HHS Public Access

Author manuscript

Wiley Interdiscip Rev Dev Biol. Author manuscript; available in PMC 2016 January 01.

Published in final edited form as:

Wiley Interdiscip Rev Dev Biol. 2015 July ; 4(4): 357–375. doi:10.1002/wdev.182.

Specification of the somatic musculature in *Drosophila*†

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Abstract

The somatic muscle system formed during *Drosophila* embryogenesis is required for larvae to hatch, feed, and crawl. This system is replaced in the pupa by a new adult muscle set, responsible for activities such as feeding, walking, and flight. Both the larval and adult muscle systems are comprised of distinct muscle fibers to serve these specific motor functions. In this way, the *Drosophila* musculature is a valuable model for patterning within a single tissue: while all muscle cells share properties such as the contractile apparatus, properties such as size, position, and number of nuclei are unique for a particular muscle. In the embryo, diversification of muscle fibers relies first on signaling cascades that pattern the mesoderm. Subsequently, the combinatorial expression of specific transcription factors leads muscle fibers to adopt particular sizes, shapes, and orientations. Adult muscle precursors (AMPs), set aside during embryonic development, proliferate during the larval phases and seed the formation of the abdominal, leg, and flight muscles in the adult fly. Adult muscle fibers may either be formed *de novo* from the fusion of the AMPs, or are created by the binding of AMPs to an existing larval muscle. While less is known about adult muscle specification compared to the larva, expression of specific transcription factors is also important for its diversification. Increasingly, the mechanisms required for the diversification of fly muscle have found parallels in vertebrate systems and mark *Drosophila* as a robust model system to examine questions about how diverse cell types are generated within an organism.

INTRODUCTION

Somatic muscle systems generate the force required for movement, allowing hatching, feeding, and locomotion. The musculature in organisms as diverse as humans and flies share many aspects, including gene expression, formation of syncytia, and establishment of the contractile apparatus. This conservation makes the study of muscle development in model organisms particularly relevant for understanding general muscle biology and disease. The fruit fly *Drosophila melanogaster* forms two discrete muscle systems throughout its life cycle: the larval body wall muscles are established during embryogenesis, while the adult

†We apologize to our colleagues for instances when, due to space constrains, we were unable to directly cite their work.

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Conflict of interest: The authors have declared no conflicts of interest for this article.

musculature is formed from cells set aside during embryogenesis that fully develop into mature muscles during metamorphosis.[1,2] This review focuses on the development of both sets of *Drosophila* somatic muscles, but is divided into two parts. In the first, we discuss the specification and diversification of the muscle fibers via signaling and transcriptional networks. In the second, we address aspects of morphogenesis, including cell fusion, nuclear positioning, attachment to tendons, and sarcomere formation. This introduction will provide a brief overview of myogenesis from the embryo to the adult, with specific topics addressed in more detail in each review.

Drosophila Myogenesis: An Overview

Myogenesis in *Drosophila* begins with the invagination of the mesoderm, which spreads along the ectoderm and is then further allocated into a number of mesodermal derivatives. After specification of the somatic mesoderm, three different types of myoblasts are formed. Muscle founder cells (FCs) and fusion-competent myoblasts (FCMs) go on to form the embryonic and larval body wall muscles, while adult muscle precursors (AMPs) are set aside as undifferentiated cells until metamorphosis.[1,2] A single FC encodes the information needed to form a muscle with specific properties, and it fuses iteratively with surrounding FCMs to make a syncytial myotube.[3] Following myoblast fusion, nuclei are then positioned correctly throughout the myotube.[4] While this distribution is taking place, the muscle also forms connections to surrounding tendon cells to establish the myotendinous junction and is innervated by motorneurons.[5,6] The contractile apparatus is then assembled and the muscles can begin to contract, leading to hatching. Although no additional fusion events occur, muscles continue to expand in size along with the growth of the larva, and the essential muscle pattern set up in the embryo does not change.

In the *Drosophila* embryo and larva, a repeated pattern of 30 distinct muscle fibers is present in each abdominal hemisegment[1] (Figure 1). Despite their similarities, such as shared expression of contractile proteins and neurotransmitter receptors, each muscle fiber can be distinguished by its size, shape, orientation, number of nuclei, innervation, and tendon attachment sites.[7] Accordingly, each muscle name conveys information on the muscle's position (D for dorsal, L for lateral, and V for ventral) and orientation (L for longitudinal, oriented from anterior to posterior; T for transverse, attached along the dorsoventral axis; O for oblique, which attach to a more dorsal position at their anterior side; and A for acute, which attach more ventrally at their anterior side). Each muscle is comprised of a single cell; in contrast, adult fly muscles are made up of bundles of fibers, analogous to mammalian muscle. There is diversity among the adult muscle fibers as well, with specific fibers dedicated to particular movements such as walking or flight.[8] This diversity is reflected in the muscle structures: the indirect flight muscles (IFMs) are fibrillar muscles, while the abdominal, leg, jump, and direct flight muscles (DFMs) are tubular muscles.[9]

Throughout development, internal and external cues guide muscles to adopt specific properties that allow those muscles to perform particular functions. In this portion of the review, we explore the mechanisms that establish somatic muscle fiber diversity in the embryo and the adult.

PATTERNING THE MESODERM

During embryogenesis, multiple tissues arise from the mesoderm, including cardiac tissue, visceral mesoderm, head musculature, gonadic mesoderm, and fat body.[7] The basic helix-loop-helix (bHLH) transcription factor Twist (Twi) can be considered a master regulator of mesodermal formation; it is also highly conserved and important for vertebrate development.[10] It is used iteratively throughout development: first, it regulates the cell behaviors necessary for gastrulation; later, it plays a role subdividing mesodermal tissues; next, it is required for the development of a subset of embryonic muscles; and finally, it marks AMPs set aside until metamorphosis.[2,11-15] The iterative use of transcriptional regulators is a hall-mark of mesodermal development and specification. In addition to Twi, a number of regulators such as the Nkx transcription factor, Tinman (Tin), and the bHLH transcription factor, Lethal of scute (L'sc), are initially expressed in broader domains that become restricted to particular cell types throughout developmental time, thereby specifying their fates.[16,17] Their patterns of expression are the result of a complex integration of these transcriptional networks with Wnt, TGFβ, RTK, and Notch signaling cascades. Like transcriptional regulators, signaling pathways also perform distinct functions at different stages of development, such as the use of Wingless (Wg) first to subdivide the mesoderm, and later to specify the fate of a muscle subset.[18-22] The combination of signaling pathways and the regulation of gene expression leads to the establishment of diverse somatic muscle fates.

