdc42</td>
<td>GTPase</td>
<td>(Dobérstein et al., 1997; Schröter et al., 2004; Jin et al., 2011)</td>
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<td></td>
<td>Crk, CrKL</td>
<td>Elmo (CG5336)</td>
<td>SH2 SH3 adaptor protein Bipartite GEF for Rac (with MBC)</td>
<td>(Schäfer et al., 2007; Vasyutina et al., 2009)</td>
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<td></td>
<td>Mbc (CG10379)</td>
<td>Dock1</td>
<td>Dock1</td>
<td>(Geisbrecht et al., 2008)</td>
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<td></td>
<td>Rac1 (CG2248), Rac2 (CG8556)</td>
<td>Dock5</td>
<td>Dock5</td>
<td>(Geisbrecht et al., 2008)</td>
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<td></td>
<td>Schizo/Loner (CG32434)</td>
<td>Brag2</td>
<td>Guanine nucleotide exchange factor</td>
<td>(Moore et al., 2007; Rushton et al., 1995; Erickson et al., 1997; Laurin et al., 2008; Pajcini et al., 2008)</td>
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<td></td>
<td>Scar (CG4636)</td>
<td>Graf1</td>
<td>Guanine nucleotide exchange factor</td>
<td>(Moore et al., 2007; Schäfer et al., 2007)</td>
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<td></td>
<td>Vrp1/Wip/Sltr (CG13503)</td>
<td>Wip</td>
<td>Wip/WASP complex</td>
<td>(O’Brien et al., 2000; Dalkilic et al., 2006; Charrasse et al., 2007)</td>
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<td></td>
<td>WASp (CG1520)</td>
<td>NWASP</td>
<td>Actin nucleation promoting factor for Arp2/3 complex</td>
<td>(Chen et al., 2003; O’Brien et al., 2000; Dalkilic et al., 2006; Charrasse et al., 2007)</td>
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<td></td>
<td>Filamin C</td>
<td></td>
<td>Muscle-specific actin crosslinking protein</td>
<td>(Dalkilic et al., 2006)</td>
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<tr>
<td>Membrane fusion: actin dynamics</td>
<td>ArpC1 (CG8978)</td>
<td>Crk</td>
<td>Arp2/3 complex</td>
<td>(Berger et al., 2008)</td>
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<td></td>
<td>Arp66B/Arp3 (CG7558)</td>
<td>Kette (CG5837)</td>
<td>Scar/Wave complex</td>
<td>(Charrasse et al., 2007)</td>
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<td></td>
<td>Non-muscle myosin 2A</td>
<td>Scar (CG4636)</td>
<td>Actin nucleation promoting factor for Arp2/3 complex Structural protein</td>
<td>(Berger et al., 2008; Gildor et al., 2009)</td>
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<td></td>
<td>Wrp1/Wip/Slr</td>
<td>Vrp1/Wip/Sltr (CG13503)</td>
<td>Wip/WASP complex</td>
<td>(Duan et al., 2001)</td>
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<td>WASp (CG1520)</td>
<td>NWASP</td>
<td>Actin nucleation promoting factor for Arp2/3 complex</td>
<td>(Kim et al., 2007; Massarwa et al., 2007)</td>
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<td></td>
<td>Filamin C</td>
<td></td>
<td>Muscle-specific actin crosslinking protein</td>
<td>(Kim et al., 2007; Schäfer et al., 2007)</td>
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1977; McLennan, 1996; Pimorady-Esfahani et al., 1997) that is crucial for normal regeneration (Grounds, 1987; Arnold et al., 2007). In response to factors found in the injured area, satellite cells begin to proliferate, differentiate and fuse with one another or with existing myofibers (Robertson et al., 1990; Robertson et al., 1993b). As in embryogenesis, no evidence exists for specialized subsets of myoblasts that seed the formation of regenerative myofibers. Multiple small myotubes form within the basal lamina sheath of the original myofiber (Schmalbruch, 1976; Snow, 1977), and fuse with one another (Robertson et al., 1990) and to the stumps of the parent myofiber (Papadimitriou et al., 1990; Robertson et al., 1993b) to regenerate the parent myofiber. Current technologies do not exist for directly visualizing myoblast fusion in vivo in mice, thus, morphological and biochemical measurements are used as an indirect readout.

Cell culture models of myoblast fusion in mice

Much of the work pertaining to myoblast fusion in mammals derives from in vitro studies using either primary muscle cells isolated from mouse, rat or human muscles, or established mouse muscle cell lines, such as C2C12 (Fig. 3). Cell culture studies demonstrate that multinucleated myotubes form in a series of ordered steps. Initially, myoblasts differentiate into elongated myocytes (see Glossary, Box 1) that migrate, adhere and fuse to one another to form small nascent myotubes (see Glossary, Box 1) that contain few myonuclei. Nascent myotubes further fuse with additional myocytes or with other myotubes to generate mature myotubes (see Glossary, Box 1) that contain many myonuclei. Such in vitro experiments are often used to complement in vivo studies of adult regenerative myogenesis in mice to examine more fully the mechanisms that regulate myoblast fusion.

