

Muscle Building: Mechanisms of Myotube Guidance and Attachment Site Selection

Review

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The complex muscle patterns of higher organisms arise as migrating myoblasts are guided toward and connect with specific attachment sites. We review here the current understanding of myotube migration, focusing on its dynamic nature and the few molecular cues that have been identified to date. Much of this knowledge comes from studies in *Drosophila*, where powerful methods for in vivo imaging and genetic manipulation can be used to tackle this important but largely unsolved problem in developmental biology.

Introduction

The dance of the ballerina, the flight of the bumblebee, the agility of the gazelle, and the less graceful but no less remarkable movements we all make during our daily life all attest to the precision with which muscle patterns are laid down during development. Whether ballerina, bumblebee, or gazelle, every muscle in the body can be uniquely identified by its shape, size, position, and attachments. The development of these muscle patterns in turn depends on a complex series of cellular events, including cell fusion, migration, and attachment, that are just as finely orchestrated as the body movements these muscles ultimately enact.

The typical muscle cell develops through the fusion of a variable number of myoblasts to form syncytial myofibers. Each myofiber attaches itself to specific tendon cells, to establish the connection with endoskeleton (in vertebrates) or exoskeleton (in invertebrates). The morphogenesis of each muscle is thus a multistep process involving myoblast specification and fusion, myotube guidance, and targeting to specific attachment sites, as well as myotube and tendon cell differentiation. The molecular mechanisms underlying many of these steps are still poorly understood. Our ignorance of the mechanisms of myotube guidance and targeting is particularly remarkable in light of the enormous effort that has been devoted in recent years to understanding the conceptually similar but far more complex process of axon guidance and targeting. Indeed, we have a much better understanding of the mechanisms that guide motor axons to specific muscles than of the mechanisms that guide muscles to specific tendon cells. In our view, myotube guidance deserves much greater attention, both in its own right and as a general model for understanding how precise patterns of cell-cell connectivity are specified during development. With the hope of stimulating such efforts, we review here the current understanding of myotube guidance in vertebrates and *Drosophila*, and

argue that the combination of genetics and in vivo imaging makes *Drosophila* a particularly powerful model system with which to explore the cellular and molecular basis of myotube guidance and attachment site selection.

Vertebrate Trunk Myogenesis

In most vertebrates, all trunk muscles originate from the dermomyotome, an epithelial sheet formed by the paraxial mesoderm that develops from the dorsal part of the epithelial somite and overlays the sclerotome (Figure 1A). The complex patterning mechanisms instructing the regionalization of the dermomyotome have been reviewed recently (Brent and Tabin, 2002). In brief, the dermomyotome is patterned by the combined action of Wnts produced from the dorsal neural tube and ectoderm and Shh produced from the notochord and floorplate. Myf5 is the first myogenic regulatory protein expressed in the skeletal muscle lineage. In concert with Pax3, Myf5 activates a network of myogenic regulatory factors, including MyoD, myogenin, and MRF4 in the muscle precursors (reviewed in Arnold and Braun, 2000). These factors trigger myotome formation initially through the delamination of muscle precursors from the dorsomedial edge of the dermomyotome (Gros et al., 2004), which then migrate inferiorly between dermomyotome and sclerotome (Figure 1A) (reviewed in Tajbakhsh and Buckingham, 2000).

After migration into the forming myotome, the round myoblasts start to elongate along the rostrocaudal axis, growing in rostral and caudal direction at the same time until they span the somite completely (Figure 1A, box I) (Denetclaw et al., 1997; Gros et al., 2004). Slightly later, myoblasts delaminate from all somite borders and elongate either in rostral, caudal, or both directions (Denetclaw and Ordahl, 2000; Gros et al., 2004). An elongated myoblast that spans a segment is called myofiber. The early myotome consists of several layers of unfused, postmitotic, differentiated myofibers. In order to facilitate its future growth, undifferentiated, mitotically active myoblast precursors enter the myotome (Kahane et al., 2001). Myoblasts from the dorsomedial and ventrolateral edges of the dermomyotome will ultimately form the epaxial (back) and hypaxial (body wall and limb) muscles, respectively (Denetclaw et al., 1997; Gros et al., 2004; Ordahl et al., 2001).

It is still unclear how early myoblasts are guided to the myotome and then instructed to elongate along the rostrocaudal axis and attach at the somite borders. An attractive scenario is that myotubes may sense polarity cues along the rostrocaudal axis that direct their elongation, as well as a “stop” signal at segment borders to terminate the elongation process. A generic stop signal may suffice in this system, since myotubes evidently do not need to search for specific tendons. Rather, it appears that the myotubes themselves instruct tendon differentiation in neighboring parts of the sclerotome close to the somite borders. This conclusion comes from elegant work showing that postmitotic myotubes in the

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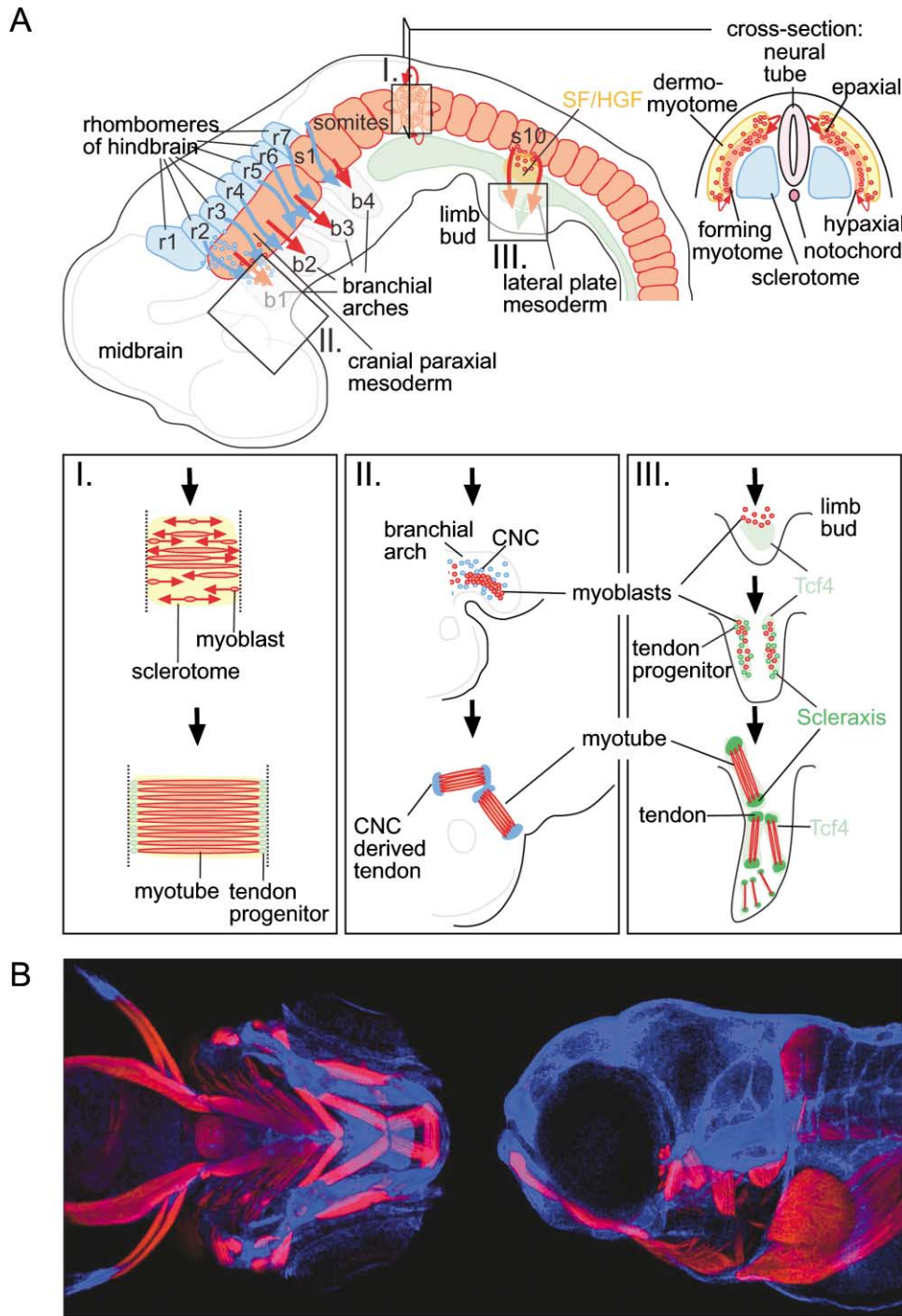


Figure 1. Developmental Origin, Migratory Behavior, and Differentiated Pattern of Vertebrate Muscles

(A) Schematic overview of myoblast migration in an avian embryo, showing the origins of back (I), craniofacial (II), and limb (III) muscles. The initial delamination of myoblasts from the somites is shown in the upper panel; the somitic organization at interlimb levels in the cross-section; the subsequent migration and targeting to tendon cells in the corresponding boxes.