Gastrulation

The process of gastrulation has been previously reviewed,[23,24] and so we present a brief overview of the process. The mesoderm arises from the ventral-most cells in the early embryo, which have high nuclear levels of Dorsal (Dl).[25] At embryonic stage 5, Dl activates the transcription factors Twi and Snail (Sna), which are required for mesodermal specification.[12,26-28] Cells expressing Twi and Sna change shape and invaginate into the ventral furrow, divide synchronously, and undergo an epithelial to mesenchymal transition.[29-32] The mesodermal cells then spread dorsally along the interior of the embryo. At the end of this migration, the cells comprise a single layer underneath the ectoderm, which is subdivided and patterned.

During gastrulation, Dl, Twi, and Sna work together to activate genes necessary for mesodermal specification.[12,26,33-36] A combination of genetic and whole genome studies has identified a large number of downstream mesodermal targets of Twi and Sna. These targets include positively regulated targets such as the mesodermal genes *Drosophila myocyte enhancing factor 2* (*Dmef2*), *tin*, *Decapentaplegic* (*Dpp*), and the FGF receptor *heartless* (*htl*), as well as negatively regulated mesectodermal genes *rhomboid* and *single minded*.[16,26,37-39] In addition to genes that promote mesodermal development, Twi also regulates a large number of genes encoding regulators of general cell behaviors such as cell adhesion, motility, and proliferation, including Cyclin E and E2F.[31,34,40-44] These functions are required in the migrating mesodermal tissue during gastrulation, and are critical for the establishment of that germ layer.

Mesodermal Subdivision

Once the internal mesodermal layer has formed, it becomes subdivided into distinct domains. It is first divided into segments, and then patterned in the anterior-posterior (A-P) and dorsal-ventral (D-V) directions. The segmentation genes *even-skipped* (*eve*) and *sloppy-paired* (*slp*) partition each segment into A and P domains by regulating the expression of mesodermal genes.[45,46] While Eve acts through Notch (N) to repress *twi* in the posterior half of the hemisegment, Wingless (Wg) activation of Slp maintains *twi* expression in the anterior half, leading to the partitioning of Twi into high and low expression domains[7,47] (Figure 2(a)). The combined Wg, Slp, and Twi activity patterns the heart and somatic muscle tissues by inducing important somatic mesodermal genes such as *slouch* (*slou*), *nautilus* (*nau*), and *muscle segment homeobox/Drop* (*msh/Dr*) while repressing visceral muscle fates[7,47-50] (Figure 2(b)). In the posterior half of the hemisegment, in contrast, a combination of extrinsic Hedgehog (Hh) activity and low Twi expression leads to expression of *bagpipe* (*bap*) and the development of the visceral musculature and the fat body.[47]

The Wg/Slp domain is patterned in the D-V direction by the signaling of the Transforming growth factor β(TGFβ) family member, Dpp, from the overlying dorsal ectoderm.[51-54] Wg and Dpp prepattern the dorsal mesoderm to respond to Ras/MAPK signaling, which is important for the later specification of promuscle clusters.[19] High levels of Dpp repress the lateral and ventral somatic muscle regulators *Drosophila Six-4* (*D-Six4*) and *pox meso* (*poxm*).[51,53,55,56] Additionally, the intersection of Wg and Dpp signaling restricts transcription of Tin to the dorsal anterior portion of the hemisegment and together with high levels of Twi results in the activation of L'sc.[17,53] Tin is a key regulator of cardiac and dorsal somatic muscle development, specifying cardioblast and dorsal somatic muscle progenitors via its regulation of genes such as *eve* and *DMef2*.[16,57-60] In the ventral half of the hemisegment, D-Six4 and Poxm regulate the expression of lateral and ventral muscle genes such as *ladybird* (*lb*) and *slou*.[34,55,56] *eve*, *lb*, and *slou* belong to a group of transcription factors known as identity genes, which are expressed in incompletely overlapping sets of cells and are required to specify particular muscle progenitor and FC fates.[7,61] One interesting possibility is that the expression of genes such as *eve*, *poxm*, and *D-Six4* subdivides each hemisegment into regional muscle groups, which are then capable of responding differently to identity genes such as *slou*. This hypothesis could provide a partial explanation for how identity genes such as *slou* and *msh*, which are expressed throughout the pattern, give rise to distinct muscles in the dorsal, lateral, and ventral regions.

PROGENITOR AND FOUNDER CELL SPECIFICATION

Promuscle Cluster Formation

Following mesodermal subdivision, Wg signaling (or a combination of Wg and Dpp in the dorsal mesoderm) integrates with receptor tyrosine kinase (RTK) signaling, particularly the *Drosophila* epidermal growth factor receptor (DER) and the fibroblast growth factor receptor Heartless (Htl), leading to Ras activation in subsets of cells.[19,21,22,62-64] This inductive RTK signaling restricts L'sc expression to smaller clusters of cells known as equivalence groups.[17] Equivalence groups first arise in the mesoderm during late stage 10

and continue appearing at stereotypic times and locations within the hemisegment until stage 12[17] (Figures 2(c) and 3(a)). Altogether there are 18 L'sc equivalence groups in each abdominal hemisegment comprised of, on average, 4–6 cells.[17] At their specification, all cells in the equivalence group have an equal potential to become a progenitor cell. A combination of Ras signaling and lateral inhibition (requiring Delta signaling via N and Suppressor of Hairless (Su(H)), directs these groups to give rise to cardiac and somatic muscle progenitors.[17,64-69] These progenitors retain high levels of L'sc expression, while the surrounding cells in the equivalence group lose it.[17] At this point, the surrounding cells can join another equivalence group, where they will have another opportunity to be a progenitor, or they can differentiate into FCMs.[17] Oddly, loss of *l'sc* only results in loss of few muscles, leading to the hypothesis that other genes must perform functions redundant to *l'sc*.[17] Evidence suggests that *poxm* may perform a similar function to *l'sc* in the lateral and ventral somatic musculature,[56] but it cannot be the only other factor regulating this process. Using temporally specific Gal4 lines to knock down the expression of *l'sc*, potentially in subsets of equivalence groups, could help teach us more about this process.

