Int. J. Dev. Biol. 42: 283-290 (1998)

Muscle patterning and specification in *Drosophila*

MAR RUIZ-GÓMEZ*

Department of Zoology, University of Cambridge, Cambridge, United Kingdom

ABSTRACT There are obvious differences in the way sense organs and muscles are generated during *Drosophila* embryogenesis. For example, all the cells that compose the final sense organ are derived from a unique cell through a well-established lineage, whereas each muscle is formed by fusion of myoblasts that belong to two different populations: a founder cell and a pool of fusion competent cells. Despite these differences, similar genes and mechanisms appear to be involved in the generation of the pattern of sense organs and in muscle development. Thus, the process of specifying individual cells and endowing them with the ability to initiate neuronal or muscle development, as well as the acquisition of alternative fates among sibling cells, appear to be under similar genetic control both in neural and muscle development.

KEY WORDS: *muscle pattern, Drosophila*

Introduction

Pattern formation is a process whereby particular cells belonging to an originally uniform population acquire unique characteristics which will allow them to differentiate and give rise to the distinct elements of a defined spatial pattern. The *Drosophila* larva provides us with an ideal system to study the process of pattern formation. For example, the elements of the Peripheral Nervous System (PNS) are arranged in a defined bi-dimensional pattern (Fig. 1A) and the somatic muscles form a three-dimensional pattern whose elements are positioned at different levels relative to the ectoderm (Fig. 1B).

Pattern formation in the PNS, both in the larva and in the adult fly, has been the subject of several studies which have led to the conclusion that the sense organs are formed step by step by a process of "progressive determination" (reviewed in Ghysen and Dambly-Chaudière, 1989; Campuzano and Modolell, 1992; Jan and Jan, 1993). PNS formation begins in the early blastula with the definition of the neuroectoderm. Within the neuroectoderm, positional information provided along the dorso-ventral and anterior-posterior axis by "prepattern genes" is translated into the local activation of "proneural genes" in clusters of ectodermal cells. Proneural genes belong to the b-HLH family of transcriptional regulators and their expression confers to ectodermal cells the competence to become neuronal precursors. Thus, loss of function of proneural genes results in a partial or complete absence of the PNS and ectopic expression produces supernumerary sensory elements. Within a proneural cluster, competence to become neuronal precursor is restricted to the one or few cells which accumulate the largest amounts of proneural proteins; this process of selection is mediated by the "neurogenic genes". Once neuronal precursors are specified, they will divide according to a fixed lineage to produce all the elements of the final sense organ.

The larval PNS consists of a relatively simple set of 40 individually identified sense organs per hemisegment, which fall into three main classes: external sense organs (es), chordotonal organs (ch) and multiple dendrite neurons (md) (Ghysen *et al.,* 1986; Bodmer *et al.,* 1989; Jan and Jan, 1993). Sense organs belonging to each class are very similar in their patterns of axon projection in the CNS (Merritt and Whitington, 1995), although es organs fall into different subtypes according to their cuticular processes (hairs or papillae) and the number of neurons that innervate them (mono- or multi-innervated). The kind of sense organ the precursors are going to give rise to, seems to be specified very early, at the time they are born (Jan and Jan, 1992). Several genes, the so called "neuron-type selector genes" are known whose function is required to confer identity to the sense organs. For example, the homeodomain protein *cut* is required to produce external sense organs (Bodmer *et al.,* 1987), and the paired domain protein *pox-neuro* is required to specify multi-innervated sense organs (Dambly-Chaudière *et al.,* 1992).

In contrast to the relative simplicity of the PNS, the muscle pattern consists of 30 elements per abdominal hemisegment which present unique characteristics clearly identifiable by morphological criteria (Bate, 1993 and Fig. 1B). The way the muscle pattern is generated poses a series of questions that seem to require different solutions to those adopted in the case of the PNS, and that make it a very attractive system to study. Firstly, muscles are

***Address for reprints**: Department of Zoology, University of Cambridge, Downing street, Cambridge, CB2 3EJ, United Kingdom. FAX: 44 1223 336676. e-mail: mr10017@hermes.cam.ac.uk