(B) Fully differentiated craniofacial muscle (red) and cartilage (blue) pattern of a day 5 zebrafish larva. Left is a ventral, right a lateral view of a zebrafish head. Courtesy of Henry Roehl.

center of the myotome secrete FGF8, which activates the FGF receptor FREK in mitotically active myoblasts close to the somite borders. These in turn signal via an unknown pathway to induce tendon cell differentiation

in adjacent sclerotome cells, as evidenced by expression of markers such as *scleraxis* (Brent et al., 2003). Thus, it is ensured that eventually all myofibers are connected to tendon cells at the somite borders.

The regular segmental arrangement of the back muscles represents a rather exceptional case of muscle and tendon development. Larger body wall muscles span many segments, implying the existence of more complex targeting mechanisms than a simple stop signal at every segment border. The situation is even more complex in case of the craniofacial muscle pattern, with around 40 muscles arranged in a precise but even more elaborate pattern (Figure 1B).

Craniofacial Myogenesis

Most of the craniofacial muscles develop from the unsegmented, cranial paraxial mesoderm anterior to the first somite (Figure 1A). These undifferentiated, branchial arch muscle precursors migrate out of the mesoderm and travel in concert with migratory cranial neural crest (CNC) into regions of the branchial arches, where muscle anlagen will form (Figure 1A, box II) (Hacker and Guthrie, 1998; Noden et al., 1999). The CNC itself gives rise to most of the skeletal elements and tendons in the head, while secreting factors such as Wnts and BMP inhibitors to induce (or at least promote) head myogenesis (Schilling et al., 1996b; Tzahor et al., 2003). CNC comprises the sole type of neural crest with a developmental capacity to produce tendon or bone tissue. It thus coordinates the differentiation of muscle, tendon, and bone development.

The mechanisms that guide these complex cell migration processes remain obscure, but the molecular analysis of a large collection of zebrafish jaw mutants may ultimately provide some insights (Piotrowski et al., 1996; Schilling and Kimmel, 1997; Schilling et al., 1996a). Markers like *engrailed*, which is expressed in a small class of developing head muscles (Degenhardt et al., 2002; Hatta et al., 1990), should allow the dynamics of individual head muscle morphogenesis to be examined using the transparent fish as a model. In the mouse, MyoR and Capsilin, two related basic helix-loop-helix (bHLH) transcription factors, are transiently expressed in a subclass of migratory paraxial mesoderm during its migration into the first branchial arch. If both genes are mutated, fewer myoblasts are found in the first branchial arch, evidently because they fail to initiate their normal differentiation program and undergo programmed cell death. As a consequence, specific head muscles derived from the first branchial arch are missing (Lu et al., 2002). These examples suggest that the precursors of distinct head muscle classes are already specified during or even before the onset of the migration process. This would enable each class to respond differentially to cues on their way and thus allow for a direct selection of their individual targets. Such a strategy, however, appears not to be used by the limb muscle precursors.

Limb Myogenesis

The migration of the vertebrate limb muscle precursors has been studied more extensively (reviewed in Birchmeier and Brohmann, 2000; Buckingham et al., 2003). These precursors delaminate from the ventrolateral part of the dermomyotome and migrate along a broad region rather than following a narrow, defined pathway into the limb bud. At delamination, the myoblasts form sustained cellular protrusions, up to 20 μm long, which are then

followed by 5–10 μm -long filopodia during the migration process (Knight et al., 2000). The delamination of muscle precursors from the dermomyotome is thought to be triggered by activation of the c-Met receptor tyrosine kinase by its ligand SF/HGF (Figure 1A, box III). c-Met is expressed at all axial levels, but SF/HGF is restricted to the mesenchyme of the limb and branchial arches, thus ensuring that c-Met activation and hence myoblast delamination occurs at the appropriate axial levels only (Bladt et al., 1995). In *c-Met* and *SF/HGF* mutants, muscle precursors are correctly specified, as evidenced by the preserved expression of the marker *Lbx1*, but they fail to delaminate (Dietrich et al., 1999). Conversely, ectopic SF/HGF is sufficient to trigger delamination of muscle precursors from ectopic positions (Brand-Saberi et al., 1996; Heymann et al., 1996).

Interestingly, SF/HGF is also expressed along the migratory route of the myogenic precursors, making it seem an attractive candidate for a guidance cue for these cells. Several observations suggest, however, that this is not the case. First, high levels of ectopic SF/HGF do not divert precursors from their normal path, as would be expected for a factor providing a directional cue (Brand-Saberi et al., 1996; Heymann et al., 1996). Second, in *c-Met* and *SF/HGF* mutants, even though myoblasts cannot delaminate, they still frequently form long cellular processes that extend along the normal direction of migration (Dietrich et al., 1999). Together, these observations suggest that SF/HGF promotes delamination of myoblasts from the dermomyotome and facilitates their migration, but some other cue or cues provide directional signals to these cells. Sensitivity to these guidance cues may depend at least in part on the homeobox gene *Lbx1*, as muscle precursors delaminate in *Lbx1* mutants but often migrate to wrong positions. Migration of muscle precursors to the hindlimbs and the dorsal forelimbs is highly abnormal, resulting in a marked reduction of these muscles in *Lbx1* mutants (Brohmann et al., 2000; Gross et al., 2000; Schäfer and Braun, 1999).

When muscle precursors enter the limb bud, they split into a dorsal and ventral muscle mass adjacent to the cartilage-forming mass (Figure 1A, box III). Both domains are progressively split into three parts along the proximal-distal axis and develop into more than 40 uniquely identifiable muscles, characteristic for tetrapod limbs. Commitment to a specific muscle fate occurs late during myogenesis, as revealed by lineage tracing experiments showing that precursors migrating through the proximal limb still have the potential to contribute to any muscle (Kardon et al., 2002). This implies that signals at or near the destination control not only muscle guidance but also muscle fate. These signaling mechanisms may be revealed by mutants that lack specific muscles. A candidate for such a gene is the homeobox gene *Mox2*, which is expressed in the migratory myoblasts and is required for the formation of certain muscle groups in the limbs (Mankoo et al., 1999). However, the interpretation that *Mox2* specifies the fate or targeting of these muscles is complicated by the fact that *Pax3* and *c-Met* expression is also affected to some extent in *Mox2* mutants. This indicates that the loss of specific muscles may result at least in part from a defect in

delamination and migration, rather than in muscle specification or targeting (Mankoo et al., 1999). No other candidate for a limb muscle fate or targeting gene has yet been identified.

The limb tendon and muscle connective tissue precursors develop from the prechordal plate. It is currently not clear at which stage tendon precursors are committed to a tendon cell fate. All differentiated tendon cells are marked by the expression of *scleraxis*, a bHLH transcription factor. At an earlier stage, *scleraxis* expression marks the putative tendon cell precursors, which are located in close proximity to migratory myoblasts (Figure 1A, box III) (Schweitzer et al., 2001). Tendon development occurs in a number of discrete steps. The initial formation of tendon precursors, as evidenced by *scleraxis* expression, is independent of myoblasts, and vice versa. However, the maturation and segregation of tendon primordia into individual tendons is induced by approaching myotubes (Kardon, 1998). Conversely, if tendon precursors are removed, myotubes enter ectopic regions, indicating that unidentified signals coordinate muscle and tendon development (Kardon, 1998). Thus, muscle and tendon precursors may search together for their proper skeletal insertion sites.

A recent study indicates that the myoblasts are attracted by *Tcf4*-positive cells shortly after their migration into the limb bud. These cells outline the position of the muscles at an early stage of limb development and also surround the myofibers during later development (Figure 1A, box III). They are putative muscle connective tissue precursors; however, it is unclear if they overlap with the *scleraxis*-expressing cell population at an early stage (Kardon et al., 2003). The *Tcf4* expression pattern is independent of muscles, indicating that myoblasts may use the *Tcf4* pattern as a template that induces their differentiation into myofibers (Kardon et al., 2003). Dominant-negative *Tcf4* interferes with muscle formation at the correct positions. If *Tcf4* is activated ectopically in limb mesoderm-derived tissue, myoblasts migrate to these ectopic locations and differentiate. This indicates that activated *Tcf4* can indeed trigger the attraction of myoblasts and thus subdivide the limb into different regions of muscle differentiation.

The Case of the Missing Guidance Cues

These examples of vertebrate muscle development illustrate the various strategies that can be employed to coordinate muscle, tendon, and bone development. Back and limb muscles may represent two extremes, with craniofacial muscles adopting an intermediate strategy. Back muscles directly induce tendon cell differentiation between themselves and bone, whereas limb muscle precursors are attracted by muscle connective tissue precursors, which are surrounded by putative tendon precursors. All three cell types together then can search for the correct skeletal attachment site. The former strategy involves an early specification of muscle fate, whereas the latter requires plasticity to be maintained at least until myoblast differentiation is induced. In all of these cases, some of the molecules controlling the early steps of muscle precursor specification and migration have been identified, but the molecular mechanisms that direct the subsequent formation of specific

muscle attachments at the correct position are still completely unknown. Until the ligands and receptors that guide myoblasts to specific tendon cells have been identified, and their molecular and cellular functions defined, we cannot even begin to understand how precise muscle patterns are established. As in so many problems of developmental biology, *Drosophila* genetics may help to guide us through this impasse.

