

Muscle pattern diversification in *Drosophila*: the story of imaginal myogenesis

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Summary

There are two phases of somatic muscle formation in *Drosophila*. During embryonic development, one phase of myogenesis generates larval muscle elements that mediate the relatively simple behavioural repertoire of the larva. During pupal metamorphosis, a diverse pattern of muscle fibres are assembled, and these facilitate the more elaborate behavioural patterns of the adult fly. In this review, we discuss the current status of understanding of the cellular, genetic, and molecular mechanisms of pattern formation during the second phase, imaginal muscle development. We briefly compare aspects of embryonic and adult myogenesis in *Drosophila* and muscle development in vertebrates and highlight conserved themes and disparities between these diverse myogenic programmes. *BioEssays* 21:486–498, 1999. © 1999 John Wiley & Sons, Inc.

Introduction

In the insect embryo, skeletal myogenesis begins soon after inception of the mesoderm in the early embryo. This involves commitment and segregation of myogenic precursors, myoblasts, from this germ layer followed by the migration of proliferating myoblasts to appropriate muscle forming sites, withdrawal from cell cycle, and their fusion to give rise to syncytial muscle fibres that form precise patterns in the mature animal. These muscle fibres, which attach to distinct sites and are uniquely recognised and innervated by distinct sets of motor neurons, differ from each other not only in these respects, but also in their patterns of gene expression and physiology. Thus, there appear to be two levels of information to which myoblasts must gain access during muscle development: a) to general cues that orchestrate events in myogenesis common to the formation of all muscles, such as cell

cycle exit, fusion and differentiation, and b), to more specialised information that will determine the distinctive morphological, physiological, and molecular properties of individual muscle fibres. Part of this information transfer could occur through intrinsic mechanisms, autonomous to the myoblasts themselves, such as lineage, and partly through inductive interactions with neighbouring tissues like the epidermis and the nervous system.

In *Drosophila*, cells that will give rise to the mesoderm and its derivatives express *twist* (*tw*) and invaginate into the embryo during gastrulation.⁽¹⁾ After invagination, mesodermal cells divide and spread as an epithelial sheet closely apposed to the developing epidermis. This physical proximity of the mesodermal cells to the developing epidermis is of great consequence: secreted products of patterning genes like *decapentaplegic* (*dpp*) and *wingless* (*wg*), active in the epidermis, signal across germ layers to pattern the mesoderm below (Fig. 1).^(2,3) This would suggest that the mesoderm is naive, and that it is dependent to a large extent on inductive cues provided by the epidermis to organise itself. However, there is accumulating evidence that several elements of the genetic programme that pattern the epidermis into developmental compartments actually function autonomously in the mesodermal cells themselves, and are involved in the specification of similar “compartments” in this germ layer (Fig. 1).^(4–6) Cells belonging to these mesodermal “compartments” or anlagen, contribute to the development of distinct mesodermal derivatives including somatic muscles,

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heart, visceral muscles, and fat body. A crucial outcome of the activities of patterning genes is the refinement of *twi* expression from uniform levels to a modulated pattern in different mesoderm anlagen: cells expressing high levels of *twi* become committed to form precursors of somatic muscles that decorate the body wall of the embryo, whereas those with relatively low levels of *twi* become precursors of cardiac muscles and of visceral muscles that line the gut.⁽⁷⁾ Figure 1A illustrates the use of the terms, “progenitor,” “precursor,” and “founder” as used in this article.

Beginning midway through embryogenesis, mesodermal cells that are destined to contribute to somatic muscles of the embryo and the larva, gradually lose *twi* expression, fuse, and differentiate into muscle fibres.⁽⁸⁾ There is now ample evidence that the development of each muscle fibre in the embryo is seeded by a founder cell, which is a division product of a muscle progenitor (Fig. 1).^(9–13) Progenitor cells are chosen from an equipotential group of mesodermal cells that are drawn from domains of high *twi* expression. Cells that are denied the fate of a progenitor, by default become fusion competent feeder myoblasts. Once the progenitors divide and give rise to founders for specific muscles, feeder myoblasts are recruited to fuse with founders and elaborate the final muscle pattern. Expression of a variety of genes has been documented in the progenitors and founders of specific muscles, and in the muscle fibres that these founders nucleate. At least some of these genes have been shown to regulate specific attributes of the muscles in which they are expressed.^(11,14)

Some *twi*-expressing cells are set aside during embryonic myogenesis to contribute to adult-specific muscles that will form at the time of pupal metamorphosis of the larva (Fig. 1B).⁽¹⁵⁾ Unlike embryonic myoblasts, these cells postpone their differentiation, persistently express *twi*, and proliferate actively during larval life. In the following sections, we will follow these persistent *twi*-expressing cells and put together the information that we presently have on how these cells are specified in the embryo, and how they assemble the pattern of imaginal muscles during metamorphosis.

Adult muscle precursors are specified in conjunction with founders of embryonic muscles. By the end of embryogenesis, *twi* expression persists in a handful of mesodermal cells that have segment-specific arrangements.⁽¹⁵⁾ In the abdomen, six such cells have been identified in each hemisegment (Fig. 1B), and they remain associated with elements of the peripheral nervous system. A single ventral cell is the precursor of myoblasts that make the ventral abdominal muscle in the adult, while a pair of lateral cells are the precursors of those that form the lateral muscles. In the dorsal aspect of the embryo, there are three cells—one dorsal and two dorso-lateral in position, that are the precursors of the dorsal and dorso-lateral adult abdominal muscles

respectively. In the thoracic segments, there is an additional abundance of persistent *twi*-expressing cells (Fig. 1B), and these contribute to the larger and more diverse sets of muscles that will form in the adult thoracic segments (e.g., flight muscles and leg muscles). Segment-specific segregation of *twi*-expressing adult muscle precursors during embryogenesis appears to be dependent on the autonomous function of homeotic selector genes, or Hox genes, in the mesodermal cells themselves. Thus, mis-expression of an abdominal Hox gene, such as *Ultrathorax (Ubx)*, in mesodermal cells of thoracic segments, can transform the pattern of adult precursor segregation from thoracic to that of an abdominal segment.⁽¹⁶⁾

Until recently, the mechanism by which adult muscle precursors are specified during embryonic development was unclear. Studies investigating the function of the *numb (nb)* and *Notch (N)* genes in embryonic muscle patterning have provided us with important clues about the mechanisms involved in this process.⁽¹²⁾ It appears that in the abdominal segments of the embryo where the process has been best investigated, adult precursors are produced as sister cells of founders of embryonic muscles, after the division of muscle progenitor cells (Fig. 1).⁽¹²⁾ Asymmetric segregation of Nb during division of progenitor cells results in the production of two sister cells with different identities. The daughter cell, which inherits Nb, and therefore prevents the N pathway from functioning, is committed to becoming a founder and assembles an embryonic muscle. Its sister, which does not inherit Nb, and which has N signalling active, continues to express *twi* and postpones differentiation as an adult muscle precursor.⁽¹²⁾ Not only are the adult precursors produced as siblings of embryonic founders, they are produced at precisely analogous geographical locations where ultimately they will organise an adult muscle pattern in the pupa.⁽¹²⁾ For instance the ventral adult precursor is a sibling cell of the founder of the embryonic ventral acute muscle 3, and during pupation it gives rise to the adult ventral muscle. Similarly, the dorsal adult precursor is a sibling of the founder of the dorsal most embryonic muscle, dorsal acute 1, and in the adult forms the dorsal-most abdominal muscles. These are very important observations; they show that aspects of adult muscle pattern are laid down at a very early stage, in the embryo, in conjunction with genetic programmes that function to pattern the embryonic muscles themselves.