0214-6282/97/$10.00

Printed in Spain

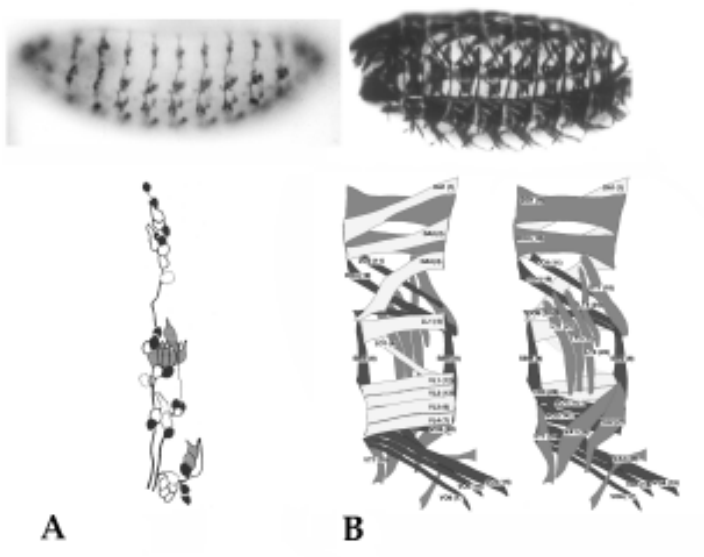

Fig. 1. Embryonic pattern of peripheral neurons (A) and somatic muscles (B). *In the upper part of the Figure both patterns are shown as revealed by staining with 22c10* **(A)** *and anti-myosin* **(B)** *antibodies. The lower part shows schematic representations of both patterns in abdominal segments. In A, external sensory neurons are represented in black, chordotonal neurons striped and multiple dendritic neurons as empty cells. In B, internal (left) and external (right) views of the larval muscle pattern are shown. Internal muscles are coloured in light grey, intermediate muscles in black and external muscles in dark grey. For muscle nomenclature see Bate (1993), Ruiz-Gómez et al. (1997).*

internal derivatives and their specification involves both information intrinsic to the mesoderm and signals from the adjacent ectoderm. How are these two factors integrated? Secondly, how are the cells that contributed to different muscles informed about their position within a three-dimensional pattern? And finally, the different cells that form a mature muscle fiber are not clonally related but the result of a fusion process, how is the information required to give rise to a particular member of the pattern implemented in those cells?

Here I will consider the problem of muscle patterning and specification. I shall highlight the fact that even though there are obvious differences in the development of the PNS and the muscle pattern, there is a conservation in terms of the genes and mechanisms that are used with only minor modifications to generate positional specificity.

Specification of the myogenic mesoderm

The mesoderm is derived from the most ventral cells in the blastoderm. These cells coexpress *twist (twi)* and *snail (sna)* and, at gastrulation, invaginate along the ventral furrow, divide twice and spread dorsally to form a monolayer in close contact with the overlying ectoderm (Leptin and Grunewald, 1990). During a third mesodermal division, some cells lose contact with the ectoderm and migrate to a more internal level. A fourth division gives rise to the final population of mesodermal cells from which all the mesodermal derivatives including the somatic muscles, the visceral muscles, the heart and the fat body will derive (Hartenstein and Jan, 1992; Bate, 1993).

Transplantation experiments have shown that, as the mesoderm invaginates, its cells have not yet been specified to give rise to the different derivatives (Beer *et al.,* 1987). At this stage patterns of gene expression reveal an apparently uniform mesodermal population, thus all mesodermal cells express uniform levels of *twi* (Thisse *et al.,* 1987), *DMEF-2* (Lilly *et al.,* 1995; Taylor *et al.,* 1995) and *tinman* (*tin*, Azpiazu and Frasch, 1993).

