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Muscle Development in *Drosophila*

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Muscle development takes place by a series of regulated steps, beginning with the specification of the muscle forming germ layer, the mesoderm. From the mesoderm originate different muscles, each one distinct in its shape, size, attachment and innervation. Muscles achieve their unique identities by utilising genetic information that is common to the development of all muscles as well as specialised information that determines their distinct properties. For this, both mesoderm autonomous information and inductive signals imparted by neighbouring tissues like epidermis and nervous system are required. To understand the genetic, the cellular and the molecular mechanisms that mediate these complex interactions, such that a functional muscle pattern emerges, is a challenge in developmental biology.

Studies in different animal systems, both vertebrate and invertebrate, have provided useful insights into myogenesis. The fruitfly, *Drosophila melanogaster*, being highly amenable to genetic dissection, has proved to be a useful tool for such studies and many significant aspects of myogenesis have been described in the fly embryo. Our laboratory has used the adult flight muscles of *Drosophila* to understand the mechanisms that govern events of myogenesis such as cell fate specification, mesoderm diversification and muscle patterning. Here we review current developments in this area.

Key Words: ***Drosophila*, Muscle development, Genetic information, Muscle pattern, Fruit fly embryo**

Cell Fate Specification and Differentiation in the Embryo

Early Mesodermal Subdivision

Very early in embryonic development, cells that will form mesoderm and its derivatives express *twist (twi)* (Thisse et al. 1988), invaginate into the embryo, spread as an epithelial sheet closely apposed to the external epidermis and divide (Leptin & Grunewald 1990). This contact is important because secreted products of patterning genes like *decapentaplegic (dpp)* signal from the epidermis to pattern the mesoderm below. *dpp* expressed in a dorsal band of ectodermal cells maintains expression of the mesodermal marker *tinman (tin)* and represses ventrally expressed genes such as *pox meso* (Staehling-Hampton et al. 1994, Frasch 1995). The mesoderm also undergoes partitioning along the anterior-posterior axis by action of segmentation genes *even skipped (eve)* and *sloppy paired (slp)* (Azpiazu et al. 1996, Riechmann et al. 1997). Consequently, a refinement of Twi expression occurs into a modulated pattern in different mesodermal anlages. Wingless (Wg) amplifies the distinctions between cells of the *eve* and *slp* domains by maintaining high levels of Twi in the *slp* domain. These cells form somatic muscles (Baylies et al. 1995). Cells of the *eve* domain become cardiac muscles and visceral muscles (Azpiazu & Frasch 1993, Bate & Rushton 1993, Yin et al. 1997, Riechmann et al. 1997).

Early Muscle Patterning: Founder and Feeders

Midway through embryogenesis, mesodermal cells destined to form somatic muscles lose Twi expression, fuse and differentiate to form muscle fibres (Bate 1990, Bate et al. 1991). Unlike vertebrates where aggregates of muscle fibres constitute a single muscle, in the *Drosophila* embryo, each muscle is a single, multinucleate fibre, unique in its position, size, sites of attachment and

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patterns of innervation (Bate 1993, Bernstein et al. 1993). The development of each muscle fibre in the embryo is seeded by a specialised myoblast called the **'Founder Cell'**. The founder cell fuses with neighbouring fusion competent myoblasts, the **'Feeder Cells'**, entraining them to its pattern of gene expression and forming a syncytial muscle (Bate 1990, 1993). Founders, thus, are privileged cells that are self sufficient in their access to genetic information required to complete myogenesis and form a specific muscle, as against the 'naive' feeders that cannot form muscles independent of the founders. In the absence of fusion, founders differentiate miniature, mononucleate muscles at proper positions, normal in all aspects of myogenesis except their size. Feeders in such a situation fail to form muscle and remain undifferentiated (Rushton et al. 1995).