Drosophila Body Wall Myogenesis

The body wall or somatic muscles of the *Drosophila* embryo display a readily visible, highly stereotyped pattern of 30 muscles in each abdominal hemi-segment from A2 to A7 (Figure 2A). These features make it an ideal model system to search for determinants of muscle attachment site specificity. Every muscle consists of only one syncytial cell which is identified by its unique size, shape, position, and characteristic insertion sites in the epidermis (Bate, 1990). The initial steps of muscle specification in *Drosophila* may be quite different from vertebrates, with myoblasts restricted in their fate almost from birth. But the later stages of muscle guidance and attachment are remarkably similar in *Drosophila* and vertebrates, giving us hope that what we learn from the fly will also be directly applicable to vertebrates.

Drosophila body muscle development has been well studied and extensively reviewed (Baylies et al., 1998; Frasch, 1999). Pioneering work by Michael Bate and colleagues led to the formulation of the founder cell hypothesis, which states that each muscle is formed by two different cell types, one muscle founder cell and several fusion competent myoblasts (FCMs) (Bate, 1990). Here, we briefly summarize the different steps of body muscle development and the evidence which led to formulation and confirmation of the founder cell hypothesis (Figure 2B). As in vertebrates, all three classes of *Drosophila* muscles (somatic, visceral, and cardiac muscles) are of mesodermal origin. At gastrulation, *twist* (*twi*) expression is uniform in all mesodermal cells and is progressively refined after germband extension. Low *twi* levels mark the visceral and cardiac primordium, whereas high *twi* demarcates the somatic muscle primordium (Baylies and Bate, 1996). From late stage 10 to stage 11, clusters of *lethal of scute* (*l'sc*)-expressing cells originate from the high *twi*-expressing cells. From each cluster, one or two cells are singled out to express high levels of *l'sc*. These cells are the muscle progenitors; the others form FCMs (Carmena et al., 1995). At late stage 11, the progenitors undergo one further, asymmetric division into two daughter cells, one of which is a muscle founder cell, while the other can be either an additional founder cell or an adult muscle precursor, or can adopt some other undefined fate (Carmena et al., 1998; Ruiz Gomez and Bate, 1997).

According to the founder cell hypothesis, each founder cell specifies all features of the developing muscle, whereas the FCMs mainly contribute to the growth of the muscle. Thus, the fate of the founder is imposed upon the nuclei of the FCMs. This implies a different fate for all the founders depending on their position within the segment. In fact, already the muscle progenitors are patterned by the concerted action of Wingless and Dpp secreted from the overlying epidermis and by

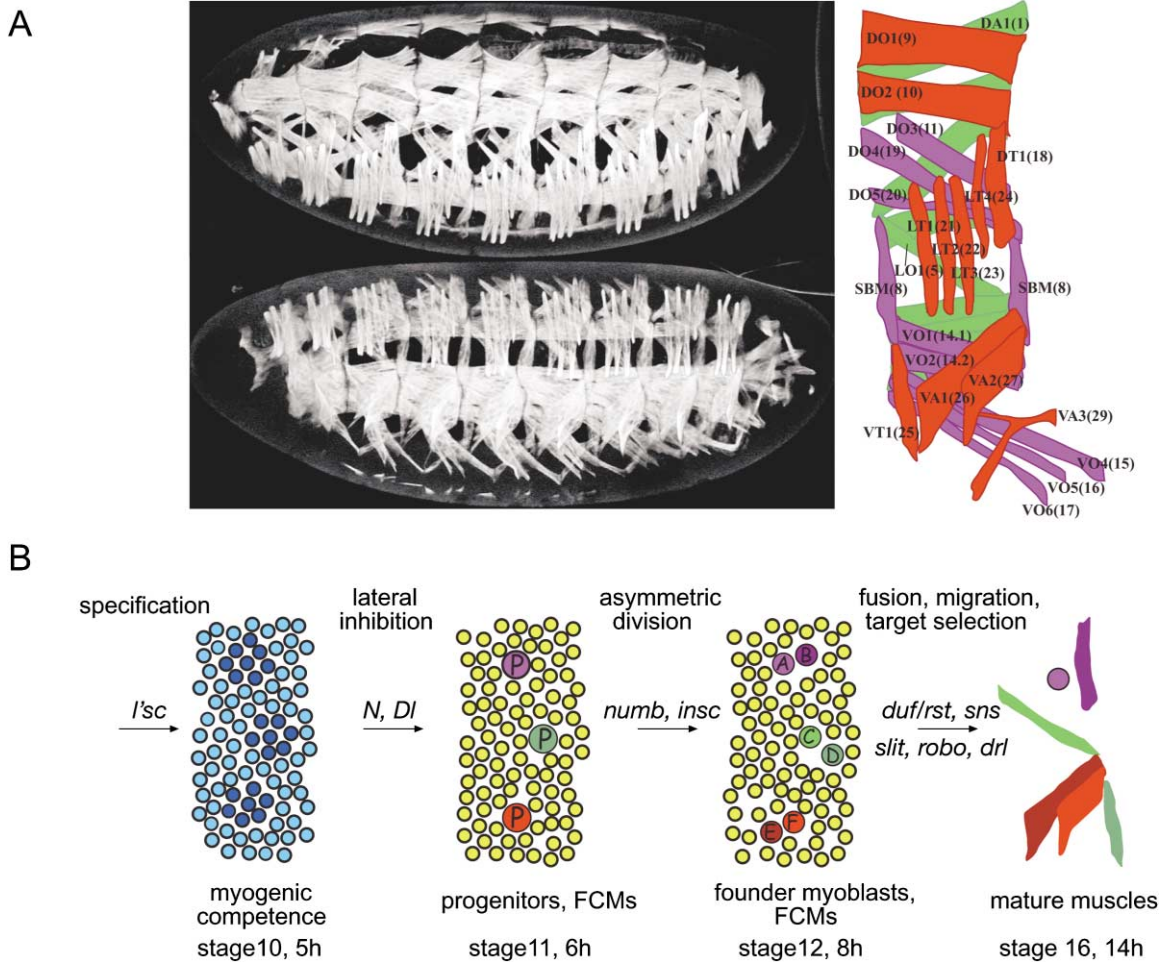


Figure 2. *Drosophila* Embryonic Body Muscle Pattern and Its Development

(A) Body muscles visualized by TauGFP in a mature, living *Drosophila* embryo viewed from dorsolateral (upper picture) and ventrolateral perspective (lower picture). Anterior is to the left; dorsal is up. The scheme on the right highlights the stereotyped muscle pattern found in segments A2 to A7, adapted from Ruiz-Gomez et al. (1997). Selected names of the 30 muscles are indicated. Red, purple, and green indicate superficial, intermediate, and interior muscles, respectively.

(B) Schematic representation of the different steps of *Drosophila* myogenesis, adapted from Baylies et al. (1998). Progenitors (P) are singled out from a field of myogenic competent cells and divide asymmetrically to generate a pair of muscle founder cells (or a founder and an adult muscle precursor [purple]). The founders fuse with the FCMs (yellow) and migrate to their specific targets at the epidermis.

subsequent RTK signaling (Baylies and Michelson, 2001; Frasch, 1999; Halfon et al., 2000). The following asymmetric division of these progenitors gives rise to founders, which express a characteristic combination of transcription factors such as *Krüppel*, *slouch*, *apterous*, *ladybird*, *vestigial*, *nautilus*, *even-skipped*, or *muscle-specific-homeobox* (Baylies et al., 1998). These genes have been proposed to function as factors selecting muscle identity, since mutants in these genes lack certain muscles, whereas others are duplicated (Knirr et al., 1999; Nose et al., 1998). In *numb* or *insc* mutants, the asymmetric division of each progenitor fails and both sister-founders express the same set of identity genes. As a consequence, two identical muscles are formed next to each other (Ruiz Gomez and Bate, 1997). These results provide strong evidence for the founder cell hypothesis.

