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Somatic muscle specification during embryonic and post-embryonic development in the nematode *C. elegans*

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Abstract

Myogenesis has proved to be a powerful paradigm for understanding cell fate specification and differentiation in many model organisms. Studies of somatic bodywall muscle (BWM) development in *Caenorhabditis elegans* allow us to define, with single cell resolution, the distinct hierarchies of transcriptional regulators needed for myogenesis throughout development. Although all 95 BWM cells appear uniform after differentiation, there are several different regulatory cascades employed embryonically and post-embryonically. These, in turn, are integrated into multiple extrinsic cell signaling events. The convergence of these different pathways on the key nodal point, that is the activation of the core muscle module, commits individual cells to myogenesis. Comparisons of myogenesis between *C. elegans* and other model systems provide insights into the evolution of contractile cell types, demonstrating the conservation of regulatory schemes for muscles throughout the animal kingdom.

INTRODUCTION

Muscle has long been a favorite tissue type for studies aiming to understand developmental mechanisms. Generally speaking, muscle is an abundant, easily defined, easily dissected tissue that is readily amenable to biochemical and physiological study. The diversity of muscle types within an organism allows for comparative studies that can reveal differences in muscle architecture and myofilament composition underlying the variety of contractile properties of this diverse tissue. Muscle was an obvious target of the new tools emerging with the molecular biology revolution that provided the means to examine gene expression, differential splicing, and characterizations of protein isoform diversity. Consequently, muscle-related biology serves as a perennial paradigm for tissue development, providing insights into the logic of both cell fate decisions and organ development.

This review will focus on the development of the bodywall musculature in *Caenorhabditis elegans*, the somatic musculature of the animal and the functional equivalent of mammalian skeletal muscle. After a brief, general introduction into *C. elegans* myogenesis, this review will focus on the cell autonomous transcriptional cascades known to be involved in the specification and differentiation of bodywall muscle (BWM). The action of these transcription factors will be integrated with cell-extrinsic signaling events that influence target gene selection and the activity of the transcriptional players. The last part of the review will briefly touch on the general developmental themes that have emerged from the

study of muscle as well as evolutionary considerations of how information from the worm informs the bigger picture view of mammalian development and evolution.

***C. elegans* Myogenesis**

There are several different types of muscle in *C. elegans* and each is considered to be mesodermal in origin (Figure 1). The muscle types are bodywall, pharyngeal, enteric, gonadal, and the sex-specific muscles used for egg laying in the hermaphrodite and mating in the male. Most of the bodywall (81 cells) and all pharyngeal and enteric muscles develop embryonically, whereas additional BWM (14 cells) and all gonadal and sex-specific muscles develop during larval stages. BWM is the most abundant muscle type, by volume and cell number, and provides the locomotive force to move the animal. This muscle is ultrastructurally and functionally equivalent to mammalian skeletal muscle. Pharyngeal muscle, a rhythmically contracting muscle comprised of 20 cells located in the head, pumps food into the gut. It is often considered the equivalent of mammalian cardiac muscle because of its contractile properties, function, and developmental specification, although this is likely an over simplification of the ontogeny and phylogeny of the pharynx. The remaining muscle types are relatively minor in terms of numbers or volume. The enteric muscles (four cells, also known as somatointestinal muscle) control defecation while the gonadal sheath cells (10 cells per hermaphrodite gonad arm) aid in oocyte maturation and movement. These muscles resemble mammalian smooth muscle types. The final muscle type is the sex-specific muscle, which in the hermaphrodite consists of 16 uterine and vulval muscle cells controlling egg laying. Whereas, the uterine muscles are morphologically and functionally related to smooth muscle cells, the vulval muscle myofilament organization resembles striated muscle. An excellent anatomical and morphological description of all muscle cell types is provided in WormAtlas <http://www.wormatlas.org/>

The somatic BWMs develop during embryogenesis as four distinct quadrants that run from the tip of the head to the tail.¹ Each quadrant is two cells wide and the individual spindle-shaped cells interdigitate within a given quadrant using tight mechanical junctions. Unlike somatic muscle in most other organisms, the BWM cells of *C. elegans* do not fuse, instead remaining as individual cells throughout development. Aside from that, most other characteristics of the BWM are analogous to mammalian skeletal muscle, including the striated myofilament lattice and general organization of the sarcomeres. Coordinated contraction of this muscle in the dorsal and ventral quadrants propagates as a wave along the body resulting in the forward and backward movement of the animal in response to environmental stimuli.