Adult muscle precursors are dividing cells associated with imaginal discs and motor nerves in the larva

During larval development, adult muscle precursors divide actively to produce small pools of myoblasts in the interstices of larval muscles, associated with peripheral nerves, and in thoracic segments they also associate with imaginal discs (Fig. 1B,C).^(15,17) These cells associate with embryonic discs, are located in specific parts of the second instar discs and

when examined in wing imaginal discs of third instar larvae are found to specifically reside in regions that will give rise to the notum of the adult fly.⁽¹⁵⁾ Thus imaginal myoblasts are not just associated with the discs, but are organised at precise positions. This specific distribution of myoblasts could involve an active migration of these cells to the presumptive notal region as the disc grows and expands during larval life, or there could be specific signals in the prospective wingblade region that prevents these cells from distributing themselves there. In larvae carrying viable allelic combinations of the *wg* gene, the prospective wingblade region is transformed into an additional notum, and in this situation, myoblasts are observed to spread into this ectopic notal tissue.⁽¹⁸⁾ What is the significance of the specific patterns of distribution of adult myoblasts on the wing and other imaginal discs? The epidermal cells that will serve as attachment sites for muscles that form from the disc-associated myoblasts are specified in third instar larvae in the notal region of the disc epithelium overlying the myoblasts and are characterised by the expression of the *stripe (sr)* gene¹⁹ and the expression of *sr* is first seen on the wing disc late in the third larval instar (A. Ghazi and K.V.R., unpublished observations). The reason for this relatively early segregation of cells that will form future insertion points for adult muscles is not clear, but the fact that myoblasts remain in close association with these cells during their residence on the prospective notum suggests the possibility that important patterning information could be exchanged between myoblasts and the *sr*-expressing cells so that these myoblasts are already informed about where to attach once they fuse and begin to form muscle fibres.

A variety of genes, most encoding regulatory proteins, have been shown to be expressed in disc- and nerve-associated adult myoblasts in third instar larvae. These are *twi*, *cut*, *vestigial*, *scalloped*, the *Drosophila* homologue of the vertebrate myocyte enhancer factor *D-Mef2* and the *Drosophila* homologue of the fibroblast growth factor receptor (FGF-1) encoded by *heartless*.^(15,18,20–24) The expression of *twi* persists in the adult precursor cells from the embryo;⁽¹⁵⁾ evolution of the expression patterns of the other genes in adult precursors from embryonic through larval instars have not been extensively investigated.

Adult muscle development during pupal metamorphosis: thoracic myogenesis

Cellular and developmental events

Much of our understanding of adult myogenesis comes from information that is available on the development of muscle fibres in the mesothoracic segment, and to some extent on muscle formation in abdominal segments. Little is known about development of muscles in other thoracic segments and in the head. We would like to believe that the overall mechanisms of muscle development in these segments are

similar to mesothoracic and abdominal myogenesis that we describe in some detail below. The bulk of the muscle mass in the mesothorax consists of the large indirect flight muscles (IFMs), which are divisible into two subsets: the dorsal longitudinal muscles (DLMs) and the dorsoventral muscles (DVMs).⁽²⁵⁾ These two groups of IFMs have distinct developmental histories: the DLMs develop using persistent larval muscles as scaffolds,^(25,26) whereas the DVMs are constructed by de novo fusion of imaginal myoblasts.⁽²⁶⁾ Clonal analysis has shown that the IFMs and most of the direct flight muscles (DFMs) are derived from adult myoblasts associated with the wing imaginal discs, whereas myoblasts associated with the mesothoracic leg discs contribute to muscles in the leg and also to a large muscle called the tergal depressor or jump muscle that remains closely associated with the DVMs.⁽²⁷⁾ However, if disc tissue with adhering myoblasts are ectopically transplanted into abdominal segments, donor myoblasts fuse promiscuously with resident abdominal myoblasts and contribute to abdominal muscles, suggesting that these cells, although on the discs, are not developmentally restricted to contribute to particular kinds of muscle fibres.^(28,29) This is in contrast to the observations of lineage tracing by clonal analysis, which suggested that disc-associated myoblasts do contribute to definite groups of muscle fibres during normal development (however, caution should be exercised in interpreting lineage data in a syncytium, particularly in the light of data obtained by direct cellular observation, discussed below in the section on abdominal myogenesis).^(27,30) Taken together with more recent experiments these data suggest that during normal development, myoblasts are channelled into particular myogenic programmes and that these programmes are precisely controlled.⁽³¹⁾ This commitment could arise through interactions with neighbouring tissues such as the epidermis and the nervous system in the course of development. When taken out of context, myoblasts can contribute to diverse muscles because they now respond to new local influences. These local influences may well be those exerted by hitherto unidentified, but proposed, adult “founder cells” that allow transplanted “feeder” myoblast to fuse but do not allow them to dictate muscle pattern.⁽²⁹⁾