Founder Cell Specification

Founder cells are characterised by the expression of specific **'muscle identity genes'** (Frasch 1999), encoding transcription factors, including *S59, apterous (ap), Kruppel (Kr), vestigial (vg), ladybird (lb)* etc., (Dohrmann et al. 1990, Bourgouin et al. 1992, Ruiz-Gomez et al. 1997, Jagla et al. 1998). The overlapping expression of these genes in different sets of muscle, and functional analysis in genetic experiments, has led to the hypothesis that individual muscles are specified by defined combinatorial codes of identity genes. Mutations in muscle identity genes have been shown to result in loss of, as well as, transformation in identities of specific muscles (Ruiz-Gomez & Bate 1997). Recently, loss of function of *nautilus (nau)*- also called *slouch* and *S59*- which is the *Drosophila* homologue of a very important family of vertebrate genes controlling multiple stages of myogenesis, has shown its requirement of a small subset of embryonic muscles (Balagopalan et al. 2001). Founder cells are division products of **'Progenitor'** cells which are chosen from equivalent groups of mesodermal cells (Carmena et al. 1995, Ruiz-Gomez & Bate 1997) and express the proneural gene *lethal of scute (l'sc)* (Carmena et al. 1995). This selection is mediated by intrinsic mesoderm encoded information as well as cues arising from regions of the epidermis overlying that part of the mesoderm. This has been clearly illustrated in the case of progenitors expressing Eve. Wg and Dpp from the epidermis activate receptor tyrosine kinase (RTK) pathways {the *Drosophila* Egf receptor (DER) and the FGF receptor encoded by *heartless (htl)*}, and thus Ras signalling, in the equivalence group. Consequently, a single cell gets selected, from this 'Equivalent Cluster' of Eve expressing cells, to continue expressing Eve. This becomes a Progenitor. The remaining cells lose Eve and adopt feeder fate (Carmena et al. 1998). This convergence of multiple signals in regulation of *eve* expression and determination of progenitor identity occurs at a single enhancer in the *eve* promoter region. It has binding sites for, and responds to, the extrinsic Wg, Dpp and RTK signals, as well as tissue specific proteins Tin and Twi, suggesting that fates in the mesoderm are determined by a combination of extrinsic cues and the developmental histories of cells (figure 1a, b, Halfon et al. 2000). Once the proneural cluster is formed, the singling out of a single progenitor is also dependent on the *Notch (N)* mediated process of Lateral Inhibition (Corbin et al. 1991, Bate et al. 1993, Baker & Schubiger 1996). Once the identity of a progenitor is determined, its asymmetric division contributes to the diversification of individual muscle fates. An important consequence of this asymmetric division appears to be the differential maintenance of expression of muscle identity genes (Kr, for instance), in only one of the two descendent founders (Ruiz-Gomez et al. 1997). The generation of this distinction depends on the cytoplasmic protein Numb (Nb) and determines founder fate (Ruiz-Gomez et al. 1997, Carmena et al. 1998). Nb is retained in one of the progenitor descendants, causes downregulation of *N* (Frise et al. 1996) and maintains expression of the muscle identity gene to form a founder for a specific muscle. It is lost from the other progeny, which allows continued N activity and subsequent loss of expression of the muscle identity gene of the progenitor, generating a founder for another muscle (figure 1c). Mechanisms controlling asymmetric segregation of Nb are not yet known, however, the product of the *inscutable (insc)* gene appears to be one of the key components. Altogether, the observed involvement of lineage genes such as *insc, nb,* and *N* may occur during the asymmetric division of all muscle progenitors.