EMBRYONIC SOMATIC MUSCLE DEVELOPMENT

The Embryonic BWM Module

The 81 embryonic BWM cells are derived from four of the five somatic founder blastomeres (AB, MS, E, C, and D).² The D blastomere gives rise to only BWM (20 cells), the C blastomere predominantly gives rise to BWM (32 cells) and hypodermis, the MS blastomere generates BWM (28 cells) and many other cell types, and a single BWM cell is descended from the AB blastomere (Figure 2). Only the clonal gut precursor founder blastomere (E) fails to generate muscle. In each founder blastomere lineage, the timing for the specification and commitment to the BWM fate can be different. However, after the completion of most cellular proliferation and migration by the comma to 1.5-fold stage of embryogenesis, all 81 BWM cells begin to execute terminal differentiation nearly synchronously.

With the exception of the lone BWM cell derived from AB, we now have a good understanding of the transcriptional events required for BWM specification and

development during embryogenesis. At the heart of somatic myogenesis is a core muscle module comprised of two transcription factors that operate zygotically: HLH-1 and UNC-120.³ Both factors are present in all BWM cells and they act in concert in BWM precursors to drive specification and differentiation. This muscle module illustrates many common developmental themes (discussed below), including redundancy, positive feedback loops, and feed-forward regulation that together ensure that once committed to myogenesis, a cell executes that fate completely.

The importance of HLH-1 and UNC-120 for myogenesis is highlighted by either the loss-of-function mutant phenotypes or artificial situations in which a given factor is ectopically expressed in uncommitted blastomeres. HLH-1, the most potent myogenic factor of the two, is the lone *C. elegans* homolog of the mammalian myogenic regulator factors (MRFs: MyoD, Myf-5, MRF-4, and Myogenin). In mammals, the MRFs regulate the specification and development of skeletal muscle by directly regulating the activity of most (if not all) downstream target genes needed for muscle specification and differentiation.⁴ In *C. elegans*, loss-of-function mutations in *hlh-1* result in severe defects in embryonic BWM development and organization.^{5,6} The *hlh-1* mutant animals are uncoordinated (Unc), appear dumpy with bulges along the body, and die in the first larval stage (L1) with high penetrance. In addition, over expression of HLH-1/MRF in early *C. elegans* blastomeres can convert essentially all cells to a BWM-like fate. Despite these potent myogenic properties, in the absence of HLH-1/MRF activity, all BWM cells are born and execute an easily identifiable BWM phenotype, including an assembly of a myofilament lattice with contractile properties.⁵ This was a clear genetic clue that other myogenic factors worked in concert with HLH-1/MRF to drive embryonic BWM development in the worm.

The second half of the core muscle module, UNC-120, is related to the MADS box-type transcriptional regulator serum response factor (SRF) in mammals. UNC-120 is one of two MADS box transcription factors in the worm, the other being MEF-2. MEF-2 is a critical somatic myogenic factor in flies and mammals,⁷ but knockouts of *mef-2* in *C. elegans* failed to reveal a role in myogenesis.⁸ In contrast, loss of function for the other MADS box protein, UNC-120/SRF, results in post-embryonic BWM dysfunction and paralysis in *C. elegans*.³ As its name implies, SRF was originally described as a general transcription factor in tissue culture cells that is upregulated in response to serum.⁹ However, subsequent studies associated SRF- and MEF-2 DNA-binding activity¹⁰ and conditional knockout studies in the mouse have shown an important role for SRF in muscle differentiation.¹¹ Like HLH-1/MRF, UNC-120/SRF is present in all embryonic blastomeres committed to the BWM fate. Moreover, ectopic expression of UNC-120 in early embryonic blastomeres is sufficient to convert most cells to a BWM-like fate.³ Thus UNC-120/SRF is a potent myogenic transcription factor and early cell type-specific marker of the BWM fate in the worm, sharing many myogenic characteristics with HLH-1/MRF.

Although not present in every BWM cell precursor, a third transcription factor needs to be considered in a description of the muscle module. This factor, HND-1, is the weakest in terms of myogenic activity and it is present in many non-muscle lineages,¹² precluding it from bona fide muscle module status. HND-1 is related to the mammalian HAND transcription factors that first became famous for their essential role in heart development.¹³ Subsequent studies have shown HAND factors to be important in many different tissue types in vertebrates.¹⁴ In *C. elegans*, HND-1/HAND is present early on in the lineage of many BWM precursors. Unlike HLH-1 and UNC-120, HND-1 activity in BWM lineages is transient and disappears prior to terminal differentiation. Mutational loss of HND-1/HAND activity has no discernable effect on BWM development, but it does have a profound effect on somatic gonad development post-embryonically, reflecting the many and diverse developmental roles of this transcription factor.¹² Despite these other roles, the presence of

HND-1/HAND in early BWM precursors and its myogenic activity in blastomere cell fate conversion assays all hinted at its important role in BWM cell fate specification. Indeed, it is HND-1/HAND-dependent activity in the MS lineage (see the following paragraphs) that results in the BWM cells present in the *hlh-1*, *unc-120* double knockout embryos.³ Only the knockout of all three transcription factors simultaneously results in an embryo devoid of all BWM cells as defined by myosin heavy chain staining (Figure 3).