Differences among mesodermal cells become evident slightly later, when the mesoderm forms a monolayer underneath the ectoderm, at the transition between stages 9 and 10. By this stage, patterns of gene expression (Azpiazu and Frasch, 1993; Bate and Rushton, 1993; Azpiazu *et al.,* 1996; Riechmann *et al.,* 1997) and morphological criteria (Dunin Borkowski *et al.,* 1995) reveal a complex spatial organization of the mesoderm into quadrants. Modulation of *twi* expression along the antero-posterior axis subdivides the mesoderm into an "anterior" sector of relatively low *twi* expression, located underneath the future tracheal pits in the embryonic segment, and a "posterior" sector of cells that express high levels of *twi*, located posterior to the invagination of the tracheal pits (Dunin Borkowski *et al.,* 1995; Baylies and Bate, 1996). This modulation of *twi* expression coincides in time with a restriction of *tin* expression to dorsal cells (Azpiazu and Frasch, 1993 and Fig. 2). By stage 10 the mesoderm is subdivided into four domains per segment, that correspond to specific patterns of gene expression. Cells belonging to any of these domains are specified to give rise to different derivatives. Thus, cells located "anterior" and dorsal that express *tin* and low levels of *twi* also express *bagpipe (bap)* and give rise to visceral muscles, whereas cells located "posterior" and ventral express high levels of *twi* and give rise to somatic muscles (Azpiazu *et al.,* 1996; Baylies and Bate, 1996; Riechmann *et al.,* 1997 and Fig. 2).

The subdivision of the mesoderm results from a combination of regulatory factors that are intrinsic and extrinsic to the mesoderm (Bate and Baylies, 1996). The patterns of expression of pair rule genes are maintained in the mesoderm as it invaginates at gastrulation and they provide intrinsic differences to the cells along the antero-posterior axis. Two of the pair-rule genes, *even-skipped (eve)* and *sloppy pair (slp),* are required for the development of the derivatives formed by the "anterior" and "posterior" mesodermal sectors respectively. Thus, in *eve* mutants the primordia of the fat body and the visceral mesoderm fail to develop (Azpiazu *et al.,* 1996; Riechmann *et al.,* 1997), whereas no heart or somatic mesoderm forms in the absence of *slp* (Riechmann *et al.,* 1997).

The input of extrinsic factors is exemplified by recent experiments that show that differences along the dorso-ventral axis depend on an inductive signal from the adjacent dorsal ectoderm (Staehling-Hampton *et al.,* 1994; Frasch, 1995). This signal is mediated by the TGF-β protein DPP and is reflected in the mesoderm by the restriction of *tin* and *bap* expression to dorsal cells (Frasch, 1995). In *dpp* mutants none of the dorsal mesoderm derivatives such as heart and midgut mesoderm develop (Staehling-Hampton *et al.,* 1994; Frasch, 1995). Another example of extrinsic signal is the requirement for *wingless (wg)* to maintain high levels of Twi in the "posterior" mesodermal sector (Bate and Rushton, 1993; Baylies *et al.,* 1995). Not all the dorsal mesodermal cells that receive DDP respond by expressing *bap* and giving rise to visceral mesoderm (Staehling-Hampton *et al.,* 1994), indicating that there is a restriction on the competence of the cells in the mesoderm. It is possible that it is the intrinsic organization of the mesoderm