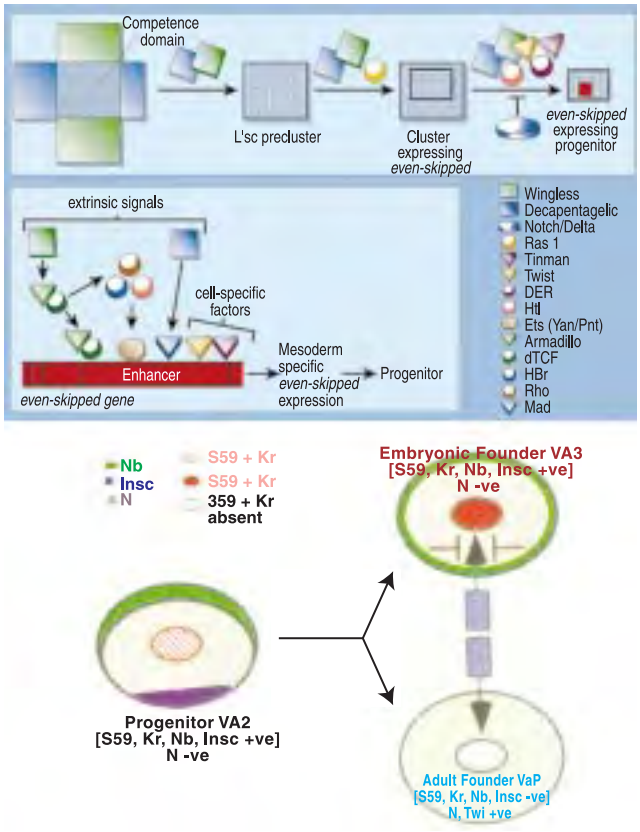

Figure 1a, b Gene regulation by combinatorial logic. Specification of Eve expressing progenitor cell which occurs through convergence of multiple signals at a single enhancer of the gene is shown in the schematic in a and b. Wg and Dpp signal along the dorsal epidermis to act on the underlying mesodermal cells that express Twi and Tin. This defines a cluster of 'competent' cells expressing the transcription factor L'sc. Signalling through the Wg and Dpp pathways, along with the RTK pathways involving DER and Htl eventually produce just one Eve expressing cell **[a].** Proteins downstream of Wg, Dpp and RTK signalling pathways combine with the intrinsic factors Twi and Tin and converge at the Eve- enhancer **[b]** to regulate its mesoderm specific expression and specification of the progenitor *(Ghazi and VijayRaghavan, 2000).* **[c].** Specification of adult muscle founders: Adult muscle founders form as siblings of embryonic muscle founders, as a consequence of asymmetric cell division of progenitors. In some instances, as in the case of the Eve- expressing progenitor in the thoracic segments shown here, when the progenitor divides, one of the progeny retains expression of Eve [red nucleus] and the other loses it [white nucleus]. This is determined by the distribution of the cytoplasmic protein Nb [green crescent] between the two progeny. The descendent that gets Nb undergoes inactivation of N activity by Nb, continues maintaining Eve expression, and becomes an embryonic muscle founder. Its sibling which does not get Nb continues to have active N signalling, loses Eve and becomes the founder of an adult muscle.

Asymmetry of Muscle Fusion

With founder cell specification, *Drosophila* myoblasts become segregated into two types of cells, founders and feeders. Founders are competent only to fuse with feeders and vice versa. The two cell types cannot fuse with members of their own class. Identification of two new genes, *dumb-founded (duf)* and *sticks-and-stones (sns),* (Ruiz-Gomez et al. 2000, Bour et al. 2000) suggest that an asymmetric distribution of cell-cell interaction molecules might be implicated in this asymmetry generation. Both Duf and Sns are novel members of the Immunoglobulin superfamily and seem to act at the earliest steps of muscle fusion. Duf is expressed in the founders but not in the feeders, whereas Sns is specifically expressed in the feeders. Both are crucial for fusion. By contrast, molecules described earlier like Drosophila Rac1 (Drac1), Myoblast city (Mbl/Dock180) and Blown fuse (Blow) are intracellular and not known to be asymmetrically expressed in the two myoblast populations (Luo et al. 1994, Doberstein et al. 1997, Erickson et al. 1997). However, how muscle identity genes influence the extent of muscle fusion is not clear.

Founder cells have also been shown to be sources of cue(s) required to trigger defasciculation and targeted growth of motor axons that innervate their unique muscles. A single founder myoblast is found to trigger the defasciculation of an entire nerve branch, suggesting that the muscle field is structured into sets of muscles each expressing a common defasciculation cue for a given nerve branch (Landgraf et al. 1999).

Adult Flight Muscle Specification and Differentiation

Specification of Adult Muscle Precursors

During the asymmetric division of progenitors, not all descendent cells get specified to form founders of larval muscles. In some regions, like the abdomen and thoracic segments, one of the progeny gets allocated to form precursors of adult muscles. The progenitor descendent that retains Nb, and the muscle identity gene expression, gets committed to become an embryonic founder. Its sibling which does not inherit Nb and which has active N signalling continues to express Twi and postpones differentiation to become an adult muscle

precursor (Ruiz-Gomez & Bate 1997). Not only are adult precursors produced as siblings of embryonic founders, they are also produced at precisely analogous geographical locations where ultimately they organise an adult pattern in the pupa.

Wing Disc Associated Myoblasts

By the end of embryogenesis, Twi expression persists in a handful of mesodermal cells, that are precursors of adult muscles and divide actively to produce pools of myoblasts in thoracic segments and adhere to the wing imaginal disc (Bate et al. 1991). Cell fate decisions in the wing disc distinguish the future wing from the body wall (notum). This decision depends on the antagonistic interactions of Wg and DER. Wg specifies the wing primordium and DER signalling, stimulated by its ligand Vein (Vn), directs cells to become notum (Wang et al. 2000, Baonza et al. 2000). Adult muscle precursors associated with the wing disc are located in the presumptive notum region and continue to remain restricted to this location till metamorphosis (figure 2a). Besides Twi, several other genes have been shown to be expressed in these myoblasts. These include *cut (ct), vg, scalloped (sd), htl* and the *Drosophila* homologue of the vertebrate *myocyte enhancer factor (Dmef- 2)* (Campbell et al. 1991, 1992, Blochlinger et al. 1993, Emori & Saigo 1993, Williams et al. 1993, 1994, Ng et al. 1996, Couso et al. 1995, Ranganayakulu et al. 1995).