Finding a fusion partner: recognition, migration and adhesion

This section examines our current understanding of how fusing myoblasts find their fusion partner(s). Although genetic loss-of-function studies in the Drosophila embryo have revealed genes essential for the early cell recognition and adhesion steps, cultured cell lines and primary mammalian cells have also allowed us to identify molecules that specifically direct migration of myoblasts to their targets. These molecules are summarized in Table 1 and discussed below in detail.

Myoblast migration, recognition and adhesion in Drosophila

In the Drosophila embryo, the FCMs and founder cells/myotubes arise in close proximity to each other, and are often adjacent cells that are in direct contact (Fig. 4). Moreover, the myotube will probably come in direct contact with additional FCMs as fusion proceeds and as the myotube becomes larger. Given these small distances, and in the absence of direct visualization of cell movements by live imaging in the Drosophila system, it is often difficult to distinguish genes involved in myoblast migration from those required for adhesion. However, indirect support for FCM migration comes from studies showing that these cells adopt a teardrop shape (Fig. 4A) with membrane processes oriented towards developing muscle fibers, consistent with their migration towards founder cells in fixed (Doberstein et al., 1997) and live (Richardson et al., 2007) embryos. Studies by Baylies et al. also revealed dramatic redistribution of FCMs to more external positions near founder cells in fixed embryos of different developmental stages (Beckett and Baylies, 2007). More direct evidence suggests that the FCMs are capable of migrating, and are dependent on transmembrane proteins of the immunoglobulin superfamily (IgSF) that include Kin-of-IrreC/Dumfounded (Kirre/~~Du~~f~~) (Ruiz-Gomez et al., 2000), Roughgest/In~~reg~~ular-optic~~chiasma-C (Rst/~~Irre-C~~) (Strunkelnberg et al., 2001) and Sticks-and-stones (Sns) (Bour et al., 2000) for this behavior (Table 2, Fig. 5). Kirre is expressed specifically in the founder cells (Ruiz-Gomez et al., 2000), where it functions redundantly with Rst (Strunkelnberg et al., 2001) to recognize and associate with FCMs. Sns is required on the surface of the FCMs (Bour et al., 2000) for their ability to recognize founder cells and myotubes. The FCMs can clearly migrate to sites of ectopically expressed Kirre or Rst (Ruiz-Gomez et al., 2000; Strunkelnberg et al., 2001), and Sns is essential for this behavior (Kocherlakota et al., 2008). These data suggest that Kirre and Rst can function as Sns-dependent attractants for the FCMs, and that signaling cascades downstream of Sns direct migration. This process could involve filopodia that extend from the FCM to probe the environment and search for founder cells, either randomly finding membrane-associated Kirre on the surface of a founder cell or moving along a gradient of secreted Kirre. The latter possibility is indirectly supported by the presence of a truncated form of Kirre protein in the media of Drosophila S2 cells transfected with full-length Kirre (Chen and Olson, 2001).
However, the presence of secreted or cleaved Kirre has not been established in vivo, and the mechanism by which FCMs find non-adjacent founder cells or myotubes is not clear.

Candidate proteins with roles in migration of other cell types, that are essential for myoblast fusion, include the small GTPase Ras-related C3 botulinum toxin substrate 1 (Rac1) and its non-conventional guanine nucleotide exchange factor complex Myoblast City and Engelmann and Cell Motility Protein (Mbc/Elmo) (Erickson et al., 1997; Ducheck et al., 2001; Geisbrecht et al., 2008). The Suppressor of cAMP Receptor/WASp family Verprolin-homologous (Scar/Wave) pathway, which is activated by Rac1 and a component of the Scar complex, but the FCMs of kette mutant embryos are still able to migrate to sites of ectopic Kirre expression (Rochlin et al., 2010) and electron-dense plaques characteristic of sites of fusion are present in these mutants (Schröter et al., 2004). Rac1 has also been implicated in stabilization of cell-cell contacts in vertebrates (Yamazaki et al., 2007), providing an alternative explanation for the loosely associated morphology of the FCMs in rac1 mutant embryos. Thus, the precise pathways regulating migration of myoblasts in flies remain to be clarified.

The Kirre, Rst and Sns IgSF proteins mentioned above, with Kirre and Rst serving redundant functions, control cell-cell adhesion between founder cells and FCMs. Hibris (Hbs), a paralog of Sns (Artero et al., 2001; Dworak et al., 2001) can direct a small amount of fusion in the absence of Sns, but is quite inefficient in comparison (Shelton et al., 2009). Whereas flies lacking Hbs are viable, muscle fibers are absent in sns mutant embryos and these embryos do not hatch as a result (Bour et al., 2000). Non-muscle Drosophila S2 cells have been valuable in dissecting the specificity of adhesive interactions between these proteins (Dworak et al., 2001; Galletta et al., 2004). Sns can interact biochemically with Rst or Kirre, in trans to mediate cell adhesion (Galletta et al., 2004). Consistent with the need for FCMs to recognize founder cells and myotubes, but not other FCMs, neither Sns nor Hbs mediates homotypic interaction of S2 cells in trans. Although Kirre and Rst can interact, cells expressing these proteins have a strong preference for those expressing Sns, possibly accounting for the apparent absence of founder cell/founder cell interactions in vivo.