Posterior Embryonic BWM Development

Although the core muscle module is critical for embryonic bodywall myogenesis, the road to activation of these two transcription factors is varied among the myogenic lineages. The simplest transcriptional cascade occurs in the posterior embryonic lineages derived from the C and D blastomeres; D clonally gives rise to BWM while C gives rise predominantly to BWM and hypodermis. In both lineages, HLH-1 and UNC-120 can be detected in nuclei of cells within one cell division of the commitment to BWM as reflected in the invariant lineage; HND-1 is also present early on. The key question is how does this zygotic muscle module get activated so early in the posterior lineages. The answer was strongly suggested by the mutant phenotype of *pal-1* that encodes a homeobox transcription factor related to Caudal in *Drosophila* and Cdx factors in mammals, all of which pattern posterior aspects of development.

In *C. elegans*, PAL-1 activity is contributed maternally, but it is also activated zygotically.^{15,16} Maternal PAL-1 activity is restricted to the posterior somatic blastomeres (C and D) through post-transcriptional control mechanisms that regulated which blastomeres will both translate this ubiquitous maternal mRNA and retain transcriptionally active PAL-1 protein.^{16,17} Maternal PAL-1 activity that is restricted to the C and D blastomeres overlaps with the subsequent transcriptional activation of zygotic *pal-1* in these two blastomere lineages, likely aided by autoregulation through a positive feed-back loop. Loss of *pal-1* activity results in an embryo completely lacking C and D descendants, revealing the critical role of PAL-1 in posterior cell fate specification. Thus PAL-1 was an obvious candidate for being the upstream activator of the muscle module in these posterior lineages. Indeed, PAL-1 has been shown to bind enhancer regions upstream of both *hlh-1* and *unc-120*, thereby mediating the handoff from maternal to zygotic control of development and directly activating the muscle module.¹⁸

In addition to its myogenic role, PAL-1 is also essential for specifying hypodermal cell fate from the C lineage. How can PAL-1 regulate both BWM and hypodermal cell fate in the posterior embryonic C lineage? The answer lies in the observation that the activity of several transcription factors in early *C. elegans* cell fate specification is bifunctional and dependent on the relative levels of the TCF/LEF factor homolog POP-1. Blastomeres that divide along the anterior–posterior (A–P) axis in the embryo yield descendants that differ in the relative levels of nuclear POP-1/TCF protein and activity; the anterior daughter always has higher nuclear POP-1 than its posterior sister cell.¹⁹ The consequence of this asymmetry in POP-1 activity is that a single transcription factor can be used to direct very different developmental outcomes, as is the case for PAL-1. In the presence of low (or absent) POP-1, PAL-1 specifies BWM fate, whereas in the presence of high POP-1 levels, PAL-1 specifies hypodermal fate.²⁰ Thus, in the D lineage that has low or no POP-1 at birth, all descendants are specified as BWM by PAL-1 activity. Within the C lineage, all BWM cells are derived from posterior daughters of A–P divisions while the anterior sisters give rise to hypodermis and other cell types. It is clear from the invariant cell lineage exactly when this decision is made in the C descendants. However, the molecular mechanisms underlying which blastomere is selected to execute cell fate commitment directed by PAL-1 and how POP-1 influences fate determination is not understood. Early embryonic asymmetries in POP-1

within the BWM lineage are regulated by non-canonical WNT signaling, suggesting it is a combination of intrinsic and extrinsic cues that distinguish cell fate choices among early C lineage descendents.

Anterior Embryonic BWM Development

Anterior embryonic BWM refers to the 28 cells derived from the MS lineage; as yet, we have no understanding of how a single BWM cell develops from the AB lineage. BWM is just one of several different cell types originating from the MS lineage that also gives rise to pharyngeal muscle, neurons, and the post-embryonic mesodermal blast cell, M (see the following paragraphs). Thus, MS is a very challenging lineage in which to dissect the intrinsic and extrinsic factors influencing cell fate specification.

Although MS BWM specification is dependent on the muscle module (HLH-1, UNC-120) with a partial assist by HND-1, the temporal-spatial pattern of deployment of these factors is complex, as is the upstream cascade of transcriptional regulators. For example, HND-1 is the earliest of the myogenic factors to be present in MS descendents, but its expression is not restricted to BWM precursors¹² (Figure 4). Several other transcription factors function redundantly with HND-1 in the MS lineage to eventually activate *hlh-1* and *unc-120*, thereby committing the cells to the BWM fate. Like HND-1, none of these other factors is restricted to the BWM lineage, suggesting it is the combinatorial effects of multifunctional transcription factors that direct myogenesis.