The entire population of wing disc myoblasts was believed to be a homogenous pool of cells, which during pupation adopted diverse muscle fates, in response to unknown autonomous or extrinsic cues. Similarly, presence of adult counterparts of embryonic muscle founders was speculated upon but not confirmed. Recent reports substantiate the presence of adult muscle founders and dispel the notion of the wing disc harbouring an identical pool of myoblasts. One of the members of the *Enhancer of split Complex {E(spl-C)}* (nuclear effectors of *N* signalling), *E(Spl-C)m6*, is found to accumulate in a small subset of wing disc myoblasts and the expression relies on *N* activity (Lai et al. 2000). This suggests that distinctions exist between myoblasts on the disc itself. There has also been evidence for the presence of a morphologically distinct class of disc associated myoblasts that prefigure the formation of some of the adult flight

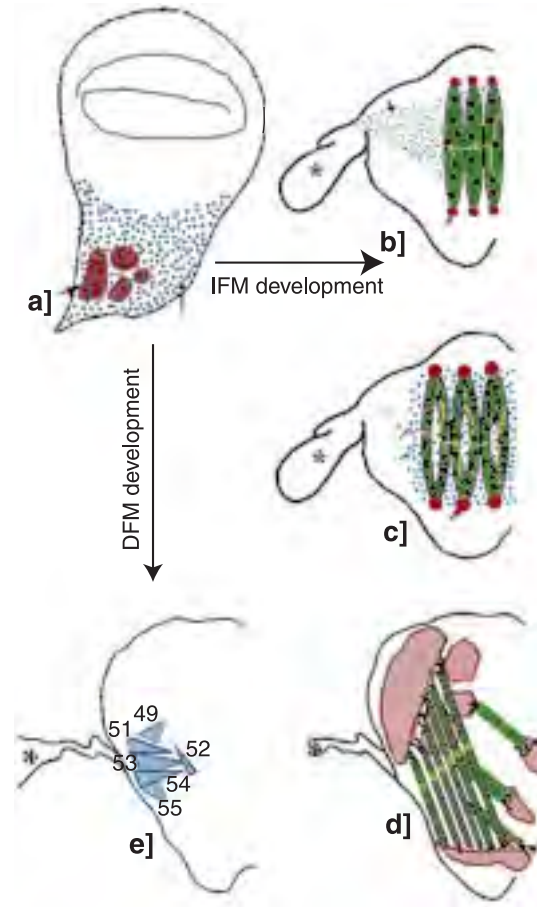

Figure 2 Schematic representation of thoracic flight myogenesis:

a] Myoblasts [blue dots] on the wing disc are associated with the presumptive notum and proliferate during larval life. *sr* expression [red regions indicated by arrows] on the presumptive notum marks the future epidermal attachment sites of thoracic muscles. IFM development is represented in B, C and D. In these panels anterior is to the top and dorsal midline to the right; **b]** Myoblasts migrate on the everting disc epithelium [schematic represents 7-12h APF] onto three larval muscles [green fibres] that escape histolysis and serve as templates that attach to *sr* expressing epidermal attachment sites [red spots]; **c]** As myoblasts fuse to the templates, they begin to split longitudinally between 14-18h APF. By 19h APF, six dorsal longitudinal muscles [DLMs] are in place; **d]** shows IFMs in an adult heminotum with their attachment sites. Six DLMs [dark green] run antero-posteriorly and attach to *sr* expressing attachment domains [red]. DVMs run dorsoventrally [light green; **e]** Direct flight muscles [DFMs] are also derived from wing disc associated myoblasts. Muscles 49 and 51-55 followed in our study are shown in blue. In panels C, D and E, the expression of *ap*, has been indicated with bright orange arrows. Wing disc shows low levels of *ap* expression through out the presumptive notum region. In all panels except a, wing bud [b, c] and adult wing nub [d, e] are indicated by asterisks. *Schematic from Ghazi et al. 2000.*

muscles too (Rivlin et al. 2000). These reports indicate that larval stages are not quiescent carriers of adult muscle precursors and that besides proliferation, active signalling and cell fate determination are undergone by these cells, before metamorphosis commences. The epidermal cells that serve as attachment sites for flight muscles are specified at the third larval instar stage itself (described ahead). The reason for this early segregation is not clear but the fact that myoblasts remain in close association with these cells during their residence on the prospective notum suggests that important patterning information could be exchanged between attachment sites and myoblasts. Besides wing disc myoblasts, there are groups of myoblasts that remain associated with peripheral nerves that innervate larval thoracic muscles (Bate et al. 1991). Whether these cells represent a special class of myoblasts that are different from the disc myoblasts and whether they contribute to definite sets of muscles is unclear.

Thoracic Flight Muscles

Two kinds of flight muscles are present in the adult notum. These are the fibrillar indirect flight muscles (IFMs; figure 2d) and the tubular direct flight muscles (DFMs; figure 2e). Both the IFMs and the DFMs derive from the wing disc myoblasts, whereas myoblasts associated with the mesothoracic leg discs contribute to a large muscle called the tergal depressor of trochanter (TDT) or jump muscle (Crossley 1978, Fernandes et al. 1991). Despite a common origin the IFMs and the DFMs differ from each other in their morphology, physiology and molecular markers displayed. But unlike the IFMs, not much is known about the development of the DFMs, because of their small size, and lack of markers that specifically label their developmental stages.