No other cell-adhesion molecules have been implicated in the adhesion process. Studies examining the loss of integrins maternally and zygotically have revealed no defects in myoblast fusion (Zusman et al., 1993; Roote and Zusman, 1995; Prokop et al., 1998), and no studies have yet revealed a role for Ca2+-dependent adhesion molecules. It is particularly notable that muscles are normal in embryos lacking N-cadherin and N2-cadherin (Iwai et al., 1997; Prakash et al., 2005), the closest proteins to mammalian M-cadherin, for which a role in mammalian myoblast fusion is well established (see below). However, it remains to be determined whether maternally provided N-cadherin masks its role in embryonic myoblast fusion, or whether another cadherin functions redundantly with N-cadherin.

Myoblast migration, recognition and adhesion in mice

Most of our knowledge about migration of mouse myoblasts arises from in vitro studies, owing to the difficulty of studying this process in vivo. The basic processes that regulate myoblast migration can be carefully controlled and dissected in vitro, and probably reflect similar mechanisms occurring in vivo. Time-lapse analyses in vitro indicate that freshly isolated satellite cells migrate extensively on their associated myofibers, consistent with their expression of receptors for chemoregulatory molecules (Siegel et al., 2009). Furthermore, myoblasts cultured on various artificial substrates are also motile and migrate in response to a variety of factors, including chemokines (Corti et al., 2001; Odemis et al., 2007; Griffin et al., 2010), growth factors (Robertson et al., 1993a; Bischoff, 1997; Lee et al., 1999; Corti et al., 2001; Villena and Brandan, 2004) and other molecules (Nedachi et al., 2009; Tokura et al., 2011). Except for Cxcr4 [chemokine (C-X-C motif) receptor 4], the receptor for the chemokine Sdf1 (stromal-derived factor 1; Cxcl12 – Mouse Genome Informatics) (Griffin et al., 2010), the effect of depleting these molecules on cell fusion has not been tested. Cell motility changes greatly during differentiation, with myocytes exhibiting less motility than myoblasts (Powell, 1973; Griffin et al., 2010) and becoming unresponsive to several potential

### Box 2. Experimental systems for studying myoblast fusion: strengths and limitations

**Drosophila**

**Strengths**
- Genetically tractable system with a rapid generation time.
- Myogenesis in the embryo occurs within 12 hours.
- Many processes are controlled by single-copy genes.
- Amenable to live imaging with tagged proteins in wild-type and mutant embryos.
- Limitations to the genetic analysis of the adult musculature are being overcome by RNAi technology.
- Amenable to ultrastructural analysis of wild-type and mutant embryos.

**Limitations**
- Limited availability of muscle-derived cultured cell lines.
- Extent of gene redundancy unknown.
- Primary cultures highly enriched for labeled myoblasts can be obtained, but are not pure and are available only in limited amounts.
- Muscle development and structure are somewhat different from vertebrates, with muscles represented by single myotubes patterned by founder cells.

**Mouse**

**Strengths**
- Long lifespan.
- Can study fusion in various contexts, such as regeneration, aging, exercise and disease.
- Muscle development and structure more closely resemble human.
- Presence of slow and fast myofibers.
- Cell culture models and approaches are well developed: can systemically control both the media and matrix of the cells; can easily manipulate cellular components with drugs, siRNA or DNA constructs; can analyze myoblast fusion in the absence of other cell types.

**Limitations**
- Documented gene redundancy that appears to be more extensive than simpler genetic model organisms.
- Limited ability to perform live cell imaging in vivo.
- Length of time to generate mutants.
- Limited number of offspring.

DEVELOPMENT
inducers of myoblast migration, such as hepatocyte growth factor (Hgf) and platelet-derived growth factor (Pdgf) (Griffin et al., 2010). This decrease in motility would increase the probability of cell-cell contact, thereby triggering differentiation (Krauss et al., 2005) and allowing myocytes to fuse with one another and with nascent myotubes (Nowak et al., 2009). As myotubes begin to form, muscle cells preferentially move into some fields and out of others (Chazaud et al., 1998), suggesting that migration is directed in response to chemotactic factors secreted by muscle cells (Bondesen et al., 2007; Griffin et al., 2010).