The cascade of transcription factors that regulate MS fates has recently come into sharper focus, revealing that they function as a transient, spatially overlapping, and sometimes functionally redundant set. At the top of the zygotically active cascade are the nearly identical factors MED-1 and -2 that are related to GATA-type transcription factors.²¹ In the absence of MED activity, the entire MS lineage fails to develop. MED-1 and -2 activity persists for two cell cycles (four MS cell stage) and each redundantly activates the T-box factor encoding gene *tbx-35* and the homeobox encoding gene *ceh-51*.²² Both of these factors are required for two of the main cell types emerging from the MS lineage, pharyngeal tissue (anterior MS lineages), and BWM (posterior lineages). Not surprisingly, the ability of these two factors to direct these two very different cell fates is dependent on POP-1/TCF levels (Fukushige and Krause, unpublished). TBX-35 is detected between the two and eight MS cell stage, whereas strong CEH-51 is detected between the four and eight MS cell stage. During this same time, HND-1 is activated in the posterior MS descendents, but perdures only in a subset of lineages that will eventually give rise to BWM; HND-1 is undetectable by the time of BWM differentiation.¹²

Two additional genes encoding transcription factors important for BWM are activated by the combinatorial activity of TBX-35, CEH-51, and HND-1. These are the homeodomain containing factors CEH-34 and UNC-39 that are both related to mammalian SIX proteins implicated in the development of several cell fates. In mammals, SIX-type transcription factors have been implicated in many aspects of myogenesis, from MRF activation²³ to differentiation.²⁴ In *C. elegans*, CEH-34/SIX has been shown to be required for post-embryonic mesoderm development²⁵ (see the following paragraphs). Current studies suggest a clear role for both CEH-34/SIX and UNC-39/SIX in embryonic somatic BWM development, although each of these factors is also present in non-BWM lineages.

On the basis of BWM phenotypes of gene loss-or reduction-of-function studies for HND-1/HAND, CEH-34/SIX, and UNC-39/SIX either alone or in combination, two themes have emerged. First, MS sub-lineages generating BWM use several different combinations of these transcription factors to activate the muscle module (HLH-1 and UNC-120) that ultimately functions to specify the BWM fate and direct differentiation. Second, persistent

activity of earlier acting factors act in concert with their downstream targets to activate myogenic genes. That is, part of the redundancy of fate specification is a feed-forward mechanism that ensures lineage appropriate downstream targets are activated. The complex, combinatorial and partially redundant cascade of transcription factors regulating MS-derived BWM development illustrates the inherent difficulties associated with interpreting knockout mutant phenotypes, even in a system with an invariant and fully defined cell lineage.

As in the posterior embryonic lineages, the relative levels of POP-1/TCF activity in daughter cells dividing along the A–P axis in the MS lineage is a key determinant of which cells will adopt a BWM fate. All BWM cells derive from a posterior daughter (low POP-1) precursor at some point within the lineage, although being a posterior daughter alone is not sufficient to adopt the BWM fate. A complication in understanding when and how POP-1/TCF functions in MS descendants is that the commitment to the BWM fate occurs at different times within the MS lineage. Some sub-lineages undergo multiple rounds of cell divisions with all descendants adopting the BWM fate. At the other extreme are lineages that segregate a single BWM cell from a sister cell that gives rise to neurons. Thus, there must exist molecular mechanisms that integrate the interpretation of POP-1/TCF activity with respect to transcription factor function during each cell cycle. This process, which is currently not understood, appears to be more sophisticated than a simple binary (high vs low) switch between sister cells of A–P divisions that appears to occur in posterior BWM versus hypodermal fate specification. Although each A–P cell division generates relative POP-1/TCF asymmetry, this process is reiterated over many cell cycles within the MS lineage. The end result is an overall gradient of POP-1/TCF that spans the length of the entire embryo, with higher levels in anterior blastomeres compared with those in the posterior. So, while we can easily assay cell fate potential of a given transcription factor in artificially induced conditions of very high POP-1/TCF, or alternatively in its absence, the natural system may be interpreting much finer scale differences in POP-1/TCF activity that modulate transcription factor function to yield multiple, rather than just binary, outcomes.

POST-EMBRYONIC SOMATIC MUSCLE DEVELOPMENT

Post-Embryonic BWMs are Derived from the M Mesoblast

In addition to the 81 BWMs that are generated embryonically, 14 BWMs are produced during post-embryonic development from the single pluripotent M mesoblast cell. As described above, the M cell is a descendant of the MS lineage. During larval development, the M cell produces 14 BWMs as well as the non-muscle macrophage-like coelomocytes (CCs) and sex myoblasts (SMs), which further divide and differentiate into vulval and uterine muscles (vms and um) required for egg laying in hermaphrodites (Figure 5(a)) and tail muscles required for mating in males.²⁶ Six of the 14-M-derived BWMs join the two dorsal quadrants (three cells/quadrant), while the remaining eight join the two ventral quadrants (four cells/quadrant) of embryonically derived BWMs. Once differentiated, the M-derived BWMs appear morphologically and functionally similar to the embryonic BWMs.