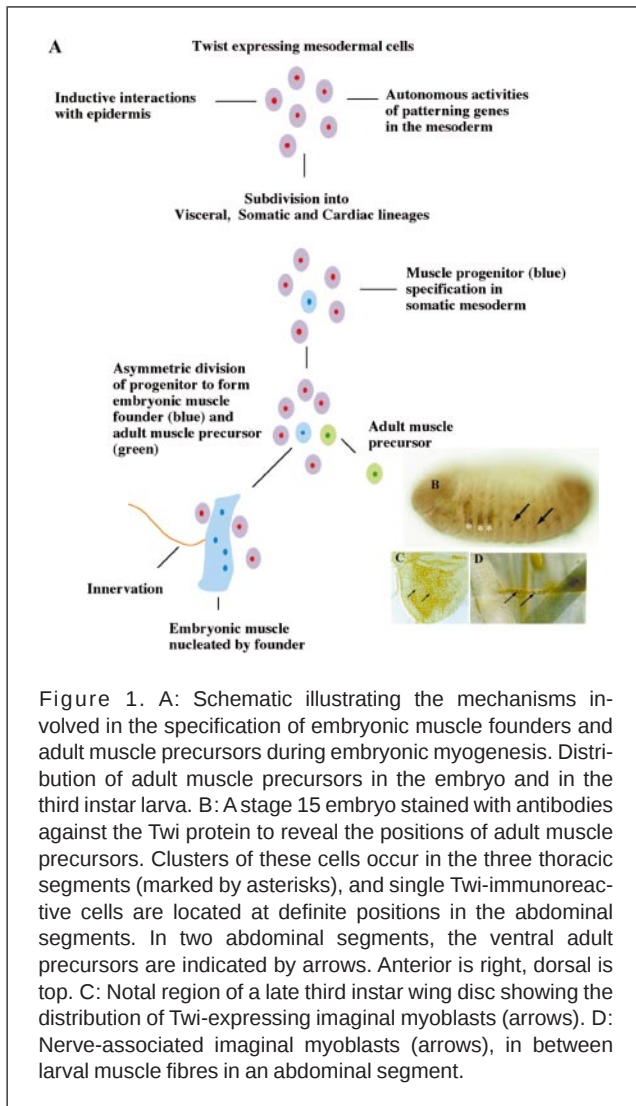
Apart from disc-associated myoblasts that contribute to diverse thoracic muscles, there are groups of myoblasts, as mentioned earlier for the abdomen, which remain associated at specific positions with peripheral nerves that innervate larval thoracic muscles.^(15,17) Whether these cells, in the thorax, represent a special class of myoblasts, that are different from the disc-associated ones in their molecular properties and developmental potentials, and whether they are channelled or committed to contribute to definite sets of thoracic muscles is presently unclear.

At the onset of metamorphosis, myoblasts from the evert-ing wing imaginal discs swarm over the persistent larval muscles that serve as substrates for the formation of the DLMs (Fig. 2A).⁽²⁶⁾ Subsequently, each larval muscle splits into two templates, and continued fusion of imaginal myoblasts with these templates results in the elaboration of the final pattern of six DLM fibres observed in the adult (Fig. 2B). Early in development, filopodial extensions are observed from the ends of developing IFM fibres and these make precise contacts at defined positions on the developing pupal epidermis (Fig. 2C).⁽¹⁹⁾ Subsequently, these regions of contact between muscle and epidermis mature into specialised junctions, the myotendon junctions.⁽³²⁾

Imaginal myoblasts are directly involved in transforming the persistent larval muscles into templates for DLM development. Direct observation of these cells during early stages of DLM development have revealed that they invade the persistent larval muscles and organise themselves into myofibres using larval muscles as positional cues.⁽³³⁾ If in larval life, wing discs are depleted of myoblasts by inhibiting their division, larval muscles fail to split, and degenerate.⁽³³⁾ The larval muscles themselves are required for regulating the proper numbers of DLM fibres that ultimately form (six in a wild-type situation); in the absence of these scaffolds, DLM developmental programme is unaffected, but the number of DLM fibres that form varies considerably.⁽³⁴⁾ In close vicinity of the developing DLMs, myoblasts fuse *de novo* and seed the pattern of the DVMs, the jump muscle, and the DFM.⁽²⁶⁾ Little is known about how pattern is organised during the development of these muscles, even though muscle formation by *de novo* fusion of myoblasts is the predominant mechanism of imaginal muscle development. The use of scaffolds in the form of persistent larval muscles in case of DLMs appears to be a superimposition on *de novo* fusion. This contention is supported by the fact that if the larval muscles are ablated, DLM myoblasts are able to fuse *de novo*, in a manner very similar to the DVMs and form DLM fibres.⁽³⁵⁾ However, an alternate explanation needs to be kept in mind. It is possible that all adult muscles were built on remnant larval muscles, with *de novo* fusion a later evolutionary modification—the DLMs would, in this scenario, be a remnant of an older mechanism. The several studies on other insects by Hinton, Lebart-Pederbas, Cifuentes-Diaz, and Tiegs (reviewed in ref. 31) need to be analysed in this context.

Unlike the *Drosophila* embryo, where muscles are patterned independently of developing innervation, muscle development in the imago presents a contrasting situation where myogenesis and development of innervation proceed in close synchrony.^{17,36–38} Metamorphic development of innervation to adult muscles have been best investigated for the DLMs.⁽¹⁷⁾ Motor neurons that innervate these muscles (and possibly also the DVMs and many other adult muscles) are likely to be

remodelled larval motor neurons.⁽¹⁷⁾ Specific metamorphic changes in DLM innervation involve withdrawal of synaptic contacts of the motor nerve from the persistent larval muscle targets, followed by elaboration of new arborisations over these muscles as they split and transform into DLM fibres (Fig. 2D).⁽¹⁷⁾ It has been suggested that cues emanating from the metamorphosing innervation could play an important role in splitting of the larval muscles into DLM templates.^(17,26) However, laser ablation of the flight muscle motor nerve does not seem to substantially affect normal development of DLMs.⁽³⁹⁾ In contrast, denervation strongly affects the development of the DVMs—though initial segregation of myoblasts to DVM formation sites and subsequent fusion is initiated in such situations, muscle development is not sustained.⁽³⁹⁾ Thus there appears to be an inherent difference in the way the DLMs and DVMs are patterned, with the DVMs requiring neural cues for development and the DLMs forming on persistent larval muscle scaffolds, which may participate actively in the process. In the absence of larval scaffolds, the DLMs adopt a DVM developmental pathway—in this situation, their development is stringently dependent on innervation.⁽³⁹⁾ The neural influence, however, could simply reflect an influence on the myoblast pool size with the DVMs being more sensitive to changes in myoblast number.⁽³⁹⁾ Although these experiments indicate a dependence on innervation for proper development of the IFMs, there is other evidence, equally persuasive, which suggest that several aspects of IFM development are independent of neural cues. For example, transplantations of whole wing discs to ectopic sites have revealed that disc-associated myoblasts can form muscle with IFM-like properties even in the absence of innervation.⁽⁴⁰⁾ Further, mutant flies that carry viable allelic combinations of regulatory mutations in the *Ubx* gene, exhibit transformations of epidermal and nervous system elements of the metathorax to the likeness of the more anterior mesothoracic segment, resulting in a duplicated mesothorax and two pairs of wings (the “triple mutant” or the “four-winged” fly (Fig. 3B,D)).⁽⁴¹⁾ Examination of myogenesis in the transformed segment has revealed that although the number of myoblasts on the transformed metathoracic haltere discs and their migration to muscle formation sites over the transformed metathoracic epidermis is altered to a mesothoracic pattern, however, the transformed nervous system (and the epidermis) is unable to induce a mature mesothoracic muscle pattern (IFMs) in the transformed metathoracic segment.^(23,42) Taken together, these observations eloquently demonstrate that although inductive cues are important in specifying certain aspects of pattern during thoracic myogenesis (for instance neural cues for DVM development and epidermal cues for regulating numbers of myoblasts and their migration patterns), intrinsic properties of the myoblasts themselves



appear to be equally crucial for the mature pattern to fully evolve.