In vitro approaches have revealed details of how some regulatory factors influence muscle cell migration. Some factors modulate the velocity or direction of cell migration, whereas others regulate the clearance of the extracellular matrix at the leading edge of migrating cells, thereby enhancing cell motility (Horsley et al., 2003; Jansen and Pavlath, 2006; Lafreniere et al., 2006; Griffin et al., 2009). Myoblast fusion in vitro is enhanced by both positive regulators [e.g. CD164 (Bae et al., 2008), interleukin 4 (Il4) (Horsley et al., 2003; Lafreniere et al., 2006), mannose receptor (MR) (Jansen and Pavlath, 2006) and mouse odorant receptor 23 (Mor23; Olfr16 – Mouse Genome Informatics) (Griffin et al., 2009)] and negative regulators [e.g. prostacyclin (Bondesen et al., 2007)] of cell migration. Whereas positive migratory factors promote cell fusion by increasing the probability of myoblasts being close to one another, negative migratory factors may enhance cell fusion by acting as a ‘brake’ on migrating cells to facilitate cell-cell contact and adhesion (Bondesen et al., 2007). Thus, the net balance between these two classes of migratory regulators would be crucial for modulating myoblast fusion.

Myoblast migration in mice in vivo has mostly been studied during the course of development (Dietrich, 1999; Birchmeier and Brohmann, 2000; Christ and Brand-Saberi, 2002) where myogenic cells often migrate long distances to form muscles in the limb and tongue. Hepatocyte growth factor and the Cxcr4/Sdf1 axis are crucial regulators of myoblast migration in mouse embryos (Brand-Saberi et al., 1996; Bandow et al., 2004; Vasyutina et al., 2005). Until recently, the ability of myoblasts to migrate in adult muscle was inferred from studies using transplantation of either genetically marked myoblasts (Watt et al., 1987; Phillips et al., 1990; Watt et al., 1993; Jockusch and Voigt, 2003) or myofibers with their associated satellite cells (Hall et al., 2010), and histological examination of these genetic markers in fixed tissue sections. However, in vivo imaging of satellite cells in regenerating muscles provided the first direct evidence for myoblast migration in adult muscles (Ishido and Kasuga, 2011).

Cell-cell adhesion molecules and their role in muscle cell fusion in mice have been mostly studied using cell culture models with some experiments in regenerating adult muscles. Many more adhesion molecules have been identified to date in mice compared with flies. Nephrin, the vertebrate homolog of Sns, is the only adhesion molecule involved in fusion in both model systems (Sohn et al., 2009). Why adhesion in mouse muscle cells is controlled by a diversity of molecules is unknown, as is the interplay among
these different molecules in preparing the cells for fusion. Such
diversity may allow for activation of specific intracellular signaling
pathways, such as Rac1 (Charrasse et al., 2007) or tyrosine kinases
(Li et al., 2004), which are activated by cell-surface engagement of
specific adhesion molecules and may be required for different
molecular events required for cell fusion.

During differentiation in vitro, muscle cells extend lamellipodia
and filopodia that contact neighboring muscle cells (Yoon et al.,
2007; Mukai and Hashimoto, 2008; Mukai et al., 2009; Nowak et
al., 2009; Studler et al., 2010) and are sites for localized adhesion
molecules (Abramovici and Gee, 2007; Mukai et al., 2009) and
signaling molecules (Abramovici and Gee, 2007; Mukai and
Hashimoto, 2008). These filopodia are reminiscent of axon growth
cones and may be necessary for recognition of other muscle cells
that are competent for fusion. In addition, filopodia may act as a
zipper mechanism by which two cells are pulled in close contact to
one another for eventual fusion (Abramovici and Gee, 2007).

Cell-contact sites are characterized by a number of molecular
changes, both extracellular and intracellular, that facilitate
recognition and adhesion. For example, molecules such as muscle-
cadherin (M-cadherin), integrins and a disintegrin and
metalloprotease 12 (Adam12) are commonly found localized at the
contact sites in both contacting muscle cells (Cifuentes-Diaz et al.,
1995; Schwander et al., 2003; Lafuste et al., 2005; Brzoska et al.,
2006). M-cadherin function is required for myotube formation in
vitro (Zeschmieg, et al., 1995; Charrasse et al., 2006) but not in vivo
(Hollnagel et al., 2002), suggesting that other cadherins or cell
adhesion molecules may functionally compensate for the lack of M-
cadherin. Eliminating the function of β1 integrin, α3 integrin and
α9 integrin decreases myoblast fusion in vitro (Schwander et al.,
2003; Lafuste et al., 2005; Brzoska et al., 2006) and in vivo
(Schwander et al., 2003). Adam12 is a transmembrane protein that
contains an integrin-binding site and can bind α9β1 integrin in
muscle cells (Lafuste et al., 2005). Antisense oligonucleotides to
Adam12 preferentially inhibit myoblast fusion with myotubes
(Lafuste et al., 2005). Adhesion molecules at these cellular contact
sites can be temporally regulated, as evidenced by N-cadherin
localization at nascent intercellular contacts but not at established
cell contacts (Abramovici and Gee, 2007). Cell-contact sites are
further characterized by changes in the cell membranes. For
example, phosphatidylserine (PS) is transiently exposed at these
sites (van den Eijnde et al., 2001) and required for myotube
formation, as inhibition of PS by annexin V inhibits fusion. Multiple
roles for this transient exposure of PS have been proposed,
including cell recognition and cell signaling. Additionally, lipid rafts
containing cholesterol transiently accumulate at cell contact sites
and are required for the accumulation of adhesion molecules (Mukai
et al., 2009). Cholesterol is proposed to help maintain the proper
rigidity of the lipid bilayers necessary for adhesion between the two
myogenic cells. Finally, molecules that associate with the
intracellular domains of specific cell-adhesion molecules, such as
β-catenin (Vasyutina et al., 2009) and kindling 2 (Dowling et al.,
2008), are also localized at cell contact sites. These intracellular
molecules probably activate signal transduction pathways that
ultimately lead to membrane fusion (Charrasse et al., 2007; Quach
et al., 2009; Vasyutina et al., 2009). Molecules that regulate
migration and adhesion in mouse muscle cells and for which a
demonstrated role in contributing to myoblast fusion has been
shown are summarized in Fig. 6 and Tables 1 and 2.