In contrast to the founders, FCMs appear naive and

do not instruct the fate of the muscle, but they are important for the growth of the muscle to its final size and shape (Rushton et al., 1995). FCMs fuse with founder cells or growing myotubes but not among themselves. To initiate fusion, founders express the Immunoglobulin domain (Ig) transmembrane protein Dumbfounded (*Duf*/Kirre and its paralog *Rst/IrreC*) which can attract the FCMs from a distance (Ruiz-Gomez et al., 2000; Strübelnberg et al., 2001). The FCMs express a different Ig transmembrane protein named Sticks and stones (*Sns*) to respond to the *Duf*-mediated signal (Bour et al., 2000). These proteins mediate cell membrane contact of founder and FCM and thus allow the membrane fusion machinery to unify the two cells (reviewed in Dworak and Sink, 2002; Taylor, 2003). The number of fusion events for each developing myotube is probably directly determined by the fate of the founder cell and correlates with the size of the mature muscle. The smallest muscles

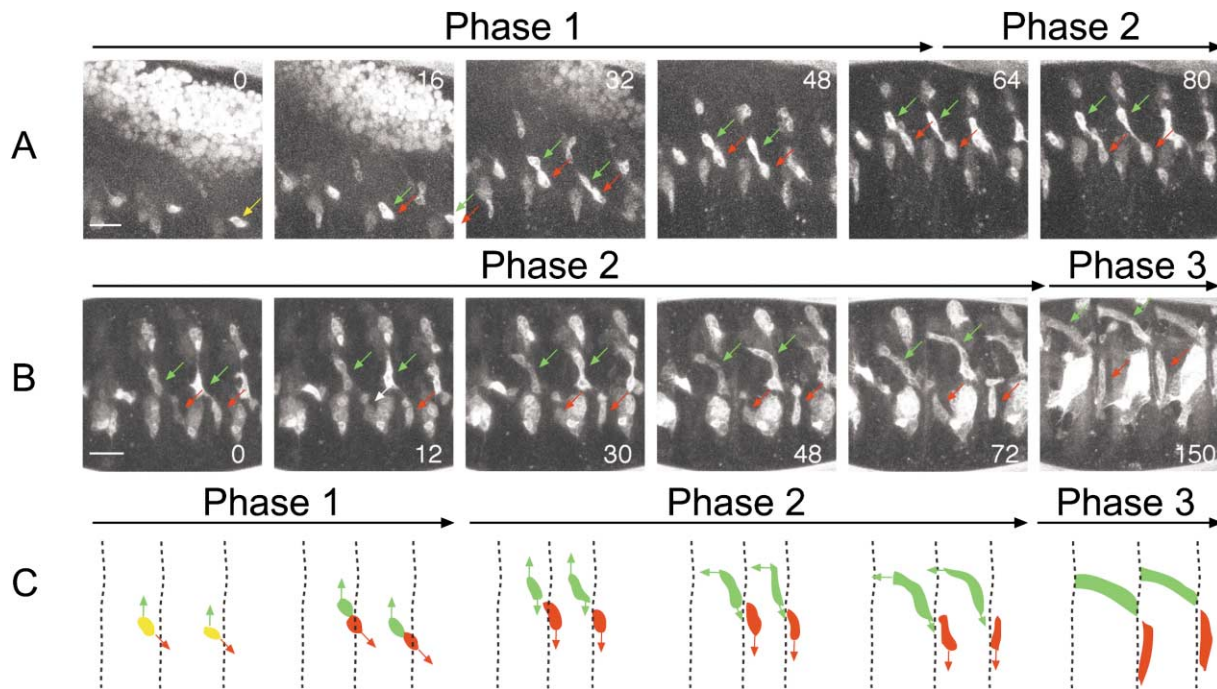


Figure 3. Three Phases of *Drosophila* Myotube Migration

(A and B) Time points taken from two time-lapse recordings (Supplemental Movie S1 in [A], Supplemental Movie S2 in [B]) showing three segments of an embryo expressing membrane-bound GFP in a subset of myotubes. Supplemental Movie S1 starts at germband retraction, the progenitor cells (yellow arrow) divide into the founders of muscle LO1 (green arrow) and VT1 (red arrow). During phase 1, the founders migrate apart from each other. In phase 2, they fuse with FCs and search for their targets. Filopodia are formed at both leading edges of each myotube (see Supplemental Movies S2 and S3). During phase 3, the targets are reached and the migration stops. Time is indicated in minutes. Scale bar represents 20 μ m.

(C) Schematic representation of the three phases with LO1 in green and VT1 in red shown in two segments according to the data in (A) and (B). Arrows mark the direction of migration or formation of filopodia.

contain 3–4 nuclei and the largest 20–25. Myotube fusion starts during stage 12 at the beginning of germband retraction. The smallest muscles appear to complete fusion when germband retraction is finished, whereas the large muscles continue fusion for several hours until stage 16 (Bate, 1990).

Three Phases of Myotube Migration

Myotubes face a tremendous challenge. As they grow in size through cell fusion, myotubes also have to find their proper attachment positions in the epidermis, the tendon cell precursors. How do myotubes know in which direction to migrate and where to stop? Before discussing possible molecular mechanisms of myotube guidance, we first need a detailed description of the dynamic events that take place during myotube migration (our unpublished data, see Figure 3), we can divide the migratory process into three distinct phases. When founder cells are born, they are ball shaped without visible polarity (Carmena et al., 1995, 1998) (Figure 3A). In the first phase of migration, which occurs during germband retraction, the muscle founder cells migrate relative to each other; for example, the founders of muscles LO1 and VT1 (see Figure 2A for nomenclature), which originate from the same progenitor, migrate apart from each other. VT1 travels in ventral-posterior direction into the adjacent posterior segment, whereas LO1 remains within

the segment but moves dorsally (Figures 3A and 3C, Supplemental Movie S1 [<http://www.developmentalcell.com/cgi/content/full/7/1/9/DC1>]; Dohrmann et al., 1990; Knirr et al., 1999). As the first fusion events occur, the myotubes become polarized and stretch to form a stable long axis. This indicates the start of the second phase at stage 13.

The second phase corresponds to the period during which the two ends of each myotube begin to search for their future attachment sites, while the center of the myotube now remains rather stable. During this phase, myotubes form extensive filopodia mainly located at the two opposite ends of the cell (Figure 3B, Supplemental Movie S2 and Figure 4, Supplemental Movie S3). These filopodia presumably sense their environment for guidance cues. Similar filopodia have also been observed in migratory myoblasts of the chick (Knight et al., 2000). In *Drosophila*, these filopodia are up to 20 μ m in length and grow and shrink up to 5 μ m per minute (Figure 4, Supplemental Movie S3). They orient primarily toward the future direction of migration or extension of the myotube. In other words, the myotubes establish dynamic leading edges at both ends, which search for their respective attachment sites. For some myotubes, one end is already close to its attachment site after the first phase of migration, whereas the other often has to move across larger distances. In case of LO1, for example, one leading edge starts from the posterior end of the segment

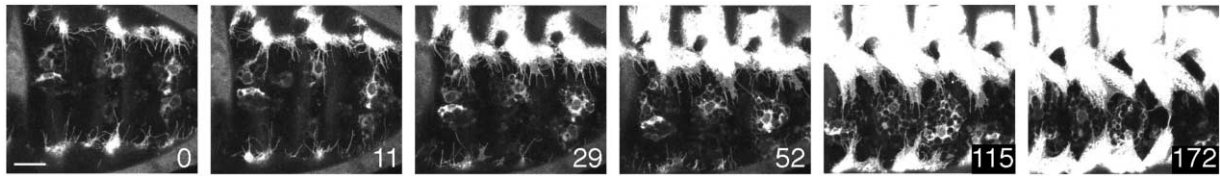


Figure 4. Myotube Leading Edges Generate Extensive Filopodia

Ventral view of myotubes expressing membrane-bound GFP. Time points taken from Supplemental Movie S3 show long, dynamic filopodia formed at the leading edges of the myotubes during the migratory phase (cell bodies are overexposed). Filopodia formation ceases as the muscles begin to attach (172 min). Scale bar represents 20 μm .

and migrates to the anterior end of the same segment (Figure 3). VO4 and VO5 migrate even further, extending from the anterior end of one segment into the next posterior segment (Figure 4).

In the third phase, myotubes have reached their target and filopodia formation ceases. The surface of the myotube facing the tendon cells becomes smooth and the myotube localizes many adhesion complex molecules toward the tendon cell in order to form a stable adhesion complex (Brown et al., 2000). The myotube now assumes the familiar rectangular shape of a muscle ready for contraction (Figures 3B, 3C, and 4; Supplemental Movies S2 and S3) (Bate, 1990). In mutants of α - or β -*integrin*, the formation of a stable adhesion complex fails and as a consequence muscles detach from their attachment sites upon contraction and round up (Brown et al., 2000).

Migration and fusion need to be coordinated during all three phases. It is remarkable that a myotube can migrate and fuse at the same time. This suggests that the guidance machinery is not perturbed by the Dumbfounded-dependent attraction of FCMs. The filopodia of the myotubes are only formed at the leading edges, whereas fusion is restricted to the central region. This spatial segregation of migration and fusion may prevent their mutual interference.

What controls formation, location, and direction of the filopodia? The filopodia could just be an intrinsic property of the highly motile and polarized myotube, or they may be induced and guided by extrinsic factors. Their striking tendency to form in the direction of growth seems to suggest that they respond at least in part to extrinsic cues. Likely sources for guidance cues are the targets of the myotubes, the future muscle attachment sites located in the overlaying epidermis.