Genetic regulation

Differential Patterns of Hox Gene Expression Specify Antero-Posterior and Dorso-Ventral Positional Information

In the *Drosophila* embryo, segment-specific diversification of muscle patterns appear to be under the direct control of Hox genes in the mesodermal cells.^(16,43,44) Hox gene expression is observed in mesodermal cells; the domains of their expression in these cells along the antero-posterior axis differ, however from their expression patterns in the overlying ectoderm.⁽³¹⁾ This appears to be a conserved phenomenon in insects: for instance, observations of the expression patterns of the *Ubx/abdominal-A* genes in grasshopper embryos have

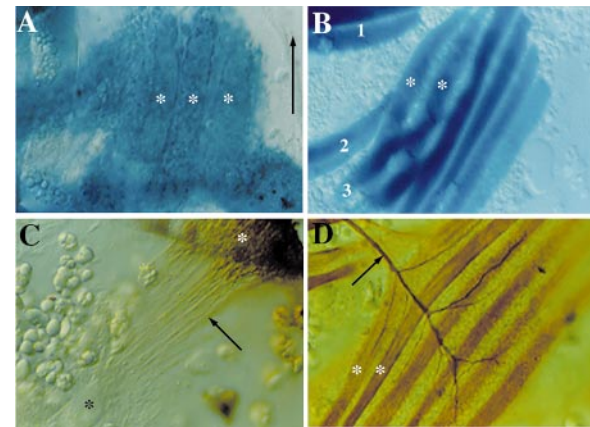
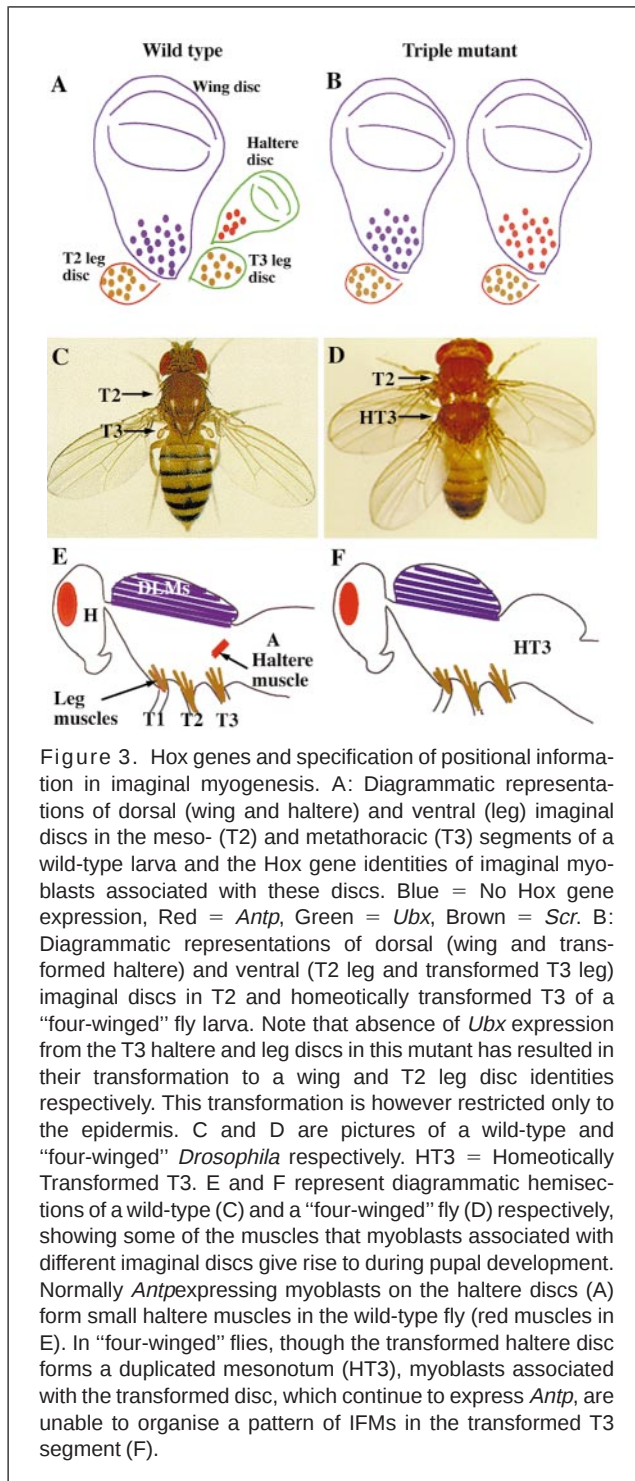
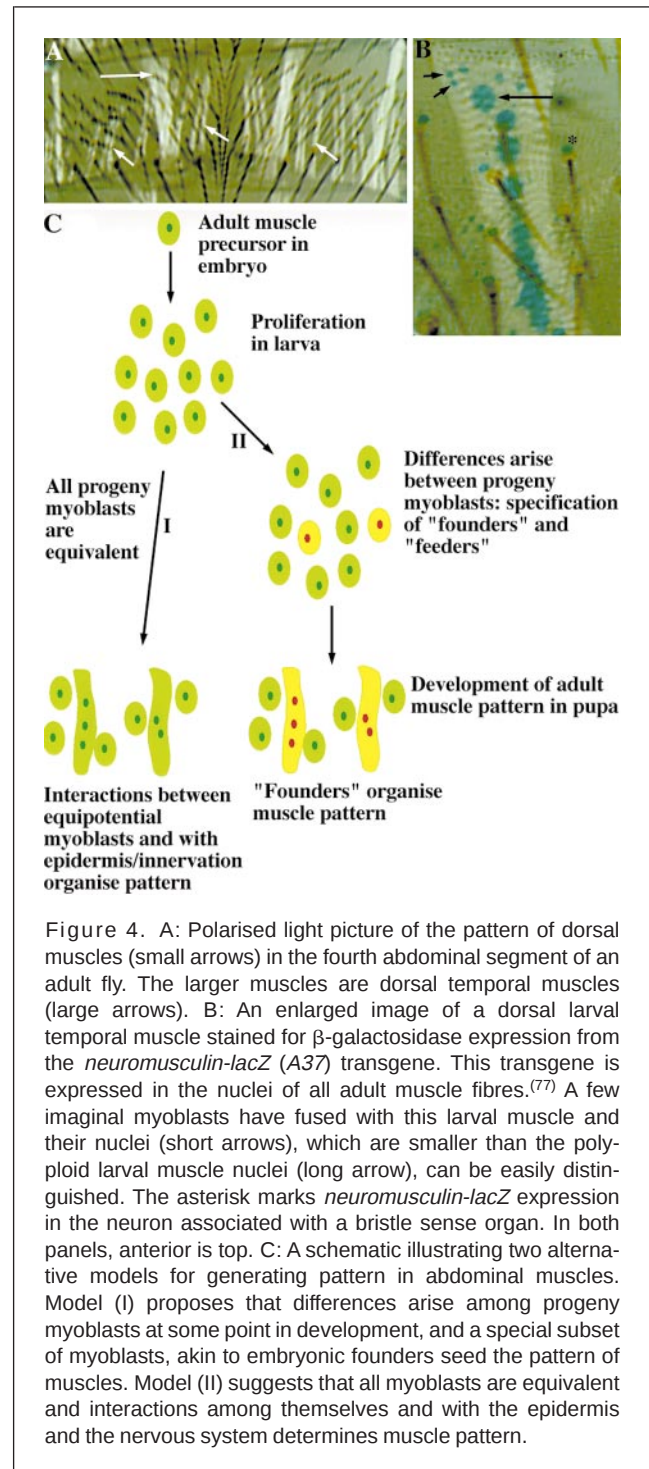