The specification of M-derived BWMs is intricately linked to the patterning events that occur during early M lineage development. In hermaphrodites, the M cell begins to divide at the mid-L1 larval stage and undergoes a series of characteristic and reproducible divisions. It first divides dorsoventrally (D–V), then left–right (L–R), and then anteroposteriorly (A–P) to produce 16 cells (Figure 5(b)). Two of the dorsal M descendants, M.dlpa and M.drpa, differentiate into CCs while their sister cells differentiate into BWMs. On the ventral side, M.vlpa and M.vrpa go through an additional round of cell division along the A–P axis. The resulting anterior cells become the SMs, while the posterior cells differentiate into BWMs.

Results over the past several years have begun to shed light on how the M lineage is diversified into the different types of muscle and non-muscle cells.

M-Derived BWM Cell Fate Specification Requires the Redundant Functions of HLH-1 and FOZI-1

Similar to the embryonic BWMs, M-derived BWMs also require HLH-1/MRF for specification. *hlh-1* is expressed in the M lineage beginning at the 2-M stage (Figure 5(a)) in both M.d and M.v, and persists till the 18-M stage, when *hlh-1* is present in all the undifferentiated M-derived BWMs and CCs, but not the SMs. On terminal differentiation, *hlh-1* expression perdures in the BWMs.²⁷ Using a temperature sensitive *hlh-1(cc561ts)* allele, or animals that completely lack M-lineage *hlh-1* expression, Harfe and colleagues discovered that in the absence of *hlh-1* expression or function in the M lineage, cell fate transformations occur. Specifically, the M-derived CCs and several random M-derived BWMs adopt the SM fate.²⁷ As the majority of M-derived BWMs are unaffected in mutant animals lacking *hlh-1* in the M lineage, additional factor(s) must be involved in M-derived BWM specification.

One of these factors is formin zinc finger protein-1 (FOZI-1).²⁸ *fozi-1* and *hlh-1* share similar M lineage expression patterns between the 2- and 18-M stage. Unlike *hlh-1* whose expression perdures in the differentiated BWMs, FOZI-1 becomes undetectable when both the BWMs and CCs differentiate. *fozi-1* null mutants also exhibit a fate transformation of M-derived CCs and several random M-derived BWMs to SMs. HLH-1/MRF and FOZI-1 do not regulate each other's expression or sub-cellular localization. Instead, they function redundantly to specify M-derived BWMs. In *hlh-1; fozi-1* double mutants, most, if not all, M-derived BWMs are transformed to SMs.²⁸

The specification of M-derived BWMs also requires the Hox protein MAB-5, an Antennapedia homolog required for posterior pattern formation.²⁹ *mab-5* is expressed throughout the M lineage and *mab-5* mutants exhibit abnormal cleavage orientation in the M lineage as well as a similar M lineage fate transformation phenotype as *hlh-1* or *fozi-1* mutants.³⁰ Double and triple mutant analysis among *hlh-1*, *fozi-1*, and *mab-5* showed that MAB-5/Antp functions in the same process as FOZI-1 in M-derived BWM specification.²⁸ How they function in this process is not clear except that they do not appear to regulate each other's expression in the M lineage.

Although FOZI-1 and MAB-5/Antp function redundantly with HLH-1/MRF to specify M-derived BWMs, these two factors do not play any role in embryonic BWM specification.²⁸ Thus, embryonic and post-embryonic BWMs require distinct sets of transcription factors that function redundantly with HLH-1/MRF for their specification, suggesting a remarkable level of complexity for the production of a simple striated musculature in *C. elegans*. An additional observation from the above studies is that HLH-1/MRF, as well as FOZI-1 and MAB-5/Antp, plays a role in specifying the M-derived, non-muscle CC fate.^{27,28,30}

HLH-1 and FOZI-1 Integrate Lineage-Intrinsic Information and Positional Information to Regulate M-Derived BWM Specification

The molecular events that lead to the M lineage expression of *hlh-1* and *fozi-1* have not been completely worked out, but they appear to involve a cascade of homeodomain proteins. One of them is the HMX homeodomain protein MLS-2, which is expressed in the M lineage from the 1- to 8-M stage and is required for activating the M lineage expression of *hlh-1*.³¹ The expression of *mls-2* in the M lineage is likely directly activated by the PBC protein CEH-20.³² CEH-20/PBC belongs to the TALE family of homeodomain proteins.³³ It has been shown to function as a cofactor for the two medial group Hox proteins MAB-5/Antp

and LIN-39/Dfd to promote the proliferation and diversification of the M lineage and to directly activate the expression of the CeTwist ortholog HLH-8 and the SoxC protein SEM-2^{34,35} (see the following paragraphs). The activation of *mls-2* by CEH-20/PBC, however, appears to be independent of the Hox factors.³² What cofactor(s) is involved in this function of CEH-20 is currently unknown. Similarly, although CEH-20/PBC has been shown to function together with another TALE homeodomain protein UNC-62/MEIS to regulate cell fate specification and cell differentiation in the M lineage,³⁶ it is not clear how these two factors regulate these processes. The M lineage expression of *fozi-1* also requires *ceh-20* function. However, the regulation of *fozi-1* by CEH-20/PBC does not appear to be mediated by MLS-2/HMX as *mls-2* null mutants exhibit normal *fozi-1* expression in the M lineage.²⁸