Attachment Sites Instruct Myotubes

The epidermal attachment sites for *Drosophila* muscles are called tendon cells, by analogy to the tendon cells that link vertebrate muscle to bone. Tendon cells are positioned within the epidermis and connect muscles to the chitinous exoskeleton of the developing embryo, the fly analog of vertebrate bones. They are located at stereotypic positions both at the segment border and within the segment, called the segmental and intrasegmental attachment sites, respectively. The earliest known marker for tendon precursors is the zinc-finger transcription factor *stripe*, which is induced in response to cues patterning the entire ectoderm during stage 11 (Piepenburg et al., 2000; Volk and VijayRaghavan, 1994). *stripe* fulfills all the criteria expected of a key regulator

for tendon cell development, being both necessary and sufficient to induce most tendon cell-specific genes, including *short stop*, *alien*, $\beta 1$ -*tubulin*, and *delilah* (Becker et al., 1997; Frommer et al., 1996; Vorbrüggen and Jäckle, 1997).

Similar to the tendon cells of vertebrate limbs, tendon cell development in *Drosophila* is biphasic. Initially, *stripe* is induced solely by epidermal cues, independently of muscle precursors. This is revealed by the normal pattern of early *stripe* expression in *twist* mutant embryos, which lack all mesoderm (Becker et al., 1997). This initial *stripe* expression pattern is rather broad and demarcates the tendon cell precursors. In order to mature properly, tendon precursors need to receive a signal from the approaching myotubes. The myotubes secrete the neuregulin-like ligand Vein, which becomes enriched at the muscle-tendon cell junctions and signals through EGF receptor to the tendon cell precursors (Yarnitzky et al., 1997). Only tendon cell precursors that receive the Vein signal will express high levels of $\beta 1$ -*tubulin* or *delilah*, which are markers for terminally differentiated tendon cells. Precursors which remain isolated from muscles appear to dedifferentiate and lose marker expression (Becker et al., 1997; Buttgerit, 1996; Yarnitzky et al., 1997).

An important factor mediating this biphasic tendon cell development is *held out wings* (*how*). *how* is expressed both in the mesoderm and in tendon precursors. It encodes two different RNA binding proteins, a long, nuclear isoform How(L) and a short isoform How(S), which is both nuclear and cytoplasmic (Nabel-Rosen et al., 1999). In early tendon precursors, only How(L) is expressed, which binds to 3'UTR of *stripe* mRNA and results in its degradation. As consequence, Stripe protein levels in the early tendon precursors remain low. In the second phase of tendon development, Vein signaling from the approaching myotubes induces How(S) in the tendon precursors. How(S) can compete with How(L) for *stripe* mRNA and results in its stabilization. Hence, Stripe protein levels are increased and tendon cells begin to terminally differentiate. In *how* mutants, Stripe levels are abnormally high in early tendon precursors. Thus, many of them start to differentiate prematurely without muscle contact (Nabel-Rosen et al., 1999, 2002).

The biphasic development of tendon cells is an interesting developmental strategy that is both precise and flexible. Only tendon cells that connect to muscle are retained, and the muscles have the option to select their appropriate tendon cells. It will be interesting to see if

a muscle always chooses the same tendon cell or if there is room for variation. In this way, a complex pattern is first established roughly and then in a second step fine tuned into its proper, final shape. Plasticity is extremely important in vertebrates and *Drosophila* muscle-tendon interactions may help us to understand its principles.

The tendon cell precursors have dramatic influence on muscle migration. The initial migration appears normal in *stripe* mutants, implying that myotubes are not significantly guided by *stripe* until stage 13, but rather start migrating on their own initiative or in response to some other unknown signal. From stage 14 on, the myotubes migrate to aberrant positions in *stripe* mutants and many fail to attach to the epidermis but adhere to other myotubes instead (Frommer et al., 1996). This demonstrates the instructive role of the tendon cell precursors for myotube pathfinding. Hence, the muscle migration process is not entirely controlled by an intrinsic myotube program but, as expected, also requires extrinsic, tendon cell-derived factors, which steer the myotubes during their second phase of migration. *stripe* alone is even sufficient to attract muscles to ectopic positions, if it is expressed ectopically from stage 11 to 14 but not later on (Becker et al., 1997; Vorbrüggen and Jäckle, 1997).

Analysis of *stripe* mutants has also revealed a second function of tendon cells. Some myotubes continue to migrate in *stripe* mutants at a time when they would normally have stopped and attached (Frommer et al., 1996). This suggests that myotubes encountering tendon cells receive a stop signal which is required to initiate phase three of migration and to terminate the travel.

Myotube Guidance Cues

What are the secreted molecules released from the tendon precursors, and how are these cues sensed and interpreted by the myotubes? Remarkably, no systematic genetic screen to identify these factors has been reported to date. The few key molecules that have been identified have come instead from studies of axon pathfinding, with their roles in myotube guidance only being revealed by further detailed analysis of the mutant phenotypes.

In the *Drosophila* CNS, the atypical receptor tyrosine kinase Derailed (Drl) regulates which commissure an axon uses to project across the midline. Axons expressing Drl prefer the anterior rather than the posterior commissure, apparently because Wnt5, the repulsive ligand for Drl, is present on the posterior commissure (Bonkowsky et al., 1999; Yoshikawa et al., 2003). Drl is also expressed in the epidermis and in a subset of developing myotubes. Its function in the epidermis is unclear, but Drl is required autonomously in the lateral-transverse muscles (LT1-3) for proper recognition of their target, the ventral intrasegmental attachment sites (see Figure 5). In *drl* mutants, muscles LT1-3 frequently pass their correct attachment sites and project too far ventrally (Figure 5) (Callahan et al., 1996). How Drl mediates tendon cell recognition remains an open question. As in neurons, Drl may transmit a repulsive signal, so that its ligand would define domains of no entry for migrating myotubes. Alternatively, Drl may detect a signal that instructs myotubes to stop migrating once they reach their correct attachment sites. Another possibility is that

the LT1-3 myotubes use Drl to detect an unknown attractive signal released from the correct tendon cell precursors. The analysis of muscle migration in *wnt5* mutants may help to clarify this important issue. Interestingly, *drl* mutant myotubes still migrate in the correct directions and often find their proper targets, suggesting that they are also guided by other cues.

Another key guidance cue, again coming from studies on axon guidance, is Slit. Slit is the key regulator of midline crossing in the CNS (Kidd et al., 1999), where it prevents axons that express Robo, the Slit receptor, from projecting across the midline. Slit is also thought to act at long range to ensure the proper axon positioning on the different lateral tracks, according to the specific combination of Robo receptors these axons express (Rajagopalan et al., 2000b; Simpson et al., 2000). Slit appears to act exclusively as a repulsive cue for Robo-expressing axons. The same does not hold true for muscles, where Slit instead has a more complex, bifunctional role, repelling myotubes at an early phase of development, but attracting them at a later phase. Kidd et al. found a few years ago that ventral muscles aberrantly cross the midline in *slit* mutants, connecting to tendon cells on the opposing side of the embryo (Kidd et al., 1999). This crossing is also seen in *robo, robo2* double mutants, whereas both *robo* and *robo2* single mutants show only very mild defects (Kramer et al., 2001; Rajagopalan et al., 2000a). The crossing defect in *slit* mutants is rescued by providing Slit just at the midline, indicating that it normally repels myotubes expressing Robo and Robo2 in much the same way as it repels axons expressing these receptors (Kramer et al., 2001).

Analysis of these midline-rescued *slit* mutant embryos revealed an additional, later function for Slit in guiding myotubes to specific attachment sites. These embryos display migration defects of the Robo expressing ventral-longitudinal muscles. Normally, these muscles extend across the entire segment, but in midline-rescued *slit* mutant embryos, they often instead make abnormal connections to the epidermis (Figure 5). As it turns out, Slit is indeed expressed from stage 13 on in the tendon cell precursors at the segment borders, but not in the intrasegmental precursors, while Robo is expressed in the ventral-longitudinal myotubes. This suggests that Slit may signal through Robo to attract these myotubes as soon as they start sending out filopodia during the second phase of migration. If Robo is expressed ectopically in the lateral-transverse muscles LT1-3, these are rerouted from their normal intrasegmental attachment sites to Slit-positive intersegmental attachment sites. Similarly, an ectopic source of Slit, expressed in stripes in the epidermis in a *slit* mutant, attracts all myotubes. This indicates that Slit has the potential to attract all myotubes, although the endogenous Slit appears to be required only for the attraction of myotubes to intersegmental attachment sites (Kramer et al., 2001).