Membrane fusion and formation of the syncytium
Although the processes of migration, recognition and adhesion are
the fundamental first steps in fusion, and perturbations in these
processes lead to obvious defects in formation of myofibers, they
are somewhat removed from the fusion process itself. Below, we discuss the genes, intracellular pathways and membrane events more intimately associated with the mechanics of myoblast fusion.

**The process of myoblast fusion in the *Drosophila* embryo**

A combination of approaches, including genetics, confocal microscopy, transmission electron microscopy (TEM) and biochemistry, has recently uncovered fundamental aspects of the fusion process itself and the molecules controlling this process. A hypothetical model describing these observations is provided in Fig. 4, with the known molecules shown in Fig. 5. Although several discrepancies remain in the morphological details, possibly as a consequence of the specific mutant alleles examined or the approach used, rapid progress is being made in deciphering the mechanism of myoblast fusion in this system.

**From the membrane to the cytoplasm**

Largely on the basis of genetic loss-of-function phenotypes associated with defects in myoblast fusion, many molecules have been identified that function downstream of the IgSF cell-surface receptors. The Sns cytodomain is essential for function, and redundant binding motifs probably link it to adaptor proteins that include the SH2-SH3 domain-containing protein CT10 regulator of kinase (Crk) (Kim et al., 2007). Crk, in turn, can recruit actin-polymerizing machinery to points of cell-cell contact [(Kim et al., 2007) see below]. The cytoplasmic domain of Kirre recruits both Rolling pebbles/Antisocial (Rol/Ans) (Chen and Olson, 2001; Menon and Chia, 2001; Rau et al., 2001), which contains numerous motifs for protein-protein interaction, and Schizo/Loner, a guanine nucleotide exchange factor (GEF) for the Arf6 GTPase (Arf51F – FlyBase) (Chen et al., 2003; Onel et al., 2004) to points of cell contact (Chen and Olson, 2001; Chen et al., 2003; Bulchand et al., 2010). Rol functions in a positive-feedback loop to stabilize Kirre at the myotube membrane, thereby ensuring its continued availability for interaction with FCMs (Menon et al., 2005). The finding that myoblasts separate in older embryos lacking *rols* is consistent with their inability to maintain adhesion (Rau et al., 2001). Though Rols can interact with Mbc biochemically, prompting the model that Rol functions to recruit Mbc to Kirre at sites of fusion (Chen and Olson, 2001), the relevance of this interaction is not clear, as Mbc does not appear to be required in the founder cells for fusion (Haralalka et al., 2001), the relevance of this interaction is not clear, as Mbc does not appear to be required in the founder cells for fusion (Haralalka et al., 2001). The relevance of this interaction is not clear, as Mbc does not appear to be required in the founder cells for fusion (Haralalka et al., 2001). Rol/Loner/Tops might facilitate Arf-mediated recruitment of Rac1 to the membrane (Chen et al., 2003), probably resulting in activation of the Scar pathway by Rac1 (see Box 3). A Schizo-activated Arf GTPase might also, like Rac1, directly activate the Scar complex, as recently shown in mammalian cells (Koronakis et al., 2011). However, the cytoplasmic domain of Kirre is not absolutely essential for fusion (Bulchand et al., 2010), and so the mechanism by which Schizo/Loner is recruited to the membrane may be more complex. Moreover, Schizo is also present in the FCMs (Richardson et al., 2007), and may mediate the same processes in these cells independently of Kirre. Finally, as Arf GTPases are also associated with vesicle trafficking and membrane recycling (D’Souza-Schorey and Chavrier, 2006), the role of Schizo and its target Arf protein in myoblast fusion might also extend to other fusion-associated processes, as discussed below.