FC and FCM Specification

Once a single progenitor has been specified, it undergoes a single asymmetric cell division to form two FCs or a single FC and an AMP[64,70] (Figure 3(b)). In the dorsal region, the progenitor can also divide to form a single FC and a pericardial progenitor. This process is similar to neuroblast specification (Box 1), in that it requires the asymmetric localization of the proteins Inscuteable and Numb (Nb).[70,71] Following division, Nb becomes localized to a single sibling, where it modulates identity gene expression via N. Progenitors express unique combinations of identity genes; upon division, expression of particular identity genes is frequently lost in one sibling FC, leading to a divergence in FC and final muscle identity (Figure 3(c)). Such is the case for the VA1 and VA2 muscles, where *slou* is expressed in the progenitor for these muscles but is only maintained in VA2.[17,72] Alternatively, sibling FCs may continue to express the same identity gene, such as *ap* expression in the LTs.[73] Thirty muscle FCs are specified, leading to the development of 30 diverse muscles in each abdominal hemisegment.[1]

After a single L'sc-expressing progenitor has been selected from the equivalence group, N-mediated lateral inhibition drives the remaining cells to adopt the FCM fate, indicated by expression of the zinc finger transcription factor Lame duck (Lmd).[74-76] While each FC gives rise to a single muscle, multiple FCMs fuse to a single FC/developing myotube, leading to a syncytial muscle fiber. Although all FCMs express Lmd, this cell population has more diversity than initially thought. For example, the Lmd target gene, *hibris*, is expressed in only a subset of FCMs. Moreover, a subset of FCMs also undergo a round of cell division, suggesting that these cells may have positional identities.[77,78] The overlying FCs and myotubes have distinct positions that can be correlated with their identities, further supporting that FCMs have diversity, as well.[78] Future work will be required to elucidate whether FCMs carry positional information important for muscle differentiation; these experiments will require the ability to 'transplant' FCMs from one part of the pattern into another or to alter gene expression in a subset of FCMs.

Whole genome studies have uncovered significant differences in the overall transcriptional landscapes of FCs and FCMs.[77,79-81] As had been previously reported, Lmd regulates genes required for myoblast fusion and muscle differentiation, including *Dmef2* and *sticks and stones*.[74] While Lmd activates expression of genes required for FCM identity, the zinc finger transcription factor Tramtrack69 (Ttk) was recently found to act in a complementary way to repress FC genes in these cells.[80] Like mutations in *lmd*, loss of Ttk function leads to an increase in FC-like cells expressing the FC-marker *rp298-lacZ*, an insertion into *dumbfounded*, which encodes an FC-specific recognition and adhesion receptor required for myoblast fusion.[50,80] Ttk overexpression significantly disrupts the muscle pattern and leads to a decrease in *twi* and *rp298-lacZ* expression. Ttk is therefore an additional key regulator of FCM fate.[80] Importantly, upon myoblast fusion, FCMs adopt the transcriptional profile of the FC or myotube to which they have fused, including expression of FC-specific identity genes.[20,72] The mechanisms that lead to FCM reprogramming upon fusion have not yet been discovered. Future work may help uncover why, for example, the FC genetic program prevails in the presence of repressors such as Ttk.

IDENTITY GENE EXPRESSION AND MUSCLE FIBER DIVERSIFICATION

Twenty identity genes have been identified thus far in the *Drosophila* embryonic somatic musculature (Figure 4 and Table 1). Identity genes share some defining characteristics: (1) they are transcription factors, (2) they are expressed in subsets of muscle progenitors and/or FCs, and (3) loss-of-function embryos display defects in the muscle subset where the identity gene is normally expressed.[7] Examination of mutant embryos has shown that identity genes regulate muscle morphogenesis, including size, shape, orientation, attachment sites, innervation, and number of fusion events.[50,72,73,87-89] While identity genes such as *eve* and *lb* are each expressed in a single lineage, DO1 and SBM respectively, most identity genes are expressed in multiple FCs.[20,87] These incompletely overlapping expression patterns have led to the combinatorial code hypothesis of muscle specification: that distinct combinations of identity genes specify final muscle properties, leading to the observed muscle diversity.

Though some identity genes, such as *collier* (*col*) in the dorsolateral region, show regional specificity in their expression patterns, other genes such as *slou* are expressed throughout the pattern[72,88] (Figure 4). It is highly likely that additional identity genes will be identified, as there are a number of muscles (several of the VOs, for example) for which there is no known identity gene. In fact, six new identity genes have been identified in just the last few years: *araucan* (*ara*) and *caupolican* (*caup*) specify a set of lateral muscles[90]; *lateral muscles scarcer* marks the four LT muscles[91]; *tailup* (*tup*) is expressed in all four dorsal muscles[92]; *optomotor-blind-related-gene-1* (*org-1*) is required for the formation of LO1, VT1, and SBM[86]; and *midline* (*mid*) specifies LT4 fate.[93] Ongoing research is investigating how these identity genes interact with one another to specify muscle fates.

BOX 1

PARALLELS TO VERTEBRATE CRANIOFACIAL MUSCULATURE DEVELOPMENT

Striking similarities have been observed between craniofacial muscle development in vertebrates and muscle specification in *Drosophila*.[82] The 60 skeletal muscles in the vertebrate head form from the cranial paraxial mesoderm (CPM), an unsegmented tissue that arises anterior to the somites. Similar to the way the dorsal mesoderm in flies develops into both cardiac and somatic muscle, cardiac and craniofacial muscle development are also intimately linked in vertebrates, relying on both extrinsic and intrinsic signals.[83] The presumptive craniofacial musculature expresses a distinct set of transcription factors, many of which are provided by orthologs of *Drosophila* identity genes, such as *Twi* and *Mid* (*Tbx20* in vertebrates).[84] In particular, the vertebrate transcription factor Islet 1 (Tup in *Drosophila*) is required for the formation of branchiomeric and cardiac muscles,[82] and the vertebrate homologue of *lb, Lbx*, specifies the lateral rectus extraocular muscle.[85] The vertebrate regulator *Tbx1* marks the head mesoderm and is required for the development of a number of muscles in the head.[84] Recently, the *Drosophila* ortholog of *Tbx1*, *Org-1*, was shown to be an FC identity gene in the dorsal musculature.[86] It is likely many other genes will be shown to have conserved roles during muscle development in flies and vertebrates.