Both *hlh-1* and *fozi-1* are expressed in the undifferentiated BWMs and CCs. In the absence of *hlh-1* and *fozi-1*, the cells that are normally fated to become BWMs and CCs adopt the SM fates. In wild-type animals, the M-derived CCs are born from the dorsal M lineage, while the SMs are derived from the ventral M lineage. How is the BWM fate distinguished from the CC and SM fates? Recent studies have identified some critical players in specifying the CC or SM fates, and begin to provide a model on how the complex and combinatorial actions of intrinsic and extrinsic cues lead to cell fate choices in the early M lineage (Figure 6).

The distinction between the dorsal CC fate and the ventral SM fate requires both the LIN-12/Notch signaling pathway and the *C. elegans* Schnurri (SHN) homolog SMA-9, which antagonizes the BMP-like Sma/Mab signaling pathway.^{37–39} Expression of the LIN-12/Notch ligands LAG-2 and APX-1 in hypodermal cells directly adjacent to the ventral M lineage cells at the 4-M stage leads to the activation of the LIN-12/Notch pathway, which functions to promote the ventral SM fate.³⁹ Loss of LIN-12/Notch signaling results in the ventral to dorsal fate transformation in the M lineage.³⁷ On the other hand, mutations in *sma-9* lead to a dorsal to ventral fate transformation in the M lineage while mutations in the Sma/Mab pathway can suppress the M lineage defects of *sma-9* mutants, suggesting that SMA-9/SHN antagonizes the Sma/Mab pathway to allow the specification of the dorsal CC fate.³⁸

In addition to the above mentioned D–V patterning mechanisms, A–P patterning by the Wnt/ β -catenin asymmetry pathway also plays a role in distinguishing the fates between the anterior CCs or SMs and their posterior sister cells, the BWMs.^{25,34,40} Like in embryonic cells described above, nuclear asymmetry of POP-1/TCF and its cofactor, the β -catenin homolog SYS-1,⁴¹ also exists in the M lineage. Specifically, SYS-1/ β -catenin is enriched in the nuclei of posterior cells, while POP-1/TCF is enriched in the nuclei of anterior cells, after each A–P cell division in the M lineage resulting in 8-, 16-, and 18-M lineage descendants. Phenotypes of reduction-of-function mutants for POP-1/TCF and SYS-1/ β -catenin suggest that the posterior BWM fate requires high SYS-1/ β -catenin while the anterior CC or SM fate requires high POP-1.²⁵

The combinatorial actions of the D–V and A–P patterning mechanisms, together with HLH-1/MRF and FOZI-1, lead to the specific expression of the forkhead transcription factor LET-381 in M.d(l/r)pa and M.d(l/r)pp.⁴⁰ LET-381 is the sole *C. elegans* FoxF-related factor, which is also the closest match for FoxC.⁴² FoxF transcription factors are important for the development of the lateral plate mesoderm, specifically the visceral/splanchnic mesoderm, in many organisms, while FoxC proteins are present, and play important roles, in multiple non-visceral mesoderm tissues.⁴² In the M lineage, LET-381/FoxF directly activates the expression of the CC specification factor, the SIX-type homeodomain protein CEH-34, in M.d(l/r)pa, which is fated to become CCs. Loss of *ceh-34* leads to a CC to BWM fate

transformation in the M lineage.²⁵ CEH-34/SIX, together with its cofactor EYA-1, then functions in a feed-forward manner with LET-381/FoxF to directly activate the expression of a number of genes involved in CC differentiation and function.⁴⁰ The expression of *hlh-1* and *fozi-1* is turned off in the differentiated CCs, but it is not known how this is achieved during normal development. Also not known is whether or not *let-381* expression is directly activated by HLH-1/MRF, FOZI-1, and/or SMA-9/SHN.

Similar combinatorial actions of the D–V and A–P patterning mechanisms on the ventral side lead to the specific expression of the SoxC transcription factor SEM-2 in M.v(l/r)pa and subsequently M.v(l/r)paa.³⁴ Moreover, the LIN-12/Notch pathway and SMA-9/SHN converge through LET-381/FoxF to inhibit the expression of *sem-2* in the dorsal M lineage. Once expressed, SEM-2 is both necessary and sufficient to promote the proliferating SM fate. Importantly, *sem-2* exhibits mutually repressive interactions with *hlh-1* and *fozi-1* to maintain their proper expression patterns in the proliferating SM cells and the differentiating BWM cells, respectively. How this mutually repressive interaction is achieved is currently unknown.