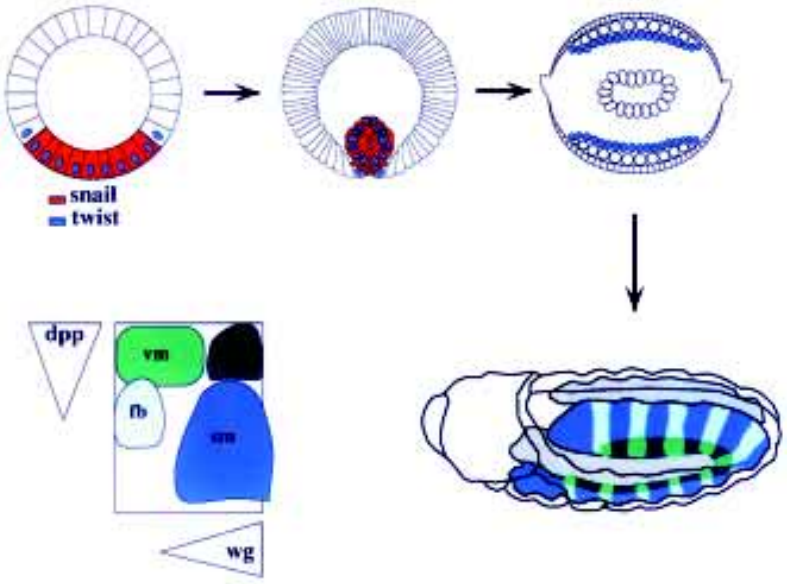

Fig. 2. Schematic representation of the allocation of mesodermal cells to form the different mesodermal derivatives. *The upper part of the Figure shows cross-sections of embryos at successive stages of development: syncytial blastoderm, stage 5 (left), germ band extension, stage 7 (centre) and fully extended germ band, stage 10 (right). At gastrulation the ventralmost cells of the blastoderm that express twist (blue circles) and snail (red) invaginate along the ventral furrow to give rise to the mesodermal population. At stage 10 the mesoderm forms a single cell layer underneath the ectoderm and mesodermal cells are allocated to give rise to the different mesodermal derivatives. This is represented in the lower part of the Figure. Modulation of Twist expression along the antero-posterior axis defines domains of high (dark blue) and low (light blue) Twist, whereas dorsal restriction of tinman (green) in response to ectodermal decapentaplegic (dpp) subdivides the mesoderm in ventral and dorsal sectors. Cells belonging to each of these sectors will contribute to different mesodermal derivatives. Thus, the visceral mesoderm (vm) derives from the dorsal low Twist domain, the heart (h) from the dorsal high Twist domain, the fat body from the ventral low Twist domain and the somatic mesoderm (sm) from the latero-ventral high Twist domain.*

which limits the ability to respond to extrinsic signals and that the combination of intrinsic and extrinsic factors dictates the specific patterns of mesodermal gene expression that give rise to the segregation of distinct derivatives.

One of these derivatives is the myogenic mesoderm that will produce the muscle pattern. It has been shown that it is the expression of high levels of Twi what propels the cells towards the myogenic pathway (Baylies and Bate, 1996). The specification of the myogenic mesoderm results from the integration of two factors that together will define the population of mesodermal cells that express high levels of Twi. The modulation of *twi* expression in high and low domains depends on an intrinsic factor, *slp* (Riechmann *et al.,* 1997) whereas the maintenance of high levels of Twi requires an extrinsic signal provided by *wingless* (Bate and Rushton, 1993; Baylies *et al.,* 1995).

The muscle pattern

Once the cells that will give rise to the myogenic mesoderm have been specified, they have to produce the distinct elements that compose the muscle pattern, such as the 30 muscles that develop in the abdominal hemisegments A1-A7 of the larva (see Fig. 1B). Muscles are syncytial and very similar to each other in terms of their physiological and structural characteristics (Bate and Rushton, 1993; Bernstein *et al.,* 1993). However, each of them can be individually identified by its position, size, orientation, insertion sites in the epidermis, patterns of gene expression and innervation by motorneurons. This implies that superimposed onto the myogenic programme are the instructions which confer individual characteristics to each member of the muscle set.

Morphological observations have revealed that the first sign of muscle development is the formation of small syncytia (2-3 nuclei) that appear at stage 12 at specific positions within the ventralmost part of the mesoderm in close contact with the ectoderm (Bate, 1990). During germ band shortening new syncytia form in the dorsal, lateral and ventral regions, in a very reproducible sequence, so that by the end of germ band retraction every muscle in the pattern is represented by a syncytium or "muscle precursor". As fusions proceed, the precursors grow and the pool of unfused myoblasts is reduced. As the precursors increase in size they project processes that grow over the surface of the epidermis towards the final attachments sites (Bate, 1990), and by stage 15 they will receive growth cones from exploring motorneurons that will establish functional neuromuscular junctions (Johansen *et al.,* 1989; Broadie and Bate, 1993).

The mechanism that underlies the specification of the muscle pattern has to account for several observations that emerge from the way muscles develop. Firstly, what triggers the sequential fusion events at particular positions in the somatic mesoderm. Secondly, how are precursors instructed about their final size (control of the number of myoblasts fusing to the precursors), their orientation (identification of their attachment sites) and the genes they have to express, including the ones coding for surface molecules required for their identification as targets for exploring motorneurons.

Formally, we could think of at least two ways of generating the muscle pattern that will fulfil these criteria. i) A mechanism that specifies single cells which will have all the information required to give rise to a particular muscle. These cells will fuse to undifferentiated myoblasts to form muscle precursors, so that as fusion proceeds the newly incorporated myoblasts are recruited to the pattern of expression characteristic of the original cells (the founder cell hypothesis, Bate, 1993). ii) A mechanism that specifies groups of cells with the specific characteristics which then will fuse together to originate the muscle precursors.