Many identity genes have iterative functions in muscle development: Eve, for example, is required for mesodermal segmentation and patterning, then expressed in promuscle clusters, then progenitors and finally FCs. Some identity genes, such as *Slou*, are expressed throughout muscle development, while others, such as *Ap*, are transiently expressed.[72,73] Identity genes belong to a variety of transcription factor families, although the majority are homeodomain-containing proteins (Table 1). The identity genes *org-1* and *mid* encode T-box containing transcription factors and represent a new identity gene class.[86,93] While all other known identity genes encode proteins containing DNA-binding domains, Vestigial (Vg) also represents a special class: it contains two activation domains, and must interact with a DNA-binding protein to function. Its partner Scalloped (Sd) serves this function in the muscle; Sd also acts as an identity gene on its own in a subset of muscles.[94]

Early mesodermal patterning is required to convey positional information to the FCs, which has been shown for the numerous signaling cascades that converge on the *cis*-regulatory modules of *eve*,[19,64,69] *slou*,[21,22,56] and *col*.[88,95,96]. Such information integrates the aforementioned D-V and A-P signaling cascades within the hemisegment, but also includes Hox transcriptional regulators such as Antennapedia (Antp), Abdominal-A (Abd-A), and Ultrabithorax (Ubx) to pattern the embryonic segments along the A-P axis.[96-99] Hox gene activity is particularly important for differentiating the thoracic from the abdominal hemisegments, though there still remains a great deal to learn about this process. While these converging pathways are important for the initial establishment of identity gene expression, much of this information must be imparted to future FCs by stage 9, prior to equivalence group formation. Primary myotubes that have been cultured from embryos

dissociated at stage 9 express at least five distinct identity genes,[100] demonstrating that ongoing context is not required to specify unique muscle fates.

Interestingly, loss or ectopic gain of identity genes only infrequently leads to direct muscle transformations, where a direct transformation is defined as a muscle in which both physical properties and gene expression have been changed to that of another muscle. Perhaps the clearest examples of a direct change are those of VA2 to VA1 and of LO1 to SBM in *Kr* and *slou* loss-of-function mutants, respectively.[49,101] In most cases, however, either intermediate defects in shape or attachment site are observed, which is the case for the other muscles that normally require Kr function.[101] Even in cases where direct transformations are observed, it is usually only a fraction of hemisegments or embryos that show the transformation. As loss of a single identity gene is rarely sufficient to cause transformations, these observations reinforce the combinatorial code hypothesis that the coordination of multiple identity genes is required to specify final muscle characteristics.

Though identity genes are, by definition, transcription factors, the identification of downstream targets is a fairly recent development. Some identity genes regulate other identity genes, particularly by repressing them in certain muscle lineages. Lb and Msh reciprocally repress their expression in the SBM and LT muscles respectively, leading to changes in muscle fate.[102] More recently, it was shown that the Iroquois gene complex members, Ara and Caup, repress *slou* and *vg* in the LT muscles.[90] In the other direction, Kr is required to positively regulate *slou* expression in VA2, and Org-1 is required for the expression of *slou*, *caup*, and *lb* in SBM, LO1, and VT1.[86,101] Finally, some identity genes autoregulate their expression, as Col does in DA3.[95]

The aberrant muscle morphologies observed in embryos with lossor gain-of-function mutations in particular identity genes suggest that these factors must also regulate downstream genes encoding modulators of cell size, shape, and attachment. One example of this class is the Kr target gene *knockout* (*ko*), which encodes a protein required for the innervation of the LT muscles.[61] Recent whole genome approaches have expanded the known catalogue of identity gene targets and have identified direct binding sites for Lb, Msh, and Slou.[103,104] Lb targets are widely representative of the many facets of morphogenesis regulated by identity genes. These include genes encoding the muscle attachment protein Inflated (if), the actin-binding protein muscle-specific protein 300 (Msp-300), the sarcomeric structural protein Sallimus (Sls), and three genes important for regulating myoblast fusion: Muscle protein 20 (Mp20), Paxillin (Pax), and M-spondin (Mspo).[89,103] As most muscles express multiple identity genes, one possible mechanism of morphogenetic regulation would be for each identity gene in a muscle to regulate a coordinated set of genes controlling a specific cell behavior or aspect of differentiation. A comparative analysis of existing whole genome data, coupled with continued identification of identity gene regulatory targets, will be important for linking FC specification to final muscle properties.

The eventual goal of work on identity genes would be to use the combinatorial code to generate muscles of specific shapes and sizes, *in vivo* and *in vitro*. Such discoveries would have far-ranging implications for stem cell transfer therapies used to treat muscle disease.

Currently, we do not know enough about the identity gene network to turn any one muscle into a muscle of our choice. There are many areas in which we need to enhance our knowledge. First, is there a hierarchy of identity gene expression? Are some identity genes required earlier, to turn on other identity genes or make cells more receptive to them? Additionally, do particular identity genes regulate clusters of genes required for specific cellular behavioral functions, such as attachment or innervation? The large body of work examining *cis*-acting mesodermal enhancers[19,34,104-106] has led to the development of synthetic genetic tools allowing expression in particular single cells or subsets of cells, and more should follow. A continued dissection of the identity gene network using these types of tools will be required to solve this problem.

Mechanisms of Identity Gene Activity

Little is known about the molecular mechanisms by which identity genes regulate the expression of their targets. No studies have identified histone modifications or chromatin rearrangements following binding of these factors, and it is not known to what extent identity genes recruit transcriptional repressors or coactivators to target genes. Recent work has shown that the SAGA transcriptional coactivator complex interacts with a large number of muscle-specific transcription factors and also binds at both the enhancers and coding sequences of muscle-specific genes.[107] Additional molecular studies will be needed to establish timing and activity of identity genes at their targets. Such experiments present challenges, as muscle subsets represent only a small percentage of cells in the total embryo, and experiments need to be tightly timed to monitor dynamic changes in gene expression.

Modifiers of chromatin structure are important in mammalian systems for muscle fiber differentiation[108] and recent work in *Drosophila* has indicated a role for chromatin modifying complexes in the regulation of muscle genes. The cofactor Akirin has been shown to be important for the expression of *DMef2* in the embryo.[109] Akirin interacts with Twi and members of the conserved Brahma chromatin remodeling complex and may function by recruiting Brahma to the *DMef2* promoter.[109] This study provided the first link between Twi and chromatin remodeling in *Drosophila*. Additionally, a novel role has been discovered for the conserved chromatin modifying complex member, Sin3A, in the diversification of muscle cell types.[110] Sin3A acts as a buffer for cell fate choice, modulating the response of muscles to the identity gene expression. Loss or gain of Sin3A expression leads to the transformation of the VA1 and VA2 muscles into one another. This role for Sin3A is very similar to that found in *Caenorhabditis elegans* neural development (Box 2).[111-113] If chromatin structure were locked-in to a particular structural conformation in distinct cell types, that would explain a significant amount of data showing that certain identity gene loss leads to changes in only a subset of muscles where that identity gene is expressed. Changes to chromatin structure may also explain the ability of *Drosophila* primary myoblasts in culture to retain identity information post dissociation. It is anticipated that additional roles for chromatin regulators will be discovered in the somatic musculature, broadening our understanding of the mechanism by which muscles are specified. To discover what makes the chromatin in one FC different from another, the field will need to make use of technologies enabling the assessment of chromatin on a single-cell level.