Figure 2. Migration, fusion and differentiation, attachment and development of innervation to the IFMs during pupal metamorphosis. In all panels, anterior is top and the dorsal midline is to the right (indicated by an arrow in panel A). A: Migration of wing disc associated myoblasts over larval muscle templates revealed by *twi-lacZ* expression (blue staining) in a 12 hour APF pupal preparation. The positions of the persistent larval muscles are indicated by asterisks. B: Onset of differentiation in the developing IFMs around 20 hours APF revealed by the activity of the *Act (88F)-lacZ* transgene. The two ventral-most DLM fibres are indicated by asterisks and the three groups of developing DVMs are indicated by corresponding numbers. C: Filopodial extensions (arrow) from the end of a developing DLM fibre (marked with a black asterisk) in a 24 hour APF pupal preparation. This preparation has been stained with antibodies against PS 1 α , which is expressed in the epidermal muscle attachment sites (brown staining, marked with a white asterisk). The filopodia from the muscle is seen to make specific contacts with PS 1 α expressing epidermal cells. D: Pattern of innervation to the developing DLMs at 24 hours APF revealed by staining with mAb 22C10, which stains the developing innervation and their target muscles. The ventral-most pair of DLMs are indicated by asterisks. The axons of motor neurons innervating the DLMs traverse through the intersegmental nerve (arrow).

revealed a similar shift in the domains of expression of these Hox genes in the mesoderm.⁽⁴⁵⁾ Consequently, in *Drosophila*, mutations in Hox genes often result in muscle transformations that do not coincide with transformations of the overlying epidermis, indicating an autonomous role for these genes in muscle pattern specification.^(29,42,43) Hox genes are also expressed in the precursors of adult muscles and function to allocate distinct identities to these cells so that they elaborate a segment-specific muscle pattern (Fig. 3A).⁽²³⁾ Interestingly however, myoblasts that reside on wing imaginal discs, and which contribute to the vast majority of dorsal muscle fibres in the mesothorax, do not express any known *Drosophila* Hox gene.⁽²³⁾ This is in contrast to the metathoracic segment, where myoblasts associated with haltere discs express *Antennapedia (Antp)* (Fig. 3A). Mis-expression of *Antp* in myoblasts



of the mesothoracic segment disrupts muscle development. In "four-winged" flies, although the numbers and migration patterns of myoblasts in the transformed metathoracic segment are altered toward the likeness of the mesothorax, their intrinsic homeotic identity remains unchanged: these myo-



blasts continue to express *Antp* (Fig. 3B).⁽²³⁾ This could explain why IFM-like muscles do not develop in the transformed segment (Fig. 3F), just as the mesothoracic mesoderm will not differentiate into IFMs on ectopic *Antp* expression.

Hox genes are not only involved in the diversification of muscle patterns along the antero-posterior axis, but in addition and in contrast to the embryo, they appear to function in the specification of dorsal versus ventral muscle patterns in the thorax during adult myogenesis. Thus the *Sex combs reduced* (*Scr*) gene of the Antennapedia Complex (ANT-C) is exclusively expressed in myoblasts associated with ventral leg imaginal discs in all thoracic segments (S. R. and K. VR., in preparation) (Fig. 3A),⁽⁴⁶⁾ and at least in the mesothorax, mis-expression of *Scr* in wing disc-associated precursors of dorsal muscles (the IFMs and DFMs), disrupts the development of most of these muscles (S. R. and K. VR., in preparation). *Scr* expression is not detected in the embryonic stages in precursors of adult muscles in the mesothorax and metathorax (S. R., unpublished observations).⁽⁴⁷⁾ It is likely therefore, that its expression in myoblasts associated with the ventral leg discs in these segments is “switched on” at a particular stage in larval development, and this could be triggered through inductive instructions derived from the disc epithelium.

Analyses of the roles played by Hox genes in individual myogenic events like migration of myoblasts to muscle formation sites and their fusion and differentiation during the development of the IFMs have been particularly revealing and has provided us with a working model of how these genes regulate individual events in myogenesis to promote muscle pattern diversification.⁽²⁹⁾ Migration patterns of myoblasts to muscle formation sites appear to be controlled not by the direct function of Hox gene expression in myoblasts, but through cues provided by the epidermis over which these cells migrate. For instance, mis-expression of the Hox gene *Ubx* (which is normally expressed in abdominal muscles and function to specify pattern in the muscles of anterior abdominal segments) in the wing disc myoblasts, does not affect the normal pattern of migration of these cells.⁽²⁹⁾ Further, and as alluded to previously, in pupae of “four-winged” flies, the migration pattern of myoblasts from the transformed haltere mimics the migration pattern of wing disc myoblasts.⁽⁴²⁾ Since in these mutants, the homeotic identity of the metathoracic epidermis is transformed to the likeness of the mesothorax without affecting the Hox gene identity of the mesodermal cells themselves, it is reasonable to believe that the altered migratory patterns of the myoblasts is instructed by the homeotically transformed epidermis. Also, when whole wing imaginal discs are transplanted into early pupal abdomen, the disc associated myoblasts tend to preferentially restrict themselves to the thoracic cuticle that differentiates from the wing disc and does not migrate out over the neighbouring abdominal cuticle, further corroborating our proposition that epidermal identity is important for myoblast migration.⁽⁴⁰⁾ The process of myoblast fusion does not appear to be stringently regulated by a Hox code as disc-associated thoracic myoblasts of different Hox identities, when transplanted into the

abdomen, fuse promiscuously with resident abdominal myoblasts and contribute to abdominal muscles.^(28,29) Hox genes, however, appear to be involved in dictating the differentiation patterns of muscle fibres. Mis-expression of the abdominal Hox gene *Ubx* in the developing IFMs suppresses expression of a thoracic muscle-specific actin gene, and conversely, partial loss-of-function of *Ubx* in specific abdominal muscles results in the transcription of the thoracic muscle-specific actin gene in these abdominal muscles.⁽²⁹⁾ Moreover, in transplantation experiments, though myoblasts of thoracic identities can fuse with abdominal myoblasts and make abdominal muscles, they are unable to execute their native differentiation programme to “switch on” transcription of the thorax-specific actin gene. This would suggest that in a syncytial myofibre, the developmental programme of the thoracic myoblast nuclei are entrained by those of the abdominal muscles, either through the domineering effect of a “founder” nucleus, or through competitive interactions between abdominal and thoracic myoblast nuclei—the former winning because of their predominance in a mosaic fibre.^(29,48)