More than a decade ago, transmission electron microscopy (TEM) using conventional chemical fixation revealed distinct morphological features at sites of cell-cell contact, as depicted in Fig. 4. Electron-dense vesicles ∼40 nm in diameter are present at sites of myoblast contact in embryos. They appear to bud from the Golgi apparatus and become coated with actin (Kim et al., 2007). Though a causative role...
has not been established, these vesicles may give rise to membrane plaques along the apposed plasma membranes (Doberstein et al., 1997). Such vesicles and/or electron-dense membrane plaques have been observed in several subsequent studies and are perturbed in a variety of mutant backgrounds (Doberstein et al., 1997; Rau et al., 2001; Schröter et al., 2004; Estrada et al., 2007; Kim et al., 2007; Massarwa et al., 2007; Berger et al., 2008; Gildor et al., 2009) (S.A., unpublished). They are also reminiscent of the vesicles observed in cultured myoblasts (Shimada, 1971; Rash and Fambrough, 1973; Engel et al., 1986). Notably, the vesicles accumulate in embryos mutant for the MARVEL domain-containing protein Singles-bar (Sing), which is essential for myoblast fusion, suggesting that it may play a role in plaque formation (Estrada et al., 2007). The vesicles are thought to be recruited in response to activation of cell-surface receptors and to deliver fusion-associated molecules to points of cell contact, perhaps through targeted exocytosis mediated by the actin nucleation-promoting factor (NPF) Wiskott-Aldrich syndrome protein (WASp) (Kim et al., 2007). However, the exact role and composition of these vesicles remain unclear.

**F-actin foci, F-actin nucleating complexes and fusion pores**

A large body of work has established that actin polymerization plays a fundamental role in myoblast fusion in the fly embryo. Dynamic F-actin foci are formed and dissolve coincident with myoblast fusion in live embryos (Richardson et al., 2007), and actin foci are found at points of cell-cell contact in fixed tissue samples (Kesper et al., 2007; Richardson et al., 2007; Kim et al., 2007). Recent studies have shown that, in founder cells, F-actin forms a thin sheath underlying points of cell contact (Sens et al., 2010). By contrast, within the limits of confocal microscopy, the dense actin focus appears to reside exclusively in the FCMs (Sens et al., 2010; Haralalka et al., 2011), as depicted in Figs 4, 5 and 7. These F-actin foci are formed by the action of the NPFs Scar and WASp on the Arp2/3 complex as outlined below.

The Scar and Kette subunits of the pentameric Scar complex (see Box 3) mediate actin polymerization via activation of Arp2/3 and are required for myoblast fusion (Schröter et al., 2004; Richardson et al., 2007; Berger et al., 2008; Gildor et al., 2009). Scar is required in both cell types, as expression in neither the founder cells nor the FCMs fully rescues the muscle defects of scar mutant embryos (Sens et al., 2010). By contrast, within the limits of confocal microscopy, the dense actin focus appears to reside exclusively in the FCMs (Sens et al., 2010; Haralalka et al., 2011), as depicted in Figs 4, 5 and 7. These F-actin foci are formed by the action of the NPFs Scar and WASp on the Arp2/3 complex as outlined below.

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Thus, Kette may contribute in some way to organization of actin the foci and/or their breakdown prior to fusion. Interestingly, in this regard, mechanistic studies in vitro have shown that the actin cytoskeleton is an impediment to expansion of a fusion pore, consistent with this suggestion (Chen et al., 2008). Notably, the fusion plaques observed by transmission electron microscopy (TEM) (see below) are also larger in the absence of kette (Schröter et al., 2004).

The WASp NPF (Schäfer et al., 2007) and associated Verprolin1/Drosophila-WASp-interacting protein/Solitary (Vrp1/D-Wip/Slr) protein (Paunola et al., 2002; Kim et al., 2007; Massarwa et al., 2007), also promote polymerization of F-actin at sites of cell-cell contact. In contrast to Scar, the Vrp1/WASp complex functions with the strong sltr allele of vrp1 (Gildor et al., 2009). Thus, other actin polymerizing proteins may be present, or some of these mutant alleles require more detailed characterization. Despite the convergence of both WASp and Scar on Arp2/3, embryos zygotically null for Arp3 exhibit some fusion (Berger et al., 2008), possibly as a consequence of maternally provided protein that complicates this analysis.

Myoblast fusion is assumed to proceed from one or more small membrane pores that expand to engulf the fusing FCM. As assayed morphologically by conventional TEM or monitored by cytoplasmic transfer of GFP, myoblast membranes are intact and pores do not form in embryos lacking kette (Schröter et al., 2004; Gildor et al., 2009). Thus, the Scar pathway is essential for pore formation. By contrast, studies examining the role of Vrp1 and WASp in pore formation have resulted in somewhat disparate results. Using GFP to monitor the transfer of cytoplasm and TEM with conventional chemical fixation, studies have reported pore formation in embryos mutant for both maternal and zygotic scar, a significant reduction in the size of the actin foci (Sens et al., 2010). These data suggest functional redundancy between the NPFs. However, foci remain in embryos mutant for other combinations of NPF-associated proteins, including a strong allele of kette and WASp (Gildor et al., 2009). Thus, other actin polymerizing proteins may be present, or some of these mutant alleles require more detailed characterization.