ADULT MUSCLE SPECIFICATION

Specific muscle groups in the adult head, thorax, abdomen and leg control walking, flying, feeding, and mating.[8] Adult muscles are formed from AMPs that were generated during FC specification earlier in development.[2] Twi-expressing AMPs persist during larval development in small clusters associated with the nerves and imaginal discs, where they are referred to as adepithelial cells.[2,118] During the larval phases, AMPs proliferate.[119] Some AMPs act as founders to seed the formation of adult thoracic, leg and body wall muscles, while others act like embryonic FCMs and fuse to developing myotubes. Like muscles formed in the embryo, adult muscles are syncytial and are formed from the fusion of mononucleate myoblasts. Adult muscles, however, are composed of multiple fibers, while the larval muscles are single fibers.

BOX 2

MYOBLAST AND NEUROBLAST SPECIFICATION SHARE A NUMBER OF CHARACTERISTICS

Somatic muscle progenitor selection and neuroblast specification in *Drosophila* are analogous processes. The similarities start with the fact that, in the segmented ventral nerve cord, there are approximately 30 neuroblasts per hemisegment, just like myoblasts. Like subdivision of the mesoderm, the neuroectoderm is patterned in both the dorsal/ventral and anterior/posterior directions by signaling proteins that include Wg and the *Drosophila* EGF receptor.[114] This patterning conveys positional information, leading to the development of distinct neuroblasts in stereotypical locations. Clusters of cells express the proneural genes of the *achaete-scute* complex (AS-C): *achaete*, *scute*, and *l'sc*. As in myoblast specification, lateral inhibition mediated by N leads to the restriction of AS-C gene expression to a single cell, which will become the neuroblast.[115] Different transcription factors are expressed within different proneural clusters and are important for the final neuroblast fate. A number of these genes, such as *eve*, function as identity genes in the somatic mesoderm.[116] Though an unsegmented tissue, neuroblasts in the brain are also specified from proneural clusters. Incompletely overlapping sets of transcription factors are expressed in the brain neuroblasts, imparting identity information to these cells.[117] Many FC identity genes play this dual role in the neuroblasts: among the factors conferring identity information to neuroblasts are *run*, *msh*, *lb*, *ap*, and *Kr*. Likewise, many neurogenic genes have roles in muscle.[66] The striking correspondence between these two processes means that findings in one tissue have strong relevance for understanding of the other.

The largest group of adult thoracic muscles are the IFMs[119] (Figure 5). In all, there are 26 IFMs: 7 pairs of dorsal-ventral muscles (DVMs), which are responsible for wing elevation, and 6 pairs of dorsal longitudinal muscles (DLMs), which antagonize the action of DVMs by depressing the wings.[119] In contrast to other thoracic, leg, and abdominal muscles, like the tergal depressor of the trochanter (TDT or 'jump muscle'), which are tubular muscles, IFMs are fibrillar and are enriched for the expression certain proteins, including flightin and Actin88F.[9,120,121] Tubular muscles have laterally aligned sarcomeres and contract

synchronously, while fibrillar muscles have nonaligned myofibrils and contract asynchronously.[122] Despite these shared properties that set them apart from the other thoracic muscles, DVMs, and DLMs follow different developmental programs: DVMs are formed completely from the fusion of AMPs, as is the case for the majority of the adult muscles, including the jump muscle. DLMs, however, develop from three pairs of larval oblique muscles.[119] These templates in the second thoracic hemisegment are actually DO muscles 1–3, which were specified during embryonic development, and undergo fiber splitting at the onset of metamorphosis.[119]

Many genes required to specify muscles in the embryo are used again to specify adult muscle properties. The formation of the abdominal and leg muscles has been reviewed elsewhere[8,123]; here we will discuss the genetic control of founder specification, as well as IFM and DFM development.

AMP Specification and Founder Selection

AMPs are set aside during embryonic development as undifferentiated, Twi-expressing cells. Like vertebrate muscle stem cells, or satellite cells, AMPs are muscle lineage-committed stem-like cells.[124] Six AMPs are specified in each abdominal hemisegment, which go on to form the adult abdominal muscles.[8] Under the control of Hox gene regulation, more AMPs are specified in the thoracic hemisegments, where they give rise to the DFMs and IFMs.[125] In addition to Twi, AMP specification and number are also regulated by EGF signaling pathway. The EGF pathway protein Rhomboid (Rho) and the EGF ligand Spitz (Spi) are both required for the specification and maintenance of the correct number of AMPs.[124] Evidence suggests that AMPs receive positional cues similar to their sibling FCs: the dorsal AMP, sibling to the DO1 FC, also forms the dorsal-most abdominal muscle in adults.[8] Moreover, during larval development, AMPs express transcription factors reflective of their positions: the embryonic identity genes *lb* and *Kr* are expressed in the lateral abdominal AMPs, *slou* and *poxm* are expressed in the ventral abdominal AMPs, and *vg* is expressed in the thoracic AMPs.[124,126]

Twi and Dmef2 are required for several events in adult muscle development, including fiber splitting, myofibrillar gene expression, and myoblast fusion.[37,127-129] Twi and N work together to maintain AMPs in a quiescent state during embryonic and larval development.[129] N is required to maintain Twi expression in the AMPs, and both proteins are responsible for keeping AMPs in an undifferentiated state. Twi and the N downstream effector Su(H) bind to the promoters of target genes, including those encoding repressors of differentiation such as *zinc finger homeodomain 1* (*zfh1*) and *Holes in muscle* (*Him*).[130] Him directly represses *Dmef2*, which is required for muscle fiber differentiation.[37,129] Twi and Su(H) also repress other genes important for FC specification and myoblast fusion, including *roughest*, *dumbfounded*/*kirre* (*duf*) and *Down syndrome cell adhesion molecule 1*.[130] During adult muscle differentiation, *twi* is downregulated, while *Dmef2*, *erect wing*, and other genes required for differentiation are upregulated.[37,129,131]

While the DLMs use the embryonic-derived muscles DO1-3 as templates for fiber formation, the other muscles in the adult are formed by the fusion of myoblasts with a founder that has been selected from the pool of AMPs.[119,132] As with myoblasts expressing

high Twi in the embryo, an appropriate number of AMPs will be specified as founders during metamorphosis.[132] Like FCs in the embryo, adult founders are marked by the expression of *duf* , which becomes restricted to a subset of AMPs.[132] The choice of which AMP becomes the muscle founder does not appear to require N-mediated lateral inhibition as in embryos. Instead, founder choice in the adult is mediated by FGF signaling via Htl.[133] Reduction of Htl or activation of the RTK-pathway antagonist, Yan, leads to a decrease in adult founders, while activation of Htl or the positive RTK regulator, Pointed, leads to an increase in founder number.[133] Htl is expressed in all Twi-expressing AMPs during third-instar larval and pupal development. Htl acts via the FGF signaling protein Heartbroken/Stumps (Hbr), which becomes localized to the *duf* -expressing adult founders during pupariation. To ensure that adult founders are not selected earlier in development, the negative regulator of FGF signaling, Sprouty, is expressed in all AMPs prior to pupariation.[133] The integration of these signals ensures that the appropriate number of adult founders is specified for differentiation into the wide variety of adult muscles types.