A Hierarchy of Gene Interactions in IFM Development: N Regulates twi Regulates D-Mef2

What are the regulatory events leading to flight muscle differentiation? In other words, what regulates the “switching off” of *twi* expression at the onset of myoblast fusion and what are the regulatory consequences of this? The *erect wing* (*ewg*) gene, which was identified as a recessive locus affecting flight,⁽⁴⁹⁾ is a DNA binding protein that is expressed in IFM myoblasts at the earliest stages of their differentiation, as *Twi* expression declines, and in developing IFM fibres on fusion.^(50,51) Although this essentially complementary pattern, of *Ewg* and *Twi* expression, suggests the possibility of negative regulation of *ewg* by *Twi*, this has not been validated. In the absence of *ewg* function, two phenotypes are noteworthy: first, the larval muscles on which the DLMs develop fail to split, illustrating a requirement for this gene in mediating this process, and secondly, there is an early degeneration of the developing DLM and some of the DVM fibres implicating a crucial role for *ewg* in proper initiation of IFM-specific differentiation programme.^(33,51) Although IFM development defects in *ewg* mutants are commensurate with its pattern of expression, the molecular mechanisms of *Ewg* function has yet to be elucidated.

Flies carrying a combination of temperature-sensitive alleles of *twi* show a reduction in the numbers and volume of the DLM fibres when grown at non-permissive temperatures.^(52,53) Other muscle fibres including the DVMs, the jump muscle and the DFMs appear not to be affected, though *Twi* is expressed in the myoblasts that contribute to all these muscles. The *twi* temperature sensitive phenotype in DLMs is very similar to phenotypes generated when *N* function is

removed during pupal development using a similar temperature sensitive *N* allele.⁽⁵²⁾ In addition, there is a premature loss of *Twi* expression when *N* function is compromised. Mosaic analysis suggests that the focus of *N* function is in the muscle cells themselves (S. Anant, unpublished observations). Over-expression of an activated variant of *N* and over expression of *Twi* produce similar phenotypes in the developing IFMs further strengthening the case that *N* regulates *Twi* expression during IFM development.⁽⁵²⁾ In both instances, mis-expression appears to inhibit proper differentiation of IFMs as revealed by the inhibition of expression of contractile protein genes like *Myosin* and *Actin*, either with antibody probes to their protein products or reporter gene expression patterns. In fact, over expression of activated *N* results in persistent expression of *Twi* in the nuclei of developing myofibres; a pattern of *Twi* expression that is never observed in normal development.⁽⁵²⁾ In addition, and as mentioned earlier, during embryonic segregation of adult muscle precursors, differential *N* activity in these cells sustains *Twi* expression, and specifies them as adult precursors.⁽¹³⁾ Taken together, all these observations suggest that *N* is a crucial signal for the maintenance of *Twi* expression in adult precursors.

The *twi* loss-of-function phenotypes in the DLMs are also mimicked by hypomorphic mutations in *D-Mef2*.^(22,53) Expression of *D-Mef2* in adult myoblasts appears to be regulated directly by *Twi*, and conditional loss of *Twi* activity results in the concomitant loss of *D-Mef2* expression in these cells, suggesting that *twi*'s function in IFM development could be mediated through its regulation of *D-Mef2* expression.⁽⁵³⁾ The *Mef* family of transcription factors in vertebrates and *D-Mef2* in flies are potent transactivators of muscle-specific differentiation genes.^(54–57) However, in embryonic as well as in pupal myogenesis in flies, *Twi* expression disappears from myoblasts just before fusion, and it is completely excluded from differentiating muscle syncytia.^(9,26,52) In contrast, the focal point of *D-Mef2* function, at least in the embryo (and by analogy in adult myogenesis), appears to be at the point of myoblast differentiation: its expression is strongly maintained in differentiating myoblasts and persists in mature muscle fibres.⁽⁵⁶⁾ Moreover, in *D-Mef2* loss-of-function mutants, muscle differentiation is strongly affected in the embryo, and expression of muscle-specific differentiation genes is completely absent.^(55,56) Thus some aspects of *D-Mef2* expression are certainly regulated by *Twi*—for instance the early induction of *D-Mef2* in the embryonic mesoderm^(58–60) and its maintenance in adult precursors;⁽⁵³⁾ these expression patterns are lost when *Twi* activity is compromised. However, since *Twi* expression is excluded from differentiating muscles, the expression of *D-Mef2* during muscle differentiation must be regulated by mechanisms other than those involving *Twi*. Persistent expression of *Twi* in differentiating embryonic muscles does not affect their development;⁽⁷⁾ however as mentioned previously, exclusion of *Twi* from differentiating

IFM fibres appears to be a crucial element for myogenesis to proceed.⁽⁵²⁾ This is a situation similar to that in vertebrate myogenesis where *Twi* is a known repressor of myogenic differentiation.^(61,62) This would suggest that though *Twi* regulates, in undifferentiated muscle precursors, expression of genes like *D-Mef2*, which directly control muscle differentiation, its absence during muscle differentiation itself is a necessary and important step for correct execution of myogenic differentiation. On mis-expression in developing IFMs, *Twi* could function like in vertebrate muscle by directly inhibiting the ability of myogenic regulators like *Mef2* from binding to their target genes and activating transcription. Mis-expression of *twi* and *D-Mef2* can result in the transcription of muscle contractile protein genes in non-mesodermal tissues.^(7,57)

sr Specifies Epidermal Cells for Muscle Attachment

A significant amount of information is available on gene expression patterns in myoblasts and developing muscles on one hand and the epidermis on the other, which function to organise muscle-epidermal attachments. As mentioned in an earlier section, there is an early onset of *sr* expression in discrete groups of cells in all imaginal discs, and this expression, if followed into pupal development, translates into the epidermal attachment sites of muscle fibres.⁽¹⁹⁾ During IFM formation, analysis of the expression patterns of the *Drosophila* homologues (Position Specific antigens) of vertebrate integrin molecules have revealed dynamic patterns of expression: a PS- β subunit (encoded by the *mysospheroid* (*mys*) locus) is expressed in myoblasts and in the filopodial ends of developing myofibres as well as in the epidermal attachment cells; the PS-1 α (encoded by *multiple edematous wing*) and PS-2 α (encoded by *inflated*) subunits are expressed on the epidermal and muscle ends respectively (see also Fig. 2C).⁽¹⁹⁾ Viable alleles of *sr* have a late detachment defect that manifests most strongly in the DLM fibres.⁽⁶³⁾ Similarly, a viable allele of *mys*, *nj42*, results in flies that lack the jump muscle, possibly again due to the failure of this muscle to attach properly.⁽⁴⁹⁾ These phenotypes are thus consistent with the roles of these molecules in regulating muscle-epidermis attachments during development. The onset of *sr* expression in the prospective attachment cells on the notal region of larval wing discs suggests a regulative role for this gene in the development of epidermal components of the myotendon junction (A. Ghazi and K.V.R., unpublished observations).⁽¹⁹⁾ A number of target genes of *sr*, most of them encoding structural components, have been identified in the embryo where *sr* has been shown to have a homologous function in specifying epidermal cells to which embryonic muscles specifically attach.⁽⁶⁴⁾ It remains to be determined whether *sr* merely regulates the expression of attachment molecules during adult muscle development, or it has a more important

instructive role in specifying pattern as its early expression in the imaginal discs prompts us to believe.