Development of the two subsets of IFMs, the dorsal longitudinal muscles (DLMs) and the dorsoventral muscles (DVMs) has been described extensively (Fernandes et al. 1991, Fernandes & VijayRaghavan 1993, Anant et al. 1998, Roy et al. 1997, Roy & VijayRaghavan 1998, 1999). The availability of several reporter genes and antibody probes that label different stages of development of these muscles, their innervation, their differentiation and their attachment, has provided insights into the mechanism of their development (Fernandes et al. 1991, Barthmaier & Fyrberg 1995). The DLMs and the DVMs have different developmental histories: DLMs develop using persistent larval muscles as scaffolds (Shatoury 1956, Crossley 1978, Fernandes et al. 1991) and DVMs develop by de novo fusion of myoblasts (Fernandes et al. 1991). During early pupation, as the wing disc evaginates, the disc myoblasts migrate on to the developing dorsal mesothorax at sites of muscle formation (Bate et al. 1991, Fernandes & VijayRaghavan 1993). At this time, when larval muscles undergo histolysis, three muscles -the dorsal oblique 1,2 and 3- persist. Myoblasts swarm over these templates, fuse with them and result in their splitting into six fibres to form the final pattern of six DLMs observed in the adult. Groups of the same myoblasts organise themselves at positions where DVMs develop and undergo fusion to form adult DVMs (Fernandes et al. 1991). Figure 2 represents the events of flight myogenesis.

Splitting of larval templates to form six fibres is an important event in IFM development and imaginal myoblasts are directly involved in the process. If wing discs are depleted of their myoblasts splitting fails and muscles degenerate (Roy & VijayRaghavan 1998). The larval muscles themselves are required for regulating the proper number of DLM fibres: on template ablation, DLM development proceeds normally but the number of fibres varies considerably (Fernandes & Keshishian 1996). Flight myogenesis also progresses in close synchrony with the development of innervation. Laser ablation of their motor nerves does not substantially affect DLM development but denervation disrupts DVM development (Fernandes & Keshishian 1998). This also indicates inherent differences in the way DLMs and DVMs are patterned, with the DVMs relying on neural cues and DLMs on persistent larval templates. The specification of DFM fate is found to depend on the *gene apterous (ap)* which gets expressed in groups of myoblasts that go onto form the DFM (Ghazi et al. 2000), whereas myoblasts that do not express ap give rise to the IFMs.

Flight Muscle Differentiation

As fusion of myoblasts to larval templates proceeds, expression of Twi from these cells declines and expression of differentiation markers, like Erectwing (Ewg), commences in developing muscles (Roy & VijayRaghavan 1998). This

downregulation of Twi is an essential step for normal differentiation, which also requires *N*. Overexpression of an activated form of *N* can result in an abnormal persistence in Twi expression in nuclei of differentiating fibres suggesting that *N* is an important signal for maintenance of Twi expression in adult precursors which in turn influences differentiation (Anant et al. 1998).

Patterning Mechanisms: Attachment of Muscles

Normal motor function requires that muscles form, and maintain, stable muscle attachments at correct skeletal locations. While little is known about mechanisms of muscle attachment in vertebrates, genetic studies in the fruitfly are beginning to reveal its cellular and molecular basis. Unlike vertebrates, where muscles attach to cartilage or bone with the help of tendons, invertebrate muscles attach to epidermal **Tendon Cells (TCs).** The ectoderm is thought to provide positional information for correct migration and arrangement of different types of myotubes. Early experiments in the mealworm *Tenebrio* had shown that rotation of pieces of the ectoderm can induce changes in somatic muscle patterning (Williams & Caveney 1980a,b, Williams et al. 1984). Tissue culture experiments in the *Drosophila* embryo illustrated that the cells along the segment borders, to which muscles attach, provide guidance cues that influence muscle migration and attachment (Volk & VijayRaghavan 1994).

TCs differentiate from early **Tendon Precursor Cells (TPCs)** (also called Epidermal Muscle Attachment {EMA} cells). Following fusion, each developing fibre extends its leading edges (filopodia) in both directions towards the TPCs (Bate 1990). Establishment of an attachment between the approaching myotube and a TPC is followed by arrest of myotube extension. Only myotube bound cells differentiate into the fusiform TCs (Becker et al. 1997). Forces exerted by the muscles are transmitted to the cuticle through a series of muscle and TC specializations at the **MyoTendinous Junction (MTJ),** where muscle and tendon cell membranes interdigitate extensively, each secured by specialized junctions to the intervening **ExtraCellular Matrix (ECM)**. Differentiation of the MTJs requires a molecular conversation between muscle and TCs, interruption of which prevents effective muscle attachment (Becker et al. 1997).