Several observations point to the founder cell hypothesis as the most plausible mechanism to generate the muscle pattern. For example, the expression of muscle marker genes characteristic of subgroups of muscles, such as *S59* (Dohrmann *et al.,* 1990) and *connectin* (Nose *et al.,* 1992) is initiated in individual mesodermal cells. Subsequently, neighboring myoblasts are recruited to specific patterns of gene expression as they fuse to these individual cells. However, the demonstration of the validity of the founder cell hypothesis came from the observation by Rushton *et al.* (1995) that muscles are formed by two kinds of myoblasts: the "founder" cell and the "fusion competent" cells. In mutants where fusion is

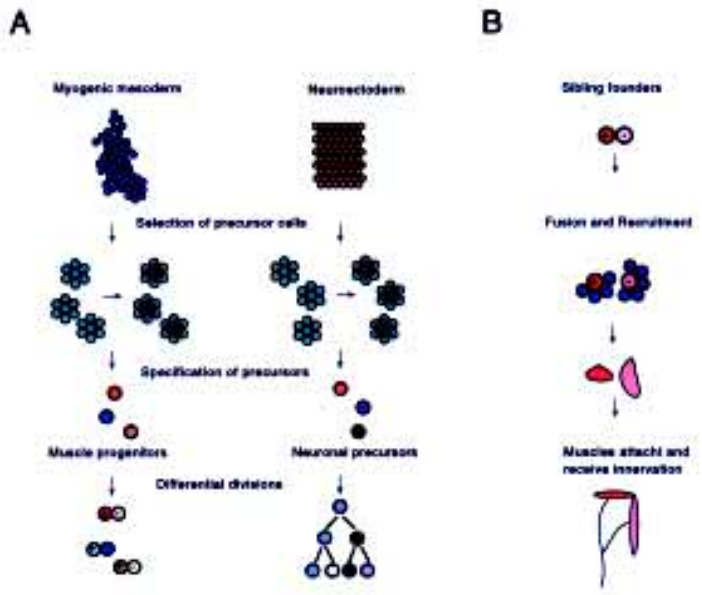

Fig. 3. The formation of muscles and sense organs (A) *Diagram showing conserved steps in the generation of muscle and sensory organ precursor cells. At specific positions of the myogenic mesoderm or the neuroectoderm groups of cells initiate the expression of proneural genes (green circles), and thus acquire the competence to become muscle or neural precursors. Competence is restricted to a single cell in the clusters, normally positioned in the centre, that accumulates higher amounts of proneural proteins (dark green circle), and prevents its neighbors from becoming precursors by a process of lateral inhibition mediated by neurogenic genes (arrowheads). By the time they are born the different precursors are individually specified and this is reflected by their patterns of gene expression. Differential divisions of the precursors will generate pairs of muscle founders or the complement of cells that will form the final sense organs.* **(B)** *Founders seed the formation of muscles. Differential divisions of the muscle progenitors produce pairs of founders that will differ in patterns of gene expression (one founder (+) will maintain the pattern of expression characteristic of its progenitor, whereas the other (-) will turn off some of the genes expressed by its progenitor). Founders fuse to surrounding myoblasts to form muscle precursors and recruit them to their patterns of gene expression. Finally muscles attach to the epidermis and receive innervation.*

blocked these two populations behave differently. Founder cells are able to differentiate and they give rise to one-celled muscles that have normal patterns of gene expression, find their normal attachment sites and receive the proper innervation. Fusion competent cells remain as an undifferentiated set of cells that express contractile proteins and end up by being engulfed by macrophages. These results clearly demonstrate that by the time the founders are born, they have all the information necessary to produce a particular muscle in the pattern and imply that the generation of muscle diversity has its origin in the specification of 30 unique founder cells.

The origin of the founder cells

Recent work has shown that founders arise in pairs from the division of muscle progenitors (Carmena *et al.,* 1995). Muscle progenitors also give rise to the precursors of the adult muscles, and in these cases one of the daughter cells will be the founder of a larval muscle, other an adult muscle precursor (Carmena *et al.,* 1995; Ruiz-Gómez and Bate, 1997). The specification of muscle progenitors takes place at reproducible positions in the most external somatic mesoderm in close contact with the ectoderm. In analogy to the selection of neuronal precursors, muscle progenitors are singled out from clusters of mesodermal cells that express the proneural gene *lethal of scute (l'sc)*. Then, by a process of lateral inhibition mediated by the "neurogenic genes", *l'sc* expression is restricted to a single cell in the cluster, the progenitor, that moves towards the ectoderm where it will divide to give rise to two founder cells (Carmena *et al.,* 1995).