How does a myotube switch its response to Slit from repulsion to attraction? This is unclear, but interestingly, the switch seems to coincide with the transition from phase one to phase two of myoblast migration. In phase one, the early myotubes, which are repelled by Slit from the midline, migrate as rather round cells relative to each other. In contrast, the phase two myotubes, which are attracted by Slit at tendon cell precursors, extend long

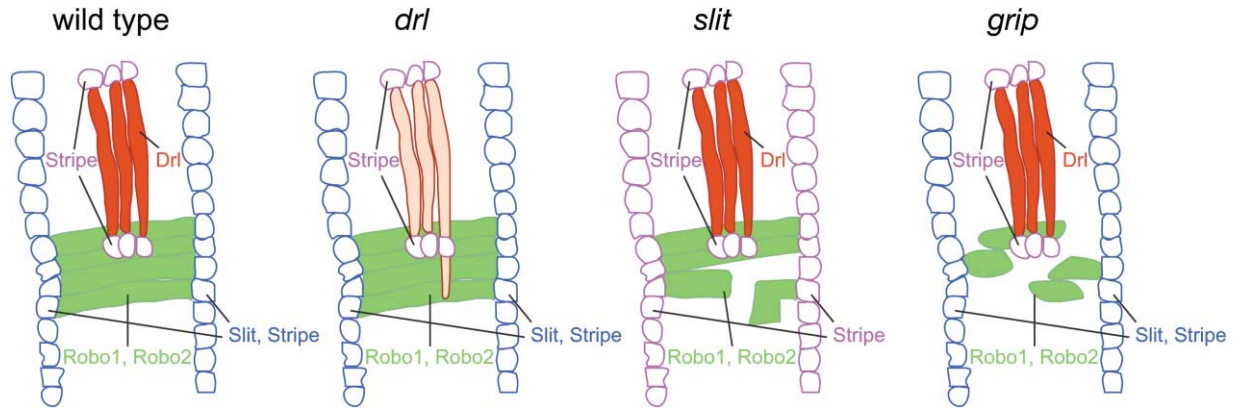


Figure 5. Genes Controlling *Drosophila* Myotube Guidance

In wild-type, the lateral-transverse muscles expressing *Drl* (red) connect to the intrasegmental attachment sites (purple). In *drl* mutants, some of these muscles ignore those attachment sites and grow further ventral. The ventral-longitudinal muscles expressing *Robo* and *Robo2* (green) connect to *Stripe* and *Slit* positive attachment sites at segment border (blue) in wild-type. However, in midline-rescued *slit* mutants, attraction of the ventral-longitudinal muscles to the segment borders partially fails, resulting in ectopic connections. This is more dramatic in *grip* mutants, in which most of the ventral-longitudinal muscles stay round or attach to other muscles.

filopodia toward their targets. One interesting possibility is that myotubes need to contact the *Slit*-expressing cells directly with their filopodia in order to be attracted, whereas the repulsive response to the midline may require a long-range signal provided by the cleaved N-terminal fragment of *Slit*.

Specificity

Thus far, *Slit* is the only muscle guidance cue known to be expressed in a subset of tendon cell precursors and hence capable of providing some specificity to attachment site selection. But *Slit* alone cannot account for the specific attachments of a particular muscle. Why are almost all myotubes attracted to the ectopic *Slit* source, if normally only a subset of them should respond to endogenous *Slit*? More generally, how is the muscle pattern set up in its very stereotyped and accurate way by such rather broadly expressed cues like *Slit*? One obvious explanation is that we still don't know most of the factors that guide myotubes to their specific targets, since no systematic screens have yet been done to find them. However, we should not think that specificity can exclusively be generated through the differential expression of various ligands and their receptors. There are at least three other ways to establish specificity, without requiring a unique combination of ligands and receptors for each muscle and its attachment sites.

One possibility is that rather common ligands and receptors can gain specificity, if myotubes differ in their responsiveness to these cues. Recently, a potential source of such differential sensitivity has been found (Swan et al., 2004). The intracellular PDZ domain protein *Grip* was shown to play an important role in the migration of the ventral-longitudinal muscles, the same ones which are attracted by *Slit*. In *grip* mutants, these muscles fail to migrate across the segment, possibly because they do not respond correctly to guidance cues from the segment border. Rather, they adopt a more round shape and attach to the epidermis at ectopic positions within the segment (Figure 5) (Swan et al.,

2004). This phenotype resembles that of the midline-rescued *slit* mutants. Moreover, as was the case for *Robo*, ectopic expression of *Grip* in the lateral-transverse muscles redirects these muscles from their intrasegmental attachment sites toward the segment border, suggesting that *Grip* is sufficient for these cells to respond to an attractive signal from the tendon cell precursors at the segment borders, such as *Slit*. However, Swan et al. found no evidence for a direct link of *Grip* to *Slit*-*Robo* signaling.

Another potential source of specificity are the intrinsic properties of the founder cells. For example, the asymmetric division that generates a founder cell may also instruct it to migrate in a specific direction. This is an appealing idea, but the evidence argues against it. For example, the oblique muscle LO1 and the dorsoventral muscle SBM arise from two adjacent progenitors, but express different markers and migrate in different directions. In *slouch* mutants, LO1 is transformed into SBM, as it expresses the SBM marker *ladybird* and attaches at the same sites as the normal SBM (Knirr et al., 1999). The initial position and asymmetric division that gives rise to the transformed LO1 founder cell is, however, still normal in these mutants, arguing against any model in which the position or initial orientation of the founder determines its subsequent migration and attachment. Nevertheless, it does seem likely that the initial position of the founder has some influence on its subsequent attachment site selection. The initial positions of founders are not totally random, but do have a broad correlation with the final muscle pattern. Given a choice of attachment sites expressing the same attractant, a myotube is presumably more likely to select the closer one, all else being equal. Thus, it would be premature on the basis of the *slouch* mutant alone to conclude that a founder cell does not receive any positional or directional cues from the location and orientation of the division in which it is born. A more careful analysis of spindle orientation and subsequent migration of more founder cells in both wild-type and mutant embryos will be required to resolve this.

Finally, competition between one myotube and its neighbors may be a relevant issue. In the ventral part of the embryo, up to three layers of muscles are formed on top of each other. All myotubes extend toward their targets and fuse with FCMs at the same time (see Supplemental Movie S3). However, genetically duplicated muscles can either both insert at the same attachment sites or insert just next to each other (Ruiz Gomez and Bate, 1997), suggesting that the guidance cues and spatial constraints are not as limiting as one may imagine. As discussed above, more than one tendon cell precursor may have the potential to be approached by the myotube and develop into the proper attachment site. Furthermore, in fusion-defective mutants, the founder cells alone extend and try to migrate to their targets, sometimes forming “mini-muscles” that attach at the correct positions and may even be innervated by the correct motoneurons (Ruiz-Gomez et al., 2000; Rushton et al., 1995). However, it is not clear if all these mini-muscles correspond to those muscles which normally insert at this position, or if the block of fusion and spatial constraints influences the pattern. The FCMs do not seem to play a major role in the migration process itself, but may provide material allowing the myotube to extend the whole way to its target. Nevertheless, there is recent evidence that FCMs do not comprise a completely uniform cell population, although a functional difference between them still remains to be demonstrated (Artero et al., 2001).

Myotube Guidance in *Drosophila* and Vertebrates: Variations on a Common Theme?

With so many of the molecular details still unknown, it is clearly premature to compare *Drosophila* and vertebrate myotube guidance at this level. At the cellular level, however, we can see both intriguing differences and striking similarities. Perhaps the most obvious difference is in the developmental potential of the early muscle precursors. In *Drosophila*, there is a clear distinction between muscle founder cells, which are restricted in their fate almost from birth, and fusion competent myoblasts, which acquire a fate only as they fuse with a specific founder cell or myotube. In contrast, there does not appear to be such distinction among vertebrate myoblasts, all of which resemble the fusion competent myoblasts of *Drosophila* in that they are initially unrestricted in their fate. These vertebrate myoblasts acquire a specific fate only much later in development—in the case of limb muscles, this does not happen until they reach the final target area. Thus, there is a much greater degree of plasticity in vertebrate muscle development, a feature that presumably allows the robust generation of much more complex muscle patterns. This distinction is also highlighted molecularly by the different roles of the myogenic regulatory factors of the *MyoD/nautilus* family: In *Drosophila*, the single *nautilus* gene (*nau*) is expressed in a specific subset of muscle founders and is required for the formation of the corresponding muscles (Balagopalan et al., 2001). The vertebrate *nautilus* orthologs, however, are globally expressed and required for the development of all muscles. This hints that some of the same molecules may regulate muscle fates in *Drosophila* and vertebrates but act in subtly

different ways that reflect the distinct strategies used to specify individual muscle fates.

Despite these well-known differences in the way muscles acquire their fate, the overall process of myotube migration and attachment site selection may be more similar in *Drosophila* and vertebrates than is generally appreciated. Here, based on our time-lapse studies, we have divided *Drosophila* myotube guidance into three distinct phases: (1) unidirectional migration; (2) bidirectional migration, and (3) attachment. Vertebrate myotubes may also progress through these three phases.

Myoblasts in the vertebrate head and limb have to travel much further than *Drosophila* myoblasts, but they also migrate as rather round, undifferentiated cells without a stable long axis. This phase thus appears as an extended version of the first phase of *Drosophila* myoblast migration. Myoblasts appear to be guided during this phase by rather global guidance cues. In *Drosophila*, one such cue is Slit protein secreted from the midline.

As they approach their target area, vertebrate myoblasts establish a stable long axis as they begin to extend in both directions. The clearest example of this is the bidirectional extension of the trunk myotubes after they enter the myotome. This resembles the second phase of *Drosophila* myotube migration. During this phase, myotubes appear to respond to more local guidance cues, for which *Drosophila* provides the examples of Slit at a subset of muscle attachment sites as well as the putative Drl ligand.