Determination of Tendon Cell Fate

Pools of epidermal cells acquire the competence to attach muscles and become TPCs by expression of the gene *stripe (sr). sr* encodes a DNA- binding protein with triple Zn finger domains. It is a *Drosophila* member of the *early growth response (egr)* family of transcription factors and is homologous to vertebrate Egr-1 and Egr-2 proteins (Volk & VijayRaghavan 1994, Lee et al. 1995, Frommer et al. 1996). *sr* is crucial and sufficient for induction of an array of tendon specific genes, including its own expression. In *sr* mutant embryos, expression of most tendon specific genes is drastically diminished and muscles ignore their normal attachment sites, insert at alternate positions and often degenerate. Conversely, generation of ectopic TCs by ectopic Sr expression leads to attraction of myotubes towards these new target cells. TPCs thus provide essential attractive cues that direct muscle extension and also arrest further filopodia formation (Volk & VijayRaghavan 1994, Frommer et al. 1996, Becker et al. 1997, Vorbruggen & Jackle 1997).

The differentiation of TCs is biphasic. The initial phase is muscle independent, characterised by expression of Sr at low levels in large groups of cells and by expression of markers like Groovin, Alien etc (Goubeaud et al. 1996, Becker et al. 1997, Strumpf & Volk 1998). These are postulated to require low levels of Sr for their induction. The second phase is muscle dependent, triggered by musculo- epithelial contact and marked by high levels of Sr in muscle attached TCs. Delilah (Del) and β-1-Tubulin are the markers of this late stage and probably require high levels of Sr for induction (Armand et al. 1994, Buttgereit 1996). Initial determination of TPC identity is induced by positional cues that pattern the entire embryonic ectoderm. Embryos mutant for the segment polarity genes like *patched, naked, lines, wg* etc., show impaired patterns of *sr* expression (Volk & VijayRaghavan 1994). At the segment borders, *sr* expressing TPCs are specified at precise locations as a result of interaction of repressive *wg* and inductive *hh* signals. A single enhancer in the *sr* promoter region has binding sites for, and responds to, both signals to determine the *sr* expression domain (Piepenburg et al. 2000).

Molecular Regulation of TC Differentiation

While TPCs are defined autonomously in the ectoderm, their terminal differentiation into TCs is induced by muscle attachment. Transmission of this signal from muscle to the epidermis involves the DER pathway and its ligand Vn. *vn* mRNA is present at high levels in all muscles before they adhere to the epidermis, but the protein is highly concentrated specifically at MTJs. This localized accumulation of Vn is critical for triggering the maturation of the single muscle bound TC and strongly activates the DER pathway in the TPC. Activation of Ras signalling follows this, followed by expression of TC specific differentiation markers like β-1-Tubulin and Del. Vn is, thus, a muscle derived signal that activate DER in TCs for their differentiation (Schnepp et al. 1996, Yarnitzky et al. 1997, 1998). Clues to the restricted localization of Vn to the MTJs emerge from studies with the gene *kakapo (kak;* earlier called *Groovin).* Kak is an intracellular protein expressed mainly along the TPC plasma membrane, presumably associated with various cytoskeletal elements within the TC and may directly regulate the extracellular localization of Vn. The precise mechanism by which this is brought about is not clear (Strumpf & Volk 1998, Gregory & Brown 1998).

The cellular interactions between muscle and tendon cell that allow the terminal differentiation of the latter involve modulating Sr levels before and after muscle binding. The mechanism that regulates the transition from TPCs to TCs by effecting Sr postranscriptionally has been shown to rely on the gene *held out wings (how).* (Zaffran et al. 1997, Baehrecke 1997). The two protein variants encoded by *how* are differentially distributed in the TPCs and TCs. How Long {How(L)} is nucleus restricted and How Short {How(S)} is present both in the cytoplasm as well as the nucleus of the tendon cells. Sr is a target of How which binds *sr* mRNA at the 3′ UTR. At the initial stage How(L) is the predominant form expressed so that Sr is maintained at low levels and the cell is maintained in a partially differentiated state. Activation of the DER pathway in the TC, on muscle binding, causes increase in levels of How(S) which presumably competes with How(L) for binding *sr* mRNA, thereby leading to increased Sr nuclear export. The resulting increase in Sr protein levels lead to a terminal differentiation of TC (figure 3; Nabel-Rosen et al. 1999). TPCs that do not undergo muscle attachment lose *sr* expression eventually. Continued muscle contact is crucial for maintenance of high levels of Sr in the TCs and a loss of muscle is known to cause an eradication of Sr from the TC (Becker et al. 1997).