Hormones and Myogenesis: Broad-Complex Genes Mediate Hormonal Regulation of Flight Muscle Development

Most developmental processes during insect metamorphosis are under hormonal regulation and imaginal myogenesis is no exception. The effects of moulting hormones on aspects of muscle development, though appreciated from a very long time in *Drosophila* and other insects, has received significant, but not the wider attention it deserves.^(65–67) The *Broad-Complex* (*BR-C*) defines an early gene induced by ecdysone, and mutations in this gene that belong to the *reduced bristles on palpus* (*rbp*) class have differential effects on flight muscle development.⁽⁶⁷⁾ Most mutations affect the development of the DVMs, with lesser affects on the DLMs and other muscles. The *BR-C* locus encodes a family of zinc-finger containing transcription factors that function in a tissue-specific manner to regulate gene expression during metamorphosis in response to ecdysone.⁽⁶⁸⁾ The rescue of flight muscle defects by the BRC-Z1 protein variant in *rbp* mutants suggests that this is the predominant isoform that mediates the effects of *BR-C* in myogenesis.⁽⁶⁹⁾ BRC-Z1 expression can be observed in the epidermal cells, in the wing disc associated myoblasts and in these cells as they assemble to form the IFMs.⁽⁶⁹⁾ DVMs detach and degenerate in *rbp* mutants suggesting that one possible function of *BR-C* in flight muscle development could be to regulate the expression of genes that are involved in the development of proper muscle-epidermal attachments. It will be important to analyse further roles of *BR-C* and of other genes directly regulated by ecdysone in adult myogenesis and to delineate the regulatory cascades that operate downstream of these genes. Exit from cell cycle, expression of differentiation genes like *ewg*, onset of myoblast fusion, contractile protein synthesis and remodelling of innervation could be among a host of functions that could possibly be regulated by *BR-C* and other ecdysone-responsive genes.

Development of abdominal muscles

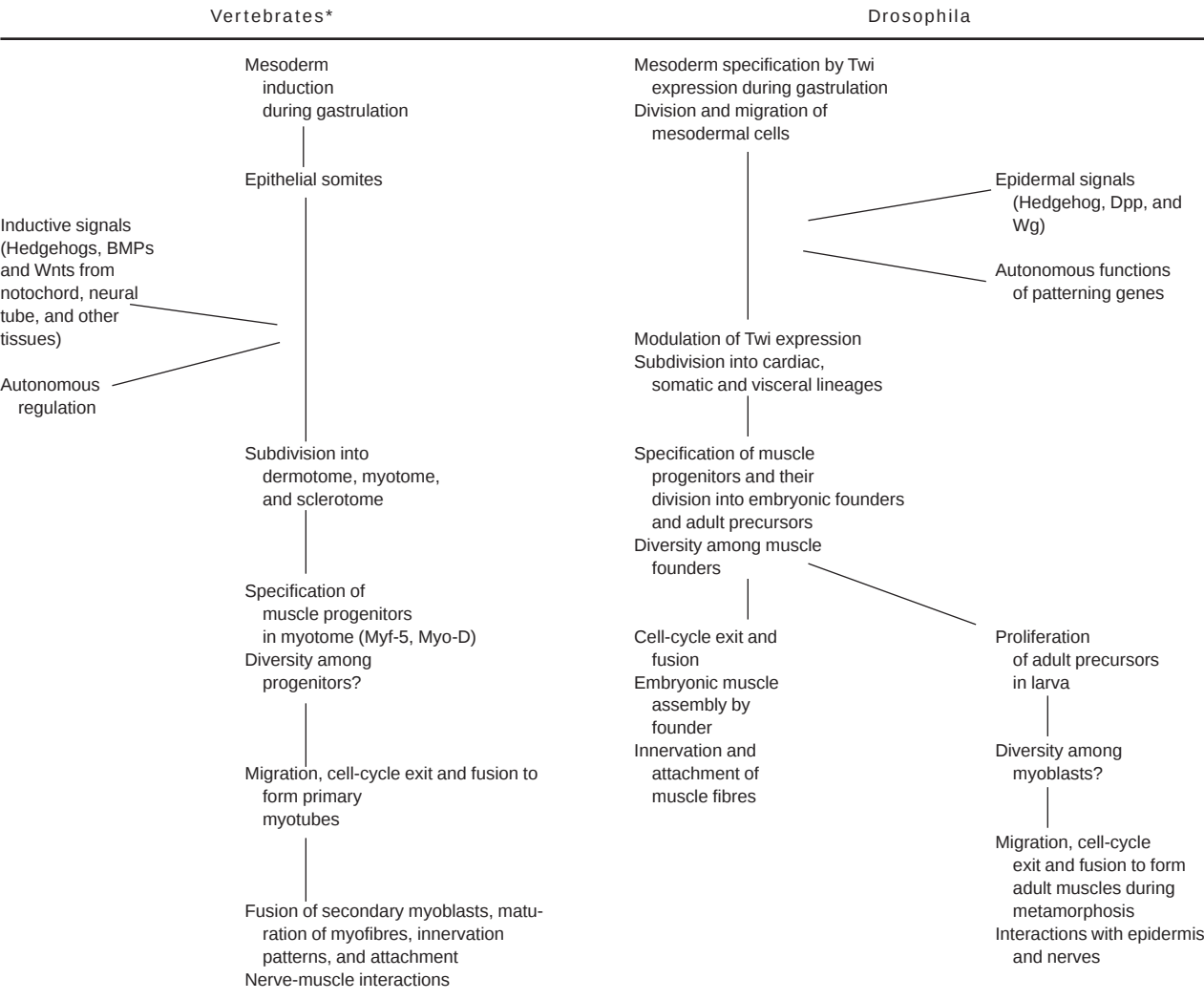
In the abdomen, adult myoblasts migrate along larval nerves to reach appropriate sites of muscle formation in the dorsal, lateral, and ventral epidermis and organise an evenly spaced and directionally oriented pattern of thin strap-like muscles (Fig. 4A).⁽⁷⁰⁾ Like in the mesothorax, *twi* expression declines in these myoblasts as they begin to fuse and is replaced by the onset of β 3-tubulin expression.⁽⁷⁰⁾ Apart from these muscles that develop de novo during pupation, some larval abdominal muscles escape histolysis and survive to adulthood (Fig. 4A,B). These temporal muscles are rapidly lost

after emergence and possibly function to allow vigorous abdominal contractions that are required during eclosion. In some sense these muscles are similar to the persistent larval muscles that function as scaffolds for DLM development in the thorax. Like the thoracic muscles, they escape histolysis during early pupation, but unlike the former, they are not remodelled into adult muscles.