Finally, the third phase of *Drosophila* myogenesis, the formation of stable attachments, also has a clear parallel in vertebrates. In both cases, this begins with the initial specification of tendon cell precursors, which is independent of the muscles, and is followed by a complex set of mutual interactions between tendon precursors and the approaching muscle that coordinates the final differentiation of both cells and the formation of a stable connection between them.

Perspectives

The analysis of myotube migration and targeting to specific attachment sites is still in its infancy, but is clearly a rich area for further exploration. Powerful new methods for time-lapse imaging of individual myotubes, combined with genetic manipulation, should ensure that rapid progress can now be made toward understanding the molecular and cellular processes that guide each myotube to its specific target. *Drosophila* myotube migration may serve as a paradigm for genetically less tractable tissues such as vertebrate limb or head muscles. Despite the greater plasticity of vertebrate muscles, the principles of myotube migration, elongation, and stereotypic attachments to the skeleton are conserved from fly to human, and provide a beautiful example for the accuracy of a complex developmental process. How satisfying it will be to one day understand the developmental processes that have endowed the ballerina with the superb musculature to execute her graceful pirouette, the bumblebee to turn on the spot in full flight, the gazelle to evade the pursuing cheetah, and our patient reader to simply turn the page.

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References

- Arnold, H.H., and Braun, T. (2000). Genetics of muscle determination and development. *Curr. Top. Dev. Biol.* 48, 129–164.
- Artero, R.D., Castanon, I., and Baylies, M.K. (2001). The immunoglobulin-like protein Hibris functions as a dose-dependent regulator of myoblast fusion and is differentially controlled by Ras and Notch signaling. *Development* 128, 4251–4264.
- Balogopalan, L., Keller, C.A., and Abmayr, S.M. (2001). Loss-of-function mutations reveal that the *Drosophila* nautilus gene is not essential for embryonic myogenesis or viability. *Dev. Biol.* 231, 374–382.
- Bate, M. (1990). The embryonic development of larval muscles in *Drosophila*. *Development* 110, 791–804.
- Baylies, M.K., and Bate, M. (1996). *twist*: a myogenic switch in *Drosophila*. *Science* 272, 1481–1484.
- Baylies, M.K., and Michelson, A.M. (2001). Invertebrate myogenesis: looking back to the future of muscle development. *Curr. Opin. Genet. Dev.* 11, 431–439.
- Baylies, M.K., Bate, M., and Ruiz Gomez, M. (1998). Myogenesis: a view from *Drosophila*. *Cell* 93, 921–927.
- Becker, S., Pasca, G., Strumpf, D., Min, L., and Volk, T. (1997). Reciprocal signaling between *Drosophila* epidermal muscle attachment cells and their corresponding muscles. *Development* 124, 2615–2622.
- Birchmeier, C., and Brohmann, H. (2000). Genes that control the development of migrating muscle precursor cells. *Curr. Opin. Cell Biol.* 12, 725–730.
- Bladt, F., Riethmacher, D., Isenmann, S., Aguzzi, A., and Birchmeier, C. (1995). Essential role for the c-met receptor in the migration of myogenic precursor cells into the limb bud. *Nature* 376, 768–771.
- Bonkowski, J.L., Yoshikawa, S., O'Keefe, D.D., Scully, A.L., and Thomas, J.B. (1999). Axon routing across the midline controlled by the *Drosophila* Derailed receptor. *Nature* 402, 540–544.
- Bour, B.A., Chakravarti, M., West, J.M., and Abmayr, S.M. (2000). *Drosophila* SNS, a member of the immunoglobulin superfamily that is essential for myoblast fusion. *Genes Dev.* 14, 1498–1511.
- Brand-Saberi, B., Muller, T.S., Wilting, J., Christ, B., and Birchmeier, C. (1996). Scatter factor/hepatocyte growth factor (SF/HGF) induces emigration of myogenic cells at interlimb level in vivo. *Dev. Biol.* 179, 303–308.
- Brent, A.E., Schweitzer, R., and Tabin, C.J. (2003). A somitic compartment of tendon progenitors. *Cell* 113, 235–248.
- Brent, A.E., and Tabin, C.J. (2002). Developmental regulation of somite derivatives: muscle, cartilage and tendon. *Curr. Opin. Genet. Dev.* 12, 548–557.
- Brohmann, H., Jagla, K., and Birchmeier, C. (2000). The role of Lbx1 in migration of muscle precursor cells. *Development* 127, 437–445.
- Brown, N.H., Gregory, S.L., and Martin-Bermudo, M.D. (2000). Integrins as mediators of morphogenesis in *Drosophila*. *Dev. Biol.* 223, 1–16.
- Buckingham, M., Bajard, L., Chang, T., Daubas, P., Hadchouel, J., Meilhac, S., Montarras, D., Rocancourt, D., and Relaix, F. (2003). The formation of skeletal muscle: from somite to limb. *J. Anat.* 202, 59–68.
- Buttgereit, D. (1996). Transcription of the beta 1 tubulin (beta Tub56D) gene in apodemes is strictly dependent on muscle insertion during embryogenesis in *Drosophila melanogaster*. *Eur. J. Cell Biol.* 71, 183–191.
- Callahan, C.A., Bonkovsky, J.L., Scully, A.L., and Thomas, J.B. (1996). Derailed is required for muscle attachment site selection in *Drosophila*. *Development* 122, 2761–2767.
- Carmena, A., Bate, M., and Jimenez, F. (1995). Lethal of scute, a proneural gene, participates in the specification of muscle progenitors during *Drosophila* embryogenesis. *Genes Dev.* 9, 2373–2383.
- Carmena, A., Murugasu-Oei, B., Menon, D., Jimenez, F., and Chia, W. (1998). Inscuteable and numb mediate asymmetric muscle progenitor cell divisions during *Drosophila* myogenesis. *Genes Dev.* 12, 304–315.
- Degenhardt, K., Rentschler, S., Fishman, G., and Sassoon, D.A. (2002). Cellular and cis-regulation of En-2 expression in the mandibular arch. *Mech. Dev.* 111, 125–136.
- Denetclaw, W.F., and Ordahl, C.P. (2000). The growth of the dermo-myotome and formation of early myotome lineages in thoracolumbar somites of chicken embryos. *Development* 127, 893–905.
- Denetclaw, W.F., Jr., Christ, B., and Ordahl, C.P. (1997). Location and growth of epaxial myotome precursor cells. *Development* 124, 1601–1610.
- Dietrich, S., Abou-Rebyeh, F., Brohmann, H., Bladt, F., Sonnenberg-Riethmacher, E., Yamaai, T., Lumsden, A., Brand-Saberi, B., and Birchmeier, C. (1999). The role of SF/HGF and c-Met in the development of skeletal muscle. *Development* 126, 1621–1629.
- Dohrmann, C., Azpiazu, N., and Frasch, M. (1990). A new *Drosophila* homeo box gene is expressed in mesodermal precursor cells of distinct muscles during embryogenesis. *Genes Dev.* 4, 2098–2111.
- Dworak, H.A., and Sink, H. (2002). Myoblast fusion in *Drosophila*. *Bioessays* 24, 591–601.
- Frasch, M. (1999). Controls in patterning and diversification of somatic muscles during *Drosophila* embryogenesis. *Curr. Opin. Genet. Dev.* 9, 522–529.
- Frommer, G., Vorbrüggen, G., Pasca, G., Jackle, H., and Volk, T. (1996). Epidermal egr-like zinc finger protein of *Drosophila* participates in myotube guidance. *EMBO J.* 15, 1642–1649.
- Gros, J., Scaal, M., and Marcelle, C. (2004). A two-step mechanism for myotome formation in chick. *Dev. Cell* 6, 875–882.
- Gross, M.K., Moran-Rivard, L., Velasquez, T., Nakatsu, M.N., Jagla, K., and Goulding, M. (2000). Lbx1 is required for muscle precursor migration along a lateral pathway into the limb. *Development* 127, 413–424.
- Hacker, A., and Guthrie, S. (1998). A distinct developmental programme for the cranial paraxial mesoderm in the chick embryo. *Development* 125, 3461–3472.
- Halfon, M.S., Carmena, A., Gisselbrecht, S., Sackerson, C.M., Jimenez, F., Baylies, M.K., and Michelson, A.M. (2000). Ras pathway specificity is determined by the integration of multiple signal-activated and tissue-restricted transcription factors. *Cell* 103, 63–74.
- Hatta, K., Schilling, T.F., BreMiller, R.A., and Kimmel, C.B. (1990). Specification of jaw muscle identity in zebrafish: correlation with engrailed-homeoprotein expression. *Science* 250, 802–805.
- Heymann, S., Koudrova, M., Arnold, H., Koster, M., and Braun, T. (1996). Regulation and function of SF/HGF during migration of limb muscle precursor cells in chicken. *Dev. Biol.* 180, 566–578.
- Kahane, N., Cinnamon, Y., Bachelet, I., and Kalcheim, C. (2001). The third wave of myotome colonization by mitotically competent progenitors: regulating the balance between differentiation and proliferation during muscle development. *Development* 128, 2187–2198.
- Kardon, G. (1998). Muscle and tendon morphogenesis in the avian hind limb. *Development* 125, 4019–4032.
- Kardon, G., Campbell, J.K., and Tabin, C.J. (2002). Local extrinsic signals determine muscle and endothelial cell fate and patterning in the vertebrate limb. *Dev. Cell* 3, 533–545.
- Kardon, G., Harfe, B.D., and Tabin, C.J. (2003). A Tcf4-positive mesodermal population provides a prepattern for vertebrate limb muscle patterning. *Dev. Cell* 5, 937–944.
- Kidd, T., Bland, K.S., and Goodman, C.S. (1999). Slit is the midline repellent for the robo receptor in *Drosophila*. *Cell* 96, 785–794.
- Knight, B., Laukaitis, C., Akhtar, N., Hotchin, N.A., Edlund, M., and