l'sc expression is common to most if not all of the mesodermal clusters which give rise to muscle progenitors. However, despite deficiencies that remove *l'sc* lack some muscles, their phenotypes are much weaker than what one would expect from its more widespread pattern of expression (Carmena *et al.,* 1995). This observation argues in favor of the existence of other genes with similar functions to *l'sc* that can partially substitute for it, as has been previously proposed in the case of the CNS (Cabrera *et al.,* 1987; Jimenez and Campos-Ortega, 1990).

Thus, although the muscles are arranged in a three-dimensional pattern they are specified in a bi-dimensional field, as are the elements of the nervous system. The way neuronal and muscle precursors are specified reveals the conservation of a general strategy to confer particular characteristics to individual cells involving a process of progressive definition of developmental potentiality (Fig. 3). The process begins with the specification of the population of cells that constitutes the neuroectoderm or the myogenic region. This is followed by a process of integration of positional information to produce a spatial landscape of groups of competent cells that express proneural genes. It is remarkable that the same gene, *l'sc,* is used to endow cells with neuronal (CNS)

TABLE 1

PATTERNS OF EXPRESSION OF MUSCLE MARKER GENES

GENE	PROTEIN MOTIF	MUSCLE EXPRESSION	DIFFERENTIALLY EXPRESSED IN FOUNDERS
even-skipped	homeodomain	DA1	yes
S59	homeodomain	DT1, VA2, VT1	yes
Krüppel	Zinc finger	DA1, DO1, LL1, LT2, 4	
		VL3, VA2, VO2, 4	yes
apterous	LIM +		
	homeodomain	LT1-4, VA2, 3	no
vestigial		DA1-3, LL1, VL1-4	no
ladybird	homeodomain	SBM	no
Toll	transmembrane		
	LRR repeats	VL4, VO3-6	no
connectin	transmembrane		
	LRR repeats	LT1-4, VA1-3	no

For muscle nomenclature see Bate, 1993, Ruiz-Gómez *et al.,* 1997. For patterns of gene expression see Frasch *et al.,* 1987, Dohrmann *et al.,* 1990, Bourgouin *et al.,* 1992, Nose *et al.,* 1992, Bate and Rushton, 1993, Halfon *et al.,* 1995, Jagla *et al.,* 1997, Ruiz-Gómez *et al.,* 1997.

and myogenic potentiality, thus behaving as a proneural and a promyogenic gene (Jiménez and Campos-Ortega, 1987; Martín-Bermudo *et al.,* 1993; Carmena *et al.,* 1995). A possible explanation of how the activity of the same gene determine different cell fates in the ectoderm and in the mesoderm could be the ability of the L'sc protein to bind DNA as heterodimers with other bHLH proteins (Cabrera and Alonso, 1991). For example, it could well be that heterodimers formed by L'sc and Daughterless (Da) would be interpreted as neurogenic and heterodimers of L'sc and Twi as myogenic by their ability to bind to different downstream genes. Finally, the proneural or promyogenic competence is restricted to single cells, neuronal precursors and muscle progenitors, by a process of lateral inhibition between competent cells that is mediated by the neurogenic genes (Corbin *et al.,* 1991; Bate *et al.,* 1993; Campos-Ortega, 1993; Carmena *et al.,* 1995).

Muscle specification

Proneural gene expression is general to all mesodermal clusters that give rise to progenitor cells, and probably reflects the acquisition by those cells of the competence to initiate the myogenic pathway (Carmena *et al.,* 1995). The entrance into myogenic differentiation is common to all muscles and is reflected by the expression of structural proteins such as muscle myosin or β3-tubulin and proteins required to make attachments to the epidermis and functional neuromuscular junctions (Bate, 1993; Abmayr *et al.,* 1995). However, as a unique member of the muscle set, each muscle has distinctive characteristics implying the existence of a mechanism of muscle diversification overimposed on the general myogenic pathway.