Ultrastructure of Myotendinous Junctions

Once in contact, muscle and tendon cells must synthesize and localize the structural components of the MTJ such that the junction can withstand mechanical stress. In the mature MTJ of the *Drosophila* embryo, membranes of the muscle and tendon cells are highly interdigitated to increase surface area and linked indirectly via hemidesmosomal or **Hemi Adherens Junctions (HAJs)** that anchor each cell to a common specialized ECM (Tepass & Hartenstein 1994). The HAJs found along the basal surface of the contacted epidermal cell have a layer of electron dense material on the inside

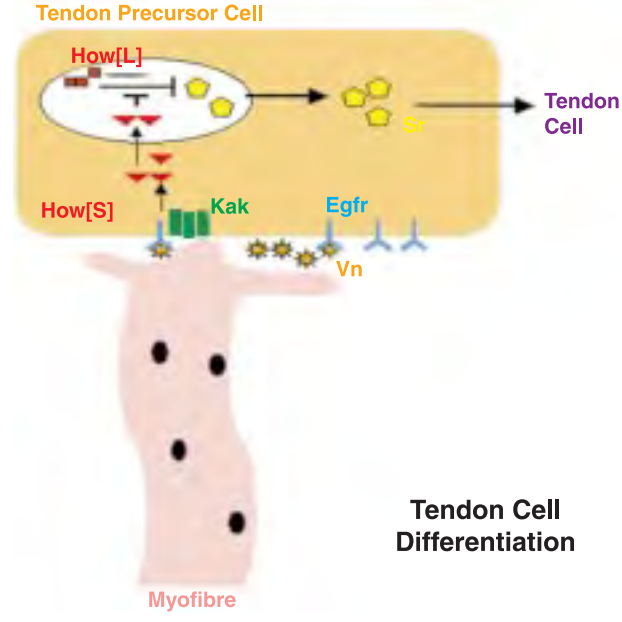

Figure 3 Differentiation of tendon precursor cells into tendon cells. Tendon precursor cells [orange] exhibit the nuclear How[L] form [red squares] of *how,* which binds *sr* mRNA and prevents its translation. On contact with a myofibre [pink], DER signalling [blue] pathway gets activated in the precursor. This is dependent on the accumulation of the DER ligand Vn [orange] at the muscle-epidermal junction. As a consequence of DER activation the levels of the nuclear cum cytoplasmic form of *how,* How[S] [red triangles] increases in the cell. How[S] competes with How[L] in binding *sr* mRNA and results in its increased export from the nucleus [yellow]. This causes an increased translation of Sr and results in differentiation of the precursor into a tendon cell. *This schematic is modified from Volk 1999.*

into which cytoskeletal elements insert: actin filaments of the contractile apparatus in the muscle and microtubules in the epidermis. The muscle membrane is linked to the microfilaments via modified terminal Z bands. The basal membrane of the TC is linked via numerous microtubules to specialized anchors (tonofibrillae) embedded in the cuticle, an apical secretion of the same cell (Caveney 1969, Prokop et al. 1998). The ECM present between the epidermal and mesodermal components of the MTJ displays a large number of proteins, which have been reported in the *Drosophila* embryo. The most well characterised of these are the Position Specific (PS) Integrins. PS integrins are dimeric- they share a common β-subunit and differ in the α-subunits. The β-subunit is encoded by *myospheroid (mys)*, mutations in which cause a muscle detachment phenotype in embryos (Newman & Wright 1981). α-PS2 is encoded by the gene *inflated (if)* and mutations do cause muscle detachment phenotypes, though not as strong as in *mys* (Brown 1994). The gene encoding α-PS1 is *multiple edematous wing (mew)* (Brower et al. 1995a,b). PS1 (αPS1βPS) is expressed at the basal surface of the TCs and PS2 (αPS2βPS) localizes to the ends of muscles where they attach to these cells (reviewed in Brown 1993). In the embryo, PS integrins are required in both layers- muscles and epidermis- to help DER mediated regulation of TC differentiation (Martin-Bermudo 2000). Besides integrins, there are other molecules like Tenascin A, Tiggrin, Laminin, Slit etc. (Baumgartner & Chiquet-Ehrisman 1993, Fogerty et al. 1994, Rothberg et al. 1990, Kidd et al. 1999).