- Horwitz, A.R. (2000). Visualizing muscle cell migration in situ. *Curr. Biol.* 10, 576–585.
- Knirr, S., Azpiazu, N., and Frasch, M. (1999). The role of the NK-homeobox gene *slouch* (*S59*) in somatic muscle patterning. *Development* 126, 4525–4535.
- Kramer, S.G., Kidd, T., Simpson, J.H., and Goodman, C.S. (2001). Switching repulsion to attraction: changing responses to slit during transition in mesoderm migration. *Science* 292, 737–740.
- Lu, J.R., Bassel-Duby, R., Hawkins, A., Chang, P., Valdez, R., Wu, H., Gan, L., Shelton, J.M., Richardson, J.A., and Olson, E.N. (2002). Control of facial muscle development by *MyoR* and *capsulin*. *Science* 298, 2378–2381.
- Mankoo, B.S., Collins, N.S., Ashby, P., Grigorieva, E., Pevny, L.H., Candia, A., Wright, C.V., Rigby, P.W., and Pachnis, V. (1999). *Mox2* is a component of the genetic hierarchy controlling limb muscle development. *Nature* 400, 69–73.
- Nabel-Rosen, H., Dorevitch, N., Reuveny, A., and Volk, T. (1999). The balance between two isoforms of the *Drosophila* RNA-binding protein *how* controls tendon cell differentiation. *Mol. Cell* 4, 573–584.
- Nabel-Rosen, H., Volohonsky, G., Reuveny, A., Zaidel-Bar, R., and Volk, T. (2002). Two isoforms of the *Drosophila* RNA binding protein, *how*, act in opposing directions to regulate tendon cell differentiation. *Dev. Cell* 2, 183–193.
- Noden, D.M., Marcucio, R., Borycki, A.G., and Emerson, C.P., Jr. (1999). Differentiation of avian craniofacial muscles: I. Patterns of early regulatory gene expression and myosin heavy chain synthesis. *Dev. Dyn.* 216, 96–112.
- Nose, A., Isshiki, T., and Takeichi, M. (1998). Regional specification of muscle progenitors in *Drosophila*: the role of the *msh* homeobox gene. *Development* 125, 215–223.
- Ordahl, C.P., Berdugo, E., Venters, S.J., and Denetclaw, W.F., Jr. (2001). The dermomyotome dorsomedial lip drives growth and morphogenesis of both the primary myotome and dermomyotome epithelium. *Development* 128, 1731–1744.
- Piepenburg, O., Vorbrüggen, G., and Jäckle, H. (2000). *Drosophila* segment borders result from unilateral repression of Hedgehog activity by Wingless signaling. *Mol. Cell* 6, 203–209.
- Piotrowski, T., Schilling, T.F., Brand, M., Jiang, Y.J., Heisenberg, C.P., Beuchle, D., Grandel, H., van Eeden, F.J., Furutani-Seiki, M., Granato, M., et al. (1996). Jaw and branchial arch mutants in zebrafish II: anterior arches and cartilage differentiation. *Development* 123, 345–356.
- Rajagopalan, S., Nicolas, E., Vivancos, V., Berger, J., and Dickson, B.J. (2000a). Crossing the midline: roles and regulation of Robo receptors. *Neuron* 28, 767–777.
- Rajagopalan, S., Vivancos, V., Nicolas, E., and Dickson, B.J. (2000b). Selecting a longitudinal pathway: Robo receptors specify the lateral position of axons in the *Drosophila* CNS. *Cell* 103, 1033–1045.
- Ruiz-Gomez, M., and Bate, M. (1997). Segregation of myogenic lineages in *Drosophila* requires *numb*. *Development* 124, 4857–4866.
- Ruiz-Gomez, M., Romani, S., Hartmann, C., Jackle, H., and Bate, M. (1997). Specific muscle identities are regulated by Krüppel during *Drosophila* embryogenesis. *Development* 124, 3407–3414.
- Ruiz-Gomez, M., Coutts, N., Price, A., Taylor, M.V., and Bate, M. (2000). *Drosophila* dumbfounded: a myoblast attractant essential for fusion. *Cell* 102, 189–198.
- Rushton, E., Drysdale, R., Abmayr, S.M., Michelson, A.M., and Bate, M. (1995). Mutations in a novel gene, *myoblast city*, provide evidence in support of the founder cell hypothesis for *Drosophila* muscle development. *Development* 121, 1979–1988.
- Schäfer, K., and Braun, T. (1999). Early specification of limb muscle precursor cells by the homeobox gene *Lbx1*. *Nat. Genet.* 23, 213–216.
- Schilling, T.F., and Kimmel, C.B. (1997). Musculoskeletal patterning in the pharyngeal segments of the zebrafish embryo. *Development* 124, 2945–2960.
- Schilling, T.F., Piotrowski, T., Grandel, H., Brand, M., Heisenberg, C.P., Jiang, Y.J., Beuchle, D., Hammerschmidt, M., Kane, D.A., Mullins, M.C., et al. (1996a). Jaw and branchial arch mutants in zebrafish I: branchial arches. *Development* 123, 329–344.
- Schilling, T.F., Walker, C., and Kimmel, C.B. (1996b). The chinless mutation and neural crest cell interactions in zebrafish jaw development. *Development* 122, 1417–1426.
- Schweitzer, R., Chyung, J.H., Murtaugh, L.C., Brent, A.E., Rosen, V., Olson, E.N., Lassar, A., and Tabin, C.J. (2001). Analysis of the tendon cell fate using *Scleraxis*, a specific marker for tendons and ligaments. *Development* 128, 3855–3866.
- Simpson, J.H., Bland, K.S., Fetter, R.D., and Goodman, C.S. (2000). Short-range and long-range guidance by Slit and its Robo receptors: a combinatorial code of Robo receptors controls lateral position. *Cell* 103, 1019–1032.
- Strunkelnberg, M., Bonengel, B., Moda, L.M., Hertenstein, A., de Couet, H.G., Ramos, R.G., and Fischbach, K.F. (2001). *rst* and its paralogue *kirre* act redundantly during embryonic muscle development in *Drosophila*. *Development* 128, 4229–4239.
- Swan, L.E., Wichmann, C., Prange, U., Schmid, A., Schmidt, M., Schwarz, T., Ponimaskin, E., Madeo, F., Vorbrüggen, G., and Sigrist, S.J. (2004). A Glutamate receptor-interacting protein homolog organizes muscle guidance in *Drosophila*. *Genes Dev.* 18, 223–237.
- Tajbakhsh, S., and Buckingham, M. (2000). The birth of muscle progenitor cells in the mouse: spatiotemporal considerations. *Curr. Top. Dev. Biol.* 48, 225–268.
- Taylor, M.V. (2003). Muscle differentiation: signalling cell fusion. *Curr. Biol.* 13, R964–R966.
- Tzahor, E., Kempf, H., Mootoosamy, R.C., Poon, A.C., Abzhanov, A., Tabin, C.J., Dietrich, S., and Lassar, A.B. (2003). Antagonists of Wnt and BMP signaling promote the formation of vertebrate head muscle. *Genes Dev.* 17, 3087–3099.
- Volk, T., and VijayRaghavan, K. (1994). A central role for epidermal segment border cells in the induction of muscle patterning in the *Drosophila* embryo. *Development* 120, 59–70.
- Vorbrüggen, G., and Jäckle, H. (1997). Epidermal muscle attachment site-specific target gene expression and interference with myotube guidance in response to ectopic stripe expression in the developing *Drosophila* epidermis. *Proc. Natl. Acad. Sci. USA* 94, 8606–8611.
- Yarnitzky, T., Min, L., and Volk, T. (1997). The *Drosophila* neuregulin homolog *Vein* mediates inductive interactions between myotubes and their epidermal attachment cells. *Genes Dev.* 11, 2691–2700.
- Yoshikawa, S., McKinnon, R.D., Kokel, M., and Thomas, J.B. (2003). Wnt-mediated axon guidance via the *Drosophila* Derailed receptor. *Nature* 422, 583–588.