Development of Specific Adult Muscle Types

Differential gene expression marks the various types of adult muscles, and embryonic identity genes are used again in adult muscle specification. The IFMs and DFMs differ from each other in significant ways, including gene expression patterns, fiber type, and location. The DFMs, for example, express the transcription factor *ap*, while the DLMs (a subset of IFMs) express *actin88F*.[120,126,134] In particular, much work has elaborated the regulatory networks specifying DLMs from DFMs, which depend upon expression of the transcription factors Vg and Cut (Ct).

DFM Development—While all AMPs express Ct,[124] the population giving rise to DFMs express much higher levels of Ct than those that will form the DLMs. Ct and Vg form a negative feedback loop in the adult musculature. In the DFM-presumptive AMPs, Ct maintains Vg in a repressed state.[126] During metamorphosis, *ap* expression is activated in these high Ct/low Vg cells.[126,134] Ap is required non-autonomously for DFM formation, where its expression regulates *stripe* (*sr*) expression in adjacent ectodermal cells and the formation of muscle attachments.[135,136] Ectopic expression of Ap in the DLMs causes muscle degeneration in these fibers, which underscores the necessity for Ap negative regulation by Vg in these muscles.[137] The identity gene *lms* was recently found to be co-expressed in the embryonic LT muscles with *ap*.[91] Interestingly, Lms is expressed in the presumptive DFM myoblasts, though earlier than Ap. Lms is required along with Ct for repression of Vg in these myoblasts.[91] Future work will be required to determine whether other embryonic identity genes play distinct roles in the development of the adult musculature.

IFM Development—Wg signaling from the notum activates Vg in the presumptive-DLM myoblasts. Vg and its cofactor Sd are coexpressed in these myoblasts, which, in contrast to DFMs, express low Ct levels. Consequently, Ap is not expressed in these cells. Instead, Vg activates expression of the fiber-specifying factor Spalt-major (Salm) in the DLMs.[138] Salm is a switch that directs muscles to adopt the fibrillar fate: ectopic expression of Salm in the abdominal or leg muscles changes those from a tubular to fibrillar structure. Salm positively

regulates IFM-specific proteins and represses genes required for tubular muscle formation.[138] Interestingly, Salm does not regulate transcription of *Actin88F* in the IFMs. Instead, the homeodomain proteins Extradenticle (Exd) and Homothorax (Hth) directly activate *Actin88F* expression.[139] Exd and Hth also positively regulate Salm expression in the DLMs and repress expression of tubular genes; whether *exd* and *hth* are themselves regulated by Vg has not yet been tested.[139] Thus, two coordinated gene networks are responsible for specifying DLM versus DFM fate and have been shown to do so by regulating downstream components of myofibrillar structure.

Salm has been shown to play another role in the specification of fibrillar muscles. In addition to regulating the transcription of IFM-specific proteins, it also affects which splice isoforms of sarcomeric structural proteins such as Stretchin (Strn-Mlck), Myofilin (Mf), and Sallimus (Sls) are expressed.[140-142] Salm itself is not a splicing factor; instead, it functions through the RNA-binding protein Arrest (Aret, also known as Bruno).[141,142] In addition to Salm, Aret expression also requires Exd and Hth in the flight muscles.[142] These pathways recall the function of the RNA-binding proteins Muscleblind (Mbl) and Hoi Polloi (Hoip), which regulate RNA splicing of sarcomeric proteins in the larval somatic musculature.[143-145] Mbl targets the alternative splicing of muscle differentiation genes, such as *troponin T*, which give rise to functional sarcomeres and myotendinous junctions,[144,145] while Hoip regulates the pre-mRNA splicing of the structural proteins Myosin heavy chain (Mhc) and Tropomyosin (Tm1).[143] Posttranscriptional regulation of gene expression is an emerging area of muscle biology, with important implications for fiber type selection. Future work will undoubtedly elucidate additional mechanisms distinguishing each type of adult muscle fiber.

CONCLUSION

An overarching question in development is how numerous tissues and cell types are derived from common origins. The larval and adult somatic muscle patterns in *Drosophila* provide examples of diverse tissues with common progenitors. They share many similarities with the somatic muscle systems of vertebrates, including cellular structure and conserved regulatory factors (Box 1). The complex patterns of muscle in the larva and adult help the organism to move and feed. The great diversity in the muscle patterns, which provides distinct muscles to perform specific functions, is laid out by both extrinsic and intrinsic influences. The integration of signaling cues from the overlying epidermis with cell autonomous transcription factors provides positional context to developing myoblasts. This information is further refined by the combinatorial coordination of multiple transcription factors, which converge on *cis*-regulatory modules to direct muscle-specific gene expression. Many of the genes and pathways important for this process are used iteratively throughout development, and the similarity between muscle and neuroblast specification highlights the way in which regulatory mechanisms can be repurposed for multiple developmental processes (Box 2).

Recent work in these systems has uncovered new insights into the process of muscle specification. Whole genome studies have explored the mechanisms by which multiple transcription factors cooperate in *cis*-regulatory networks to control gene expression. Additionally, several new FC identity genes have been discovered, as well as a new FCM-

specific transcription factor. Finally, work in adult muscles has revealed the regulatory mechanisms that govern fiber-type choice. Despite these advances, researchers are still searching for the overarching code that would allow them to convert one muscle into any other type. It will be exciting to see what future studies have to show us about the generation of muscle diversity.