It is not known how adult myoblasts fuse and organise the spaced pattern of muscle fibres in the abdomen. Denervation does not affect this pattern, but in such situations the muscles formed are thinner, possibly due to the requirement of innervation for myoblast proliferation.⁽³⁸⁾ This effect of innervation on myoblast proliferation is also seen in flight muscle development, and appears to be a conserved theme in pupal myogenesis in insects.^(39,71) Although there are some segment-specific differences in the arrangement and numbers of fibres, molecular differences among abdominal muscles is not so conspicuous as in the case of the thorax. A notable example of differential gene expression among abdominal muscles is that of the *79B Actin* gene, which is expressed at varying levels in several fibres, notably in the genital muscles in the terminal segments, and in a special muscle in fifth abdominal segment in male flies called the Muscle of Lawrence (MOL).^(72,73) This particular muscle is a unique muscle in the fly because the sex and segmental identity of the motor neuron (and not the genetic identity of the epidermis or the myoblasts) is crucial for its development and molecular identity (expression of *79B Actin*).⁽³⁶⁾ Thus, unlike other abdominal muscles, ablation of innervation to this muscle arrests its development.⁽³⁸⁾ The molecular nature of the signal that is provided by the motor nerve to organise pattern in this muscle remains unidentified. Mutations in the *fruitless* (*fru*) locus affect male sexual behaviour, and affects the development the MOL. At least one function of this gene, which encodes a putative transcription factor,⁽⁷⁴⁾ in the development of this muscle appears to be in the recruitment of extra numbers of myoblasts during muscle development, a process that makes this muscle much larger than its neighbours.⁽⁷⁵⁾ In *fru* mutants, this process of recruitment is aborted, and consequently, the development of this muscle is affected.⁽⁷⁵⁾

Specific ablation of abdominal muscle precursors have illustrated that these cells are organised into quantal units, each unit committed to give rise to a particular element of the final pattern.⁽⁷⁶⁾ Thus there are three dorsal, two lateral, and one ventral unit, corresponding to the three dorsal, two lateral, and one ventral persistent *twi*-expressing cells in the late embryo that have been identified as precursors of the abdominal muscles.⁽¹⁵⁾ In reality then, the muscles of the adult abdomen are in all likelihood made from clonal descendants of single precursors specified in the embryo. This is certainly true for the single ventral abdominal muscle that forms in each hemisegment—a single persistent *twi*-express-

TABLE 1.



*Summary of vertebrate muscle development has been adapted from Ref. 78.

ing adult precursor in the ventral abdomen of the embryo is the clonal precursor of this entire muscle.^(13,15,70,76) The precursor cell divides to produce a group of myoblasts that cooperatively generates this ventral muscle—a singular element of the adult muscle pattern (Fig. 4C). All the progeny myoblasts could be endowed with an equal share of molecular information that the precursor inherited when it was specified as a sister cell of an embryonic founder (Fig. 4C).⁽¹³⁾ On the other hand, differences could arise among these cells, in the form of “founders” and fusion competent “feeders”, at some point in post-embryonic development, and this could occur through a reiteration of events that function to generate differences among embryonic myoblasts (Fig. 4C). Thus it appears that in the abdomen, similar to the situation in the

thorax, either myoblasts are strictly committed to form a particular set of muscles or that their fates are plastic, but they are very precisely channelled during development to specific sites and they assemble a muscle only at this particular site. Although there is no conclusive answer to the first possibility, careful analysis of normal development of these muscles, coupled with ablation studies suggest that the precursors for each group of muscles are indeed strictly channelled to specific muscle formation sites and there is no migration between these groups in a segment and between groups across segments.^(70,76) Of particular significance in this respect is an additional observation that small numbers of imaginal myoblasts in the abdomen fuse with the persistent larval temporal muscles (S. R., unpublished observations;

Fig. 4B). The small size of adult myoblast nuclei allow them to be distinguished unequivocally in the muscle syncytium from the larger polyploid nuclei of the larval muscles themselves. The pattern of imaginal myoblast fusion with the temporal muscles is variable—it differs from muscle to muscle and segment to segment, which suggests that this process is quite random. This could be a direct indication that adult abdominal myoblasts, like disc-associated myoblasts in the thorax, are not committed to make a particular muscle, but can fuse promiscuously and contribute to diverse muscles. Whether this is a property of all abdominal myoblasts or of a special subset akin to feeder myoblasts in embryos is not clear. If this property is shared by all myoblasts, then there is a strong reason to believe that all adult abdominal myoblasts form an equivalent pool of cells (model I in Fig. 4C), not committed to form any particular muscle, but during development are precisely shunted to appropriate muscle forming sites where they interact with themselves and neighbouring tissues to organise a specific muscle pattern.

Concluding remarks

In this review we have discussed how, in *Drosophila*, cells are set aside during embryonic development as precursors of adult muscles, and how these cells cooperate and organise a pattern of muscle fibres during pupal metamorphosis. Comparison with adult myogenesis allows one to directly appreciate similarities and differences between these two episodes of myogenesis in the fly. It is apparent that many mechanisms involved in adult myogenesis are reiterations of those that operate during diversification of embryonic muscles. There are also instances where new principles are deployed, however; new genes are involved and genes that function in one context during embryonic myogenesis are recruited for new functions during adult myogenesis. Major tasks in the years ahead should focus on elucidating the molecular basis of many events in adult myogenesis that we have been able to describe here only in cellular terms. We have little insight into processes such as cell cycle regulation in myoblasts, regulation of myoblast fusion, growth, regeneration, and size regulation in muscle fibres, mechanisms of patterning of adult motor innervation, and the roles of hormones in coordinating myogenesis. Interestingly, even from the little that we know, both embryonic and imaginal muscle development in *Drosophila* show striking similarities with elements of pattern formation in vertebrate muscle (see Table 1) and there is reason to believe that a better understanding of myogenic mechanisms in *Drosophila* will further our understanding of muscle patterning in higher organisms. It is in this light that the information we have, and which we hope to garner in the coming years on myogenic mechanisms in *Drosophila*, will be even more informative than many exciting developments of the recent past.

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