Evolutionary Considerations—The study of somatic myogenesis in *C. elegans* provides important clues that inform our more general understanding of myogenesis, development, and evolution. At the big picture level, the results from *C. elegans* support a unified theory of animal muscle development.³ That is, it is likely that all muscle types arose through evolution from a common ancestral contractile cell type. This is not obvious given the clear separation in transcriptional programs regulating modern vertebrate skeletal, cardiac, and smooth muscle development. In mammals, each of these muscle types has a distinct and characteristic set of transcription factors that are critical for myogenesis. For example, skeletal muscle is wholly dependent on the MRFs,⁴ whereas cardiac and smooth muscle are not. Moreover, cardiac muscle is reliant on Nkx-2.5, GATA-4,-5,-6, and HAND-1,-2,^{43,44} factors with no known role in skeletal myogenesis. The study of somatic muscle development in *C. elegans* has shown that transcription factors important for each of these different mammalian muscle types act cooperatively to drive BWM myogenesis in the worm. This strongly suggests that an ancestral contractile cell type used a core set of transcriptional regulators that subsequently were segregated and restricted to specific muscle types during animal evolution.

The term somatic muscle in mammals is often used generically, implying a uniform tissue type that develops from a single developmental program; that is not the case. The skeletal muscle in the trunk of the animals utilizes transcriptional and intercellular signaling processes that are distinct from those of the limb skeletal muscle.⁴⁵ Moreover, the craniofacial muscles have a completely different embryonic origin than the trunk and limb muscle, in addition to differences in cell intrinsic and extrinsic control mechanisms.⁴⁶ In a similar way, the apparently identical BWM cells in *C. elegans* execute their developmental program quite differently, with different transcriptional regulators and cell signaling events deployed in the anterior versus posterior of the embryo as well as post-embryonically. It is likely that these regional or temporally distinct BWM programs in *C. elegans* are evolutionarily linked to the variety of skeletal programs present in modern mammals. Understanding the complex interplay of these factors in a model system with a defined cell lineage will be informative in teasing apart the myogenic (and other) roles of these evolutionarily conserved factors in mammals.

CONCLUSION

We can now describe the core set of transcription factors and intercellular signaling events needed to specify and differentiate both embryonic and post-embryonic BWM in *C. elegans*

from the single cell zygote onward. However, our knowledge of BWM myogenesis is far from complete and many important questions remain. Some of these questions are applicable across development. For example, how are cell proliferation, migration, and fate specification coordinated? What is the relationship between cleavage orientation and cell fate specification? What regulatory relationships are direct and which ones are indirect? What other intrinsic and extrinsic factors are involved in fine-tuning cell fate decisions? Answers to these questions will drive research during the next chapter of myogenic inquiry, giving us a more holistic view of myogenesis in the context of a developing organism.

In addition to these general questions, each lineage has more narrowly defined questions of interest that underscore our naïveté in understanding these developmental processes. For embryonic BWM development, it might be how PAL-1 discriminates between the muscle and hypodermal cell fate programs in response to POP-1 differences. Or, it might be how an MS-derived BWM is specified from a complex mesoectodermal lineage in which its sister cell yields a pair of neurons. In the post-embryonic lineage, dramatic cell fate transformations revealed by mutants show we still have much to learn. For example, previous work by Basson and Horvitz⁴⁷ have shown that mutations in the zinc finger protein SEM-4 affect both the dorsal and the ventral M lineage, causing a CC to BWM fate transformation on the dorsal side and a SM to BWM fate transformation on the ventral side. How is SEM-4 related to D–V and A–P patterning mechanisms and to the factors involved in BWM, CC, and SM fate distinctions? Future work will be geared toward identifying additional players and deciphering the complex relationships that allow the precise and invariable formation of the 95 BWM cells that are an essential part of the evolutionary success of this organism.

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FIGURE 1.

The major muscle groups of *Caenorhabditis elegans* hermaphrodites. The major muscle cell types in the hermaphrodite are shown schematically in this lateral view of the animal with anterior to the left and dorsal to the top. At top, are the 95 bodywall muscles (BWMs), the somatic muscle providing locomotive force for the animal. Dark red cells reflect the 81 embryonically derived cells with lighter red shading used to indicate the approximate location of the 14 post-embryonically derived cells. The BWM cells run in four quadrants along the length of the animal; only one side is schematized. At the bottom are several additional muscle cell groups. The pharynx (orange) pumps food into the gut while the enteric muscles (blue) control defecation. The egg-laying muscles are comprised of those controlling the opening of the vulva (vms, in green) and extrusion of zygotes by contraction of uterine muscles (purple). The only muscles of the hermaphrodite not depicted are the gonadal sheath cells.

FIGURE 2.