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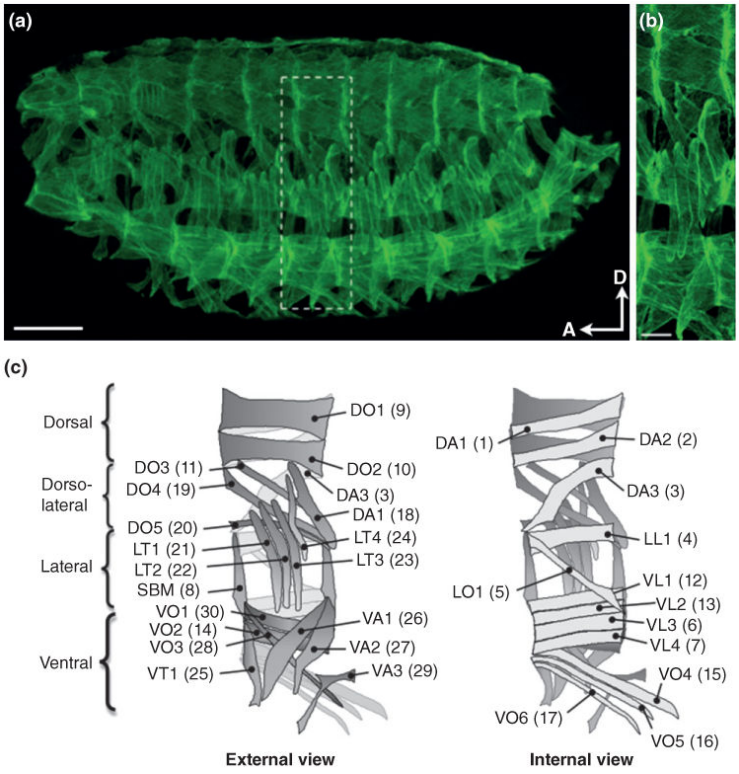

FIGURE 1.

The embryonic and larval muscle pattern. (a) Lateral view of a stage 16 embryo stained with an antibody against myosin heavy chain to reveal the segmentally repeated muscle pattern. Scale bar, 50 μm. For this and all subsequent images, dorsal is up and anterior is left. (b) Close-up of a single abdominal hemisegment from (a), showing the 30 unique muscles in the hemisegment. Scale bar, 10 μm. (c) External (left) and internal (right) schematics of the abdominal muscle pattern. Muscles can be grouped into four regions: dorsal (DA1, DA2, DO1, DO2), dorsolateral (DO3, DO4, DO5, DA3, DT1, LL1), lateral (LT1-4, LO1, SBM), and ventral (VA1-3, VL1-4, VO1-6, VT1). The alternate muscle numbering system is shown in parentheses after the muscle name. (Reprinted with permission from Ref 146. Copyright 2010 Elsevier)

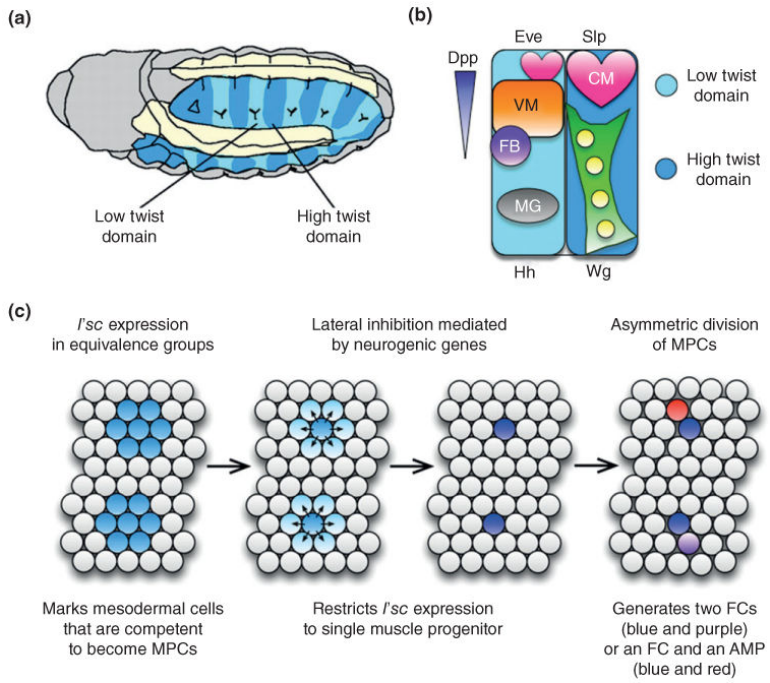

FIGURE 2.

Mesodermal subdivision and equivalence group formation. (a) Cartoon of a stage 10 embryo showing Twi expression modulated into high (dark blue) and low (light blue) domains. Cells of the high Twi domain give rise to all somatic muscles. (b) Cartoon of a single hemisegment, showing mesodermal subdivision and the tissues that arise from those regions. CM, cardiac muscle (pink); VM, visceral muscle (orange); FB, fat body (purple); MG, mesodermal glia cells (gray). The somatic muscle is colored green with yellow nuclei. (c) Expression of L'sc marks groups of mesodermal cells that are competent to become muscle progenitors. Lateral inhibition restricts l'sc expression to a single progenitor. Cells that lose L'sc expression become FCMs. Asymmetric division of progenitors generate two FCs (blue and purple) or an FC and an AMP (blue and red, respectively). (Reprinted with permission from Ref 7. Copyright 1998 Cell Press)

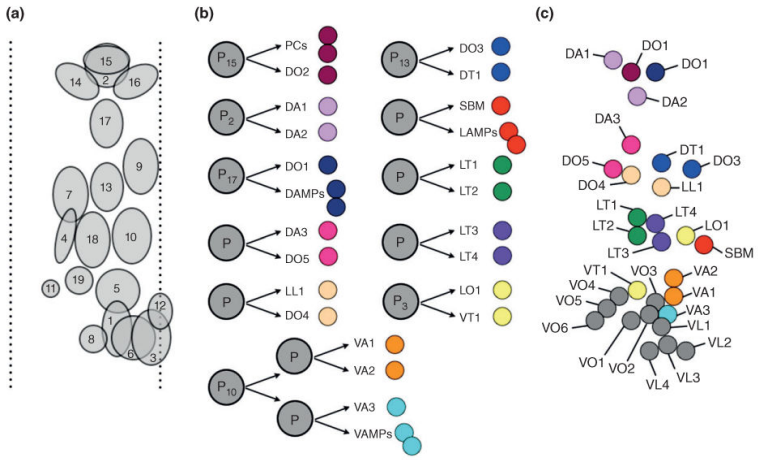

FIGURE 3.