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As evidenced by identification of the FCM-specific Vrp1/WASP complex (Kim et al., 2007; Massarwa et al., 2007) and by FCM-exclusive expression of Blow (Jin et al., 2011), studies have established the molecular asymmetry between the founder cells and FCMs. Recent studies have also shown that, despite ubiquitous expression (Erickson et al., 1997), the Rac1 GEF Mbc and active Rac1 are asymmetrically distributed in the FCMs at points of cell-cell contact (Haralalka et al., 2011). As mentioned earlier, Mbc is one subunit of a highly conserved bipartite guanine nucleotide exchange factor for the small GTPase Rac1 (Erickson et al., 1997; Balagopalan et al., 2006). Like its partner Cell death abnormality 12/Engulfment and cell motility protein (Ced12/Elmo) (Geisbrecht et al., 2008) and target Rac1 (Luo et al., 1994; Hakeda-Suzuki et al., 2002), Mbc is essential for myoblast fusion. It is required in the FCMs, but is not required in the founder cells for their fusion with these FCMs (Haralalka et al., 2011). Activated Rac1 is sufficient in the FCMs to rescue fusion in mbc mutant embryos (Haralalka et al., 2011), suggesting that the sole function of mbc is to activate Rac1 in these cells. As in other systems (Kobayashi et al., 1998; Lebensohn and Kirschner, 2009), the function of Rac1 may be to mediate the local activation of Scar prior to myoblast fusion (Fig. 5). Notably, in addition to its role in formation of the actin focus discussed earlier, Scar is also responsible for the thin sheath of actin present in the founder cells (Sens et al., 2010).

Given the high levels of Mbc and active Rac1 in the FCMs at sites of cell-cell contact, it remains to be determined how Scar is activated in the founder cells to generate this sheath (Sens et al., 2010), and whether undetectable levels of Rac1 are sufficient. Moreover, as Mbc is not required in the founder cells, a GEF other than Mbc must accomplish this goal (Haralalka et al., 2011). Interestingly, studies in mammalian cells have shown that Arf GTPases can, like Rac GTPases, also directly activate the Scar complex (Koronakis et al., 2011). Thus, perhaps the Arf GEF Schizo, described above, serves this purpose in the founder cells.

Analysis with high-pressure freezing TEM has revealed that sites of cell contact are also highly asymmetric morphologically, with finger-like projections from the FCM invading the syncytium (Sens et al., 2010). It seems likely that the dynamic F-actin foci seen in live embryos (Richardson et al., 2007; Sens et al., 2010; Haralalka et al., 2011) and in the actin core bounded by Sns and Kirre (Kesper et al., 2007; Sens et al., 2010; Haralalka et al., 2011) are associated with these invasive finger-like projections, which seem to be devoid of cytoplasmic content and filled with filaments (Sens et al., 2010). These protrusive structures precede the formation of fusion pores (Sens et al., 2010) and, like the WASp-dependent actin-rich podosomes defined in macrophages and osteoclasts (Murphy and Courtneidge, 2011), depend on the FCM-specific WASp-associated protein Vrp1 (Sens et al., 2010). Though membrane projections are present in embryos mutant for vrp1 or WASp (Sens et al., 2010), consistent with the presence of F-actin in these mutant embryos (Gildor et al., 2009), the finger-like projections appear to collapse, suggesting that WASp-dependent F-actin contributes to their rigidity but perhaps not their actual formation (Sens et al., 2010). Interestingly, studies in other systems have suggested that the protrusive activity associated with podosomes and invadopodia involves the coupling of actin polymerization with membrane deformation (Gimona et al., 2008; Murphy and Courtneidge, 2011). Moreover, WASp-dependent actin polymerization can be activated by proteins that cause membrane curvature, a feature commonly associated with destabilization of the membrane and membrane fusion (Albiges-Rizo et al., 2009).
Thus, the mechanism responsible for initial formation of these invadopodia might involve unidentified membrane bending proteins at sites of fusion.

In contrast to the collapsed fingers in embryos mutant for the WASp complex noted above (Sens et al., 2010), the fingers are present and appear to be normal with regard to depth and rate of G-actin exchange in embryos lacking the pentameric Scar complex (Jin et al., 2011). These studies, in combination with additional studies described above that examine pore formation (Schröter et al., 2004; Kim et al., 2007; Gildor et al., 2009), suggest that Vrp1/WASp drives formation of the invasive fingers prior to pore formation, and that Vrp1/WASp and Kette/Scar function redundantly or sequentially in pore formation. Sens and colleagues (Sens et al., 2010) proposed that a single pore forms at the tip of one invasive projection and expands to engulf the FCM. Although these investigators have observed macro-pores ranging from 300 nm to 1.5 μm (Sens et al., 2010), smaller micropores have not been described. Thus, it remains to be determined whether fusion is accomplished by a single 25-50 μm micropore that expands too rapidly to be observed, or whether multiple micropores converge to form this large opening. Perhaps, as seen in mutations of other fusion systems (Gattegno et al., 2007), analysis of Drosophila mutants that block complete fusion will allow this issue to be addressed.