Attachment of Adult Flight Muscles

During pupation, as the adult epidermis arising from the evaginating imaginal discs gradually replaces the larval epidermis, the larval connections are replaced with new attachments to the adult cuticle. In the adult, like in the embryo, these attachment sites are specified by the gene *sr* (Fernandes et al. 1996). On the presumptive notum region of the wing disc, *sr* is expressed in a discrete set of domains, in the epidermal cells. There are four regions in the anterior notum and one thin line in the posterior notum (Figure 4a), which prefigure attachment sites of flight muscles. *sr* is the earliest marker known to specify these domains, at the disc stage itself. During pupation, these regions form the tendon cells of flight muscles (figure 4b). The development of these attachment sites with respect to the developing muscles has been described, and correlation of different IFM fibres to specific subsets of *sr* domains has been mapped in detail (Fernandes et al. 1996). The anterior clusters serve as insertion points for the DLMs, the DVMs and the TDT, whereas the posterior region is adhered to by the posterior DLM ends and the ventral end of DVM III. Ventral attachments of DVM I and II and TDT arise from the leg discs (figure 4b). It is conceivable that matching of muscle fibres and specific *sr* subsets occurs and *sr* may act in conjunction with other molecules to ensure that correct muscle pattern develops. Mutations at the *sr* locus cause defects in IFMs, especially in the DLMs. There is DLM loss characterised by normal early steps of myogenesis followed by detachment of the fibres, which leads to 'curling up' and eventual degeneration (Costello & Wyman 1986). Information on the function of *sr* in adult muscle attachment comes from expression pattern analysis, partial loss of function mutants and from data from the embryo, but the details of its action are not clear.

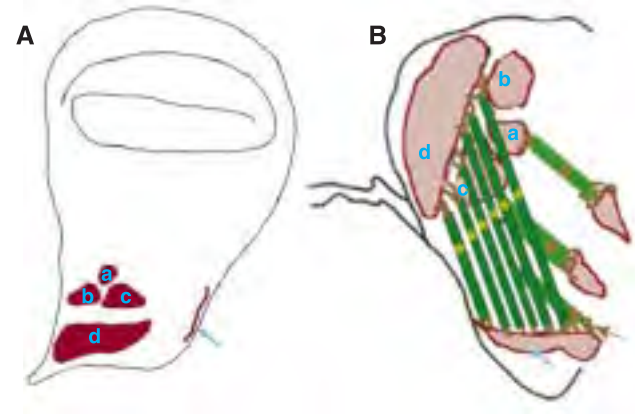

Figure 4 Flight Muscle Attachment Sites. **a,** Schematic representation of a wing imaginal disc with the *sr*-expressing attachment sites shown in red. The four domains 'a-d' give rise to the anterior dorsal attachment sites of the IFMs while the thin posterior stripe [arrow] gives rise to the posterior attachment sites; **b,** Schematic of an adult heminotum that forms from the presumptive notum region of a wing disc. DLMs are shown as dark green fibres, indicated by yellow asterisks. DVMs are shown in lighter green and indicated by red asterisks. The attachment sites of these muscles, derived from the *sr*- expressing cells of the wing disc, are shown in red. Different domains [a-d] that correlate with the attachment of different muscle subsets are indicated in the figure. Posterior attachment site is marked by a blue arrow. The brown arrow marks the epidermal extensions, apodemes, which connect the muscle to the tendon cells. In **a**, anterior is to the left. In **b**, anterior is to the top.

Besides *sr*, a few other molecules are known to show remarkable expressions in adult TCs. Integrins display a dynamic temporal expression profile during flight muscle attachment. They are not expressed when muscle fibres first make their appearance (12-20h APF) but following muscle-epidermis contact are detected at the attachment sites. PS1 is at the muscle ends and also in the fibres that connect the developing muscles to their attachment sites, while PS2 is restricted to ends of larval muscles (Fernandes et al. 1996). A few mutants that show attachment specific defects have been reported. These include the Broad Complex (BR-C) transcriptions factors that are induced by the hormone 20-hydroxyecdysone (20E). Mutations of the *reduced bristles on palpus (rbp)* complementation group, which corresponds to the BRC-Z1, the isoform expressed in pupal tendon cells, reduce or eliminate DVMs selectively and disrupt muscle attachments. Mosaic analyses have revealed that *rbp+* function is required in dorsal TCs for normal DVM attachment. Presumably BR-C Z1, under control of 20E regulates target genes whose products control specific features of TC maturation (Sandstorm et al. 1997, Sandstorm & Restifo 1999). A Type I Ser/Thr Protein Phosphatase 1(PP1) that is encoded by the gene *flapwing (flw)* also functions in maintenance of IFM attachments (Raghavan et al. 2000). Ultrastructure of adult flight muscle MTJs has been described and resembles the embryonic description. A recent study however, has described the presence of an additional sheath of overlapping flattened cytoplasmic extensions surrounding the TC processes (Sandstorm & Restifo 1999).

While much is now known about the mechanisms governing different aspects of muscle development, both in the embryo, and in the adult *Drosophila*, there is much more that needs to be deciphered. This includes the genetic and molecular aspects of muscle size control, muscle diversity etc. The importance of signalling networks interacting with each other in controlling myogenic targets and transcriptional heirarchies are beginning to be unravelled now. Greater insights into the complexities of these interactions and feedback networks, along with discovery of novel factors whose functions remain to be examined will help elucidate the details of myogenesis. The adult flight muscle system continues to remain an exciting model to ask such questions of wide biological significance and will surely contribute in providing answers to many of them.

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