Origin and position of the embryonic somatic muscles of *Caenorhabditis elegans*. (a) Lineage diagram of the early cell divisions leading to the establishment of the five somatic founder blastomeres (AB, MS, E, C, and D) and single germline precursor (P4). (Reprinted with permission from Ref 2. Copyright 1983 Society of Developmental Biology) Bodywall muscle (BWM) cells are derived from all somatic founder blastomeres (colored font) except E that gives rise clonally to the gut. The number of BWM cells from each founder lineage is indicated parenthetically. (b) BWM cells from different lineages (colored as in (a)) are schematically superimposed on a Nomarski image of an embryo at the 1.5-fold stage of development. In this lateral view, only the left two BWM quadrants are represented with anterior to the left and dorsal to the top.

FIGURE 3.

A comparison of bodywall muscle (BWM) cells in a wild-type and muscle module mutant embryo. (a) Schematic diagram of the left lateral view of an embryo at the 1.5-fold stage of development with the BWM cells (blue lines) and nuclei (filled circles) indicated. (Reprinted with permission from Ref 2. Copyright 1983 Society of Developmental Biology) (b) Wild-type embryonic BWM differentiation at the 1.5-fold stage of development. Antibodies recognizing myosin heavy chain protein highlight the cytoplasm of BWM beginning to undergo terminal differentiation with nuclei positions visible as unmarked areas near the center of each cell. (c) Absence of detectable BWM in embryos lacking the transcriptional muscle module factors. Myosin heavy chain staining of an embryo at the 1.5-fold stage of development in which the transcription factors HLH-1/myogenic regulator factor (MRF) and UNC-120/serum response factor (SRF) have been eliminated by mutation and the factor HND-1/HAND has been knocked down by *hnd-1* RNAi. Loss of all three transcription factors completely abolishes BWM specification and differentiation.

FIGURE 4.

Transcriptional hierarchy for embryonic bodywall muscle (BWM) specification. The known zygotic transcriptional cascade is shown for all embryonically derived BWM with the exception of the single BWM cell from the AB lineage. There are at least three distinct pathways resulting in the activation of the core muscle module factors HLH-1/myogenic regulator factor (MRF) and UNC-120/serum response factor (SRF) (red box), which once activated, ensures BWM specification and differentiation. Note the variations within the MS lineage that employs two different SIX-type factors in different sub-lineages. In addition, HND-1/HAND is present in one sub-lineage, but absent from the other.

FIGURE 5.

The *Caenorhabditis elegans* hermaphrodite post-embryonic M lineage. (a) The M lineage showing all differentiated cell types that arise from M. (Reprinted with permission from Ref 26. Copyright 1977 Society of Developmental Biology) (b) A schematic lateral view of the M lineage through larval development. Times are indicated post-hatching at 25°C. A, anterior; D, dorsal; L, left; P, posterior; R, right; V, ventral.

FIGURE 6.

Gene regulatory networks involved in bodywall muscle (BWM) specification in the post-embryonic M lineage. Thick blue lines represent likely direct regulatory relationships, whereas black lines represent relationships based solely on genetic data, and do not distinguish direct versus indirect. Gray lines and text indicate lack of expression in indicated cell. (a) The dorsal M lineage at the 16-M stage showing a left/right pair of cells that will adopt either a coelomocyte (CC) or BWM fate. LET-381/FoxF/C directly regulates the expression of the CC specification factor CEH-34/SIX2, and both genes function in a feed-forward manner to regulate a number of CC differentiation genes and suppress the BWM fate. LET-381/FoxF also inhibits the expression of the sex myoblast (SM) specification factor SEM-2/SoxC to prevent SM production on the dorsal side. Expression of *let-381* is regulated by POP-1/SYS-1 asymmetry, dorsoventral (D–V) signaling mechanisms (LIN-12/Notch and SMA-9/SHN) and mesoderm intrinsic factors [HLH-1/myogenic regulator factor (MRF), FOZI-1, and MAB-5/Antp], which may also regulate *ceh-34* expression independently of LET-381/FoxF. (b) The ventral M lineage at the 16- and 18-M stages showing a left/right pair of cells that will adopt either a SM or BWM fate. SEM-2/SoxC is likely directly activated in the M lineage by the HOX/PBC complex, and becomes restricted to the SM mother cells due to a combination of POP-1/SYS-1 asymmetry and the lack of LET-381/FoxF on the ventral M lineage. SEM-2/SoxC is then retained in the SM cells and the SM lineage, while the SM sister cells express HLH-1/MRF and FOZI-1 and become BWMs. The initiation of the asymmetric expression of SEM-2/SoxC versus HLH-1/MRF and FOZI-1 may be due to POP-/SYS-1 asymmetry. Once initiated, SEM-2/SoxC and HLH-1/MRF and FOZI-1 appear to mutually repress each other to maintain their proper expression.