Relevant to this issue, TEM using conventional chemical fixation originally suggested the presence of multiple pores and extensive membrane vesiculation, and implicated several molecules in pore expansion (Doberstein et al., 1997; Massarwa et al., 2007; Berger et al., 2008). However, this membrane breakdown is not observed in samples processed by high-pressure freezing (Sens et al., 2010), leaving unresolved the issue of whether multiple pores can form and which genes mediate pore expansion.

Myoblast fusion in mammals

Much of the work in this area has been geared towards determining whether the specific molecules identified in flies that have a role in actin cytoskeletal rearrangements display conserved functions in mouse muscle cells in vitro and in vivo.

Actin cytoskeletal remodeling in mouse myoblasts

Extensive cytoskeletal reorganization occurs before and after fusion in cultured mouse myoblasts (Fulton et al., 1981). Visualization of the actin cytoskeleton revealed dynamic changes in fusing mouse myoblasts in vitro, as described for flies (Swailes et al., 2006; Duan and Gallagher, 2009; Nowak et al., 2009; Stadler et al., 2010). A dense actin wall forms in one cell, paralleling the long axis of aligned myoblasts (Duan and Gallagher, 2009), and is hypothesized to provide the membrane rigidity needed for cell fusion. As fusion proceeds, gaps appear in this actin wall at sites of vesicle accumulation, vesicles pair in both cells along the membrane, and fusion pores then form. Similar vesicles are also observed in regenerating muscles (Robertson et al., 1990). Non-muscle myosin 2A is associated with the actin wall and is required for its formation, as well as for the appearance of vesicles at the membrane and for myoblast fusion (Duan and Gallagher, 2009). In the absence of proper actin cytoskeletal remodeling, F-actin structures accumulate at the site of cell-cell contact and are correlated with a decrease in myoblast fusion (Nowak et al., 2009). Together, these studies demonstrate a functional role for the actin cytoskeleton in myoblast fusion in mice.

Signaling to the actin cytoskeleton

Similar to the situation in flies, actin regulatory molecules also play key roles in fusion of mouse myoblasts. Mutations in these proteins lead to defects in myoblast fusion in vitro and in vivo (Dalkilic et al., 2006; Pajcini et al., 2008; Vasyutina et al., 2009; O’Brien et al., 2006; Charrasse et al., 2007; Laurin et al., 2008). For example, the functions of several signaling molecules involved in actin dynamics in flies are evolutionarily conserved in mice. These include four GEFs: brefeldin A-resistant Arf GEF (Brag2; Iqsec1 – Mouse Genome Informatics); Dedicator of cytokinesis 1 and dedicator of cytokinesis 5 (Dock1 and Dock5); and Trio. Loss of either Brag2 (the mammalian ortholog of Schizo/Loner) or Dock1 (the mammalian ortholog of Mbe, also referred to as Dock180), results in myotubes with few nuclei in vitro (Pajcini et al., 2008). Furthermore, Dock1/Dock5 loss impairs primary myofiber formation during mouse embryonic development (Laurin et al., 2008). In addition, Trio is essential for secondary myofiber formation during development (O’Brien et al., 2000) as well as myoblast fusion in vitro (Dalkilic et al., 2006; Charrasse et al., 2007). Other signaling molecules with conserved functions in mouse myoblast fusion are the small GTPases Arf6 (Chen et al., 2003) and Rac1 (Charrasse et al., 2007; Vasyutina et al., 2009). Conserved roles also exist for actin regulatory molecules such as Nck-associated protein 1 (Nap1; Nckap1 – Mouse Genome Informatics) (the ortholog of Kette) (Nowak et al., 2009). Knockdown of Nap1 in cultured mouse myoblasts impairs cell fusion but not cell migration as observed in kette mutants in flies (Rochlin et al., 2010). Furthermore, defects in neural Wiskott-Aldrich syndrome protein [NWASP; Wasl – Mouse
membrane as fusion proceeds. The morphology and behavior of the cells themselves, and events at points of cell-cell contact, would benefit from advances in live-imaging on a rapid time scale with greater resolution. Finally, the mechanisms by which the process is regulated, preventing excess fusion, are poorly understood.

Additional questions remain unresolved from an evolutionary perspective. Among these is why mice, and probably humans, require so many additional molecules for processes such as migration and adhesion that precede the actual membrane fusion event. Certainly mammalian muscles are more complex (integrating both fast and slow fibers) and larger (requiring the fusion of many thousands of myoblasts in a single 30 cm myofiber). The myoblasts also migrate further distances than just one or two cell diameters. The scale of this process dwarfs that occurring in the fly, possibly accounting for additional complexity as well as redundancy. In this context, the increasing commonalities between flies and mice are surprising at the very least and, at best, an integrative approach using multiple model organisms will aid in identifying the fundamental basics of the process.

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