Progenitor and founder cell specification. (a) Cartoon showing the locations of the L'sc-expressing equivalence groups within a single abdominal hemisegment. (Reprinted with permission from Ref 17. Copyright 1995 Cold Spring Harbor Laboratory Press) (b) Progenitors (P) divide to give rise to FCs for particular muscles as well as pericardial cells (PCs) and dorsal, lateral, and ventral adult muscle precursors (DAMPs, LAMPs, and VAMPs, respectively). Sibling FCs are depicted, and the number of the equivalence group (subscript) from which the progenitor arose is indicated if known. (c) Stereotypical arrangement of FCs in a single abdominal hemisegment at stage 12. Sibling FCs share the same color. (Reprinted with permission from Ref 78. Copyright 2007 Elsevier)

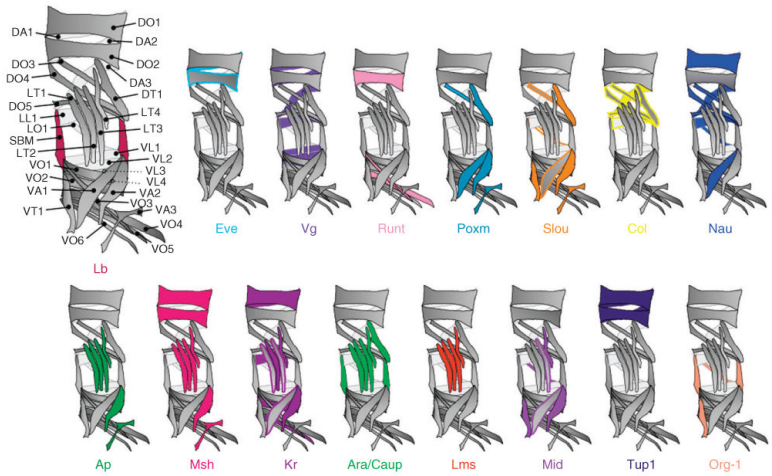

FIGURE 4.

Identity genes are expressed in incompletely overlapping subsets of muscles. Schematic of the muscle pattern in a single abdominal hemisegment at stage 16. Muscles are color-coded to show identity gene expression patterns. A colored outline marks identity gene expression in progenitors that is lost from FCs. Note that some identity genes, such as ap, are expressed in FCs at stage 13 but lost in the final muscle at stage 16. (Reprinted with permission from Ref 7. Copyright 1998 Cell Press)

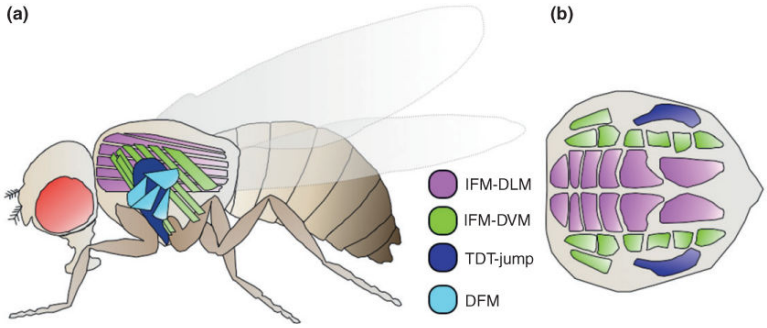

FIGURE 5.

Adult muscle pattern. Schematic depicting the arrangement of muscles in the adult thorax as seen in a lateral view (a) and a cross-section through the thorax (b). Anterior is to the left in both panels; dorsal is up in panel (a) and (b) represents a dorsal view. Both groups of indirect flight muscles (IFMs) are shown: 6 pairs of dorsal longitudinal muscles (DLMs, magenta) and 7 pairs of dorsoventral muscles (DVMs, green). Direct flight muscles (DFMs) are shown in light blue, and the tergal depressor of the trochanter (TDT, also known as the jump muscle) is shown in dark blue. (Reprinted with permission from Ref 126. Copyright 2001 Cell Press. Reprinted with permission from Ref 147. Copyright 2010 Frontiers)

TABLE 1

Identity Genes and their Expression Patterns during *Drosophila* myogenesis

Identity Gene	Transcription Factor Type	Progenitor Expression	FC and Muscle Expression	Vertebrate Ortholog
Apterous [ap][73]	LIM homeodomain	LTs1-4, VA2, VA3	LTs1-4, VA2, VA3	Lhx2, Lhx9
Collier (col)[88,148]	COE	DO3, DO4, DO5, DA3, DT1, LL1	DA3	Ebf4
Even-skipped (eve)[20,149]	Homeodomain	DA1, DO2	DA1	Evx1, Evx2
Muscle segment homeobox/Drop (msh/Dr)[50]	Homeodomain	DO1, DO2, LTs1-4, VA2, VA3	DO1, DO2, LTs1-4, VA2, VA3	Msx
KRÜPPEL (Kr)[101]	Zinc finger	DA1, DO1, LTs1-4, LL1, VA1, VA2, VO2, VO5	DA1, DO1, LT2, LT4, LL1, VA2, VO2, VO5	KLF
Twist (twi)[11,14]	bHLH	DO1, DO3, LT2, LT4, VAs1-3	DO1, DO3, LT2, LT4, VAs1-3	Twi
Nautilus (nau)[48,66]	bHLH	DO1, DA3, DOs3-5, LL1, LO1, VA1	DO1, DA3, DOs3-5, LL1, LO1, VA1	MyoD
Slouch/S59 (slou)[49,72]	Homeodomain	DO3, DT1 LO1, VAs1-3, VT1	DT1, VA2, VA3, VT1	Nkx1
Runt (run) [150]	RUNT	VO3, VO4	DO2, VO3, VO4	Runx
Ladybird (lb)[87]	Homeodomain	SBM	SBM	Lbx
Pox meso (Poxm)[56]	Paired	DO3, DT1, VAs1-3	DO3, DT1, VAs1-3	Pax1, Pax9
Vestigial (vg)[151]		DA1, DA2, DA3, LL1, VLs1-4	DA1, DA2, DA3, LL1, VLs1-4	VGLL
Scalloped (sd)[94]	TEF-1	Throughout pattern	Throughout pattern, becomes restricted to ventrals	TEAD
Ptx1[152]	Homeodomain	Ventral muscles	Ventral muscles	Pitx
Lateral muscles scarcer (lms)[91]	Homeodomain	LTs1-4	LTs1-4	
Tailup (tup)[92]	LIM Homeodomain	DA1, DA2, DO1, DO2	DA1, DA2, DO1, DO2	Islet-1
Optomotor-blind-related-gene-1 (Org-1)[86]	T-box	SBM, LO1, VT1	SBM, LO1, VT1	Tbx1
Midline [mid][93]	T-box	LT3, LT4, VA1, VA2, LO1	LT4, LO1, VA2, VT1	Tbx20
Araucan (ara)[90]	Homeodomain	LTs1-4, SBM, DT1	LTs1-4, SBM, DT1	Irx4b
Caupolican (caup)[90]	Homeodomain	LTs1-4, SBM, DT1	LTs1-4, SBM, DT1	Irx

Genes with recently identified functions in muscle specification are highlighted in gray.