The earliest sign of muscle diversification is the particular combination of genes that each progenitor express (Table 1). Some of these genes are expressed in individual progenitors like *even-skipped (eve)* (Frasch *et al.,* 1987) and *ladybird (lb)* (Jagla *et al.,* 1997), and others in subsets of them, e.g., *Krüppel (Kr)* (Ruiz-Gómez *et al.,* 1997) and *S59* (Dohrmann *et al.,* 1990), generating a landscape of partially overlapping patterns of expression. In some cases the patterns of gene expression initiated in the muscle progenitors are maintained in both founder cells resulting from their division, as in the case of the progenitors that express Connectin (Nose *et al.,* 1992). The expression of other genes, however, may be differentially regulated in the two sibling founders. Thus, a progenitor expressing both *Kr* and *S59* will give rise to sibling founders that differ in their patterns of gene expression: one maintains the expression of both genes, the other loses it (Ruiz-Gómez *et al.,* 1997). Since sibling founder cells give rise to muscle precursors that differ in patterns of gene expression and that eventually give rise to muscles with different characteristics, it is very likely that the regulated expression of transcription factors such as *Kr* and *S59* conditions the development of some or all the characteristics of individual muscles.

Although this suggestion has been made many times, it has not been addressed until recently. Loss- and gain-of-function analysis of two transcription factors expressed in subsets of muscles, *apterous* (*ap*, Bourgouin *et al.,* 1992) and *nautilus* (*nau*, Keller *et al.,* 1997) has shown that it is possible to produce partial loss or duplication of the muscles that normally express these transcription factors. These results, however, do not demonstrate that altering patterns of gene expression in muscle precursors leads to predictable changes in muscle characteristics.

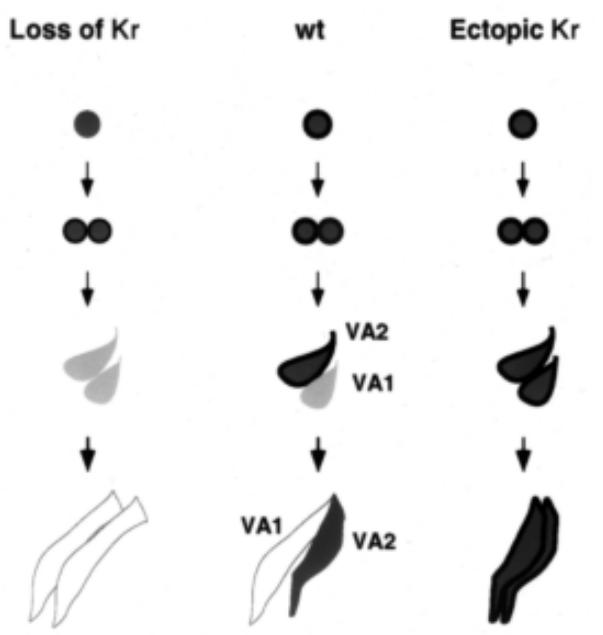

Fig. 4. Diagram showing the effects of loss and ectopic expression of *Krüppel* in the development of muscles VA1 and VA2. *Light and dark shading indicates levels of S59, Kr expression is represented by a black outline. During normal development (central column) a progenitor that co-expresses Kr and S59 gives rise to the S59-positive founders that will seed the formation of muscles VA1 and VA2. Kr is lost in VA1 founder and S59 decays in the VA1 precursor, whereas both S59 and Kr are maintained in the VA2 precursor. In the absence of Kr (left column) the segregation of S59-positive progenitors and founders is not affected. However, S59 expression declines in the VA2 precursor by stage 13, indicating that the maintenance and not the initiation of S59 expression in VA2 is dependent on Kr. In these conditions muscle VA2 is transformed towards its S59-non expressing sibling VA1. When Kr is ectopically expressed in the mesoderm (right column), the segregation of S59 cells is unaffected, confirming that Kr is unable of initiate S59 expression. However, it can maintain S59 in VA1 precursor and muscle, that now appears transformed towards the S59-expressing VA2 fate. Thus differential maintenance of Kr in the VA1/VA2 lineage is responsible for the diversification of muscles VA1 and VA2.*

Similar analysis with *Krüppel (Kr)*, a gene encoding a nuclear protein that acts as a transcriptional regulator during the process of embryonic segmentation (Gaul *et al.,* 1987), have specifically demonstrated that it is possible to transform individual muscle phenotypes by switching patterns of gene expression from those characteristic of one precursor to those typical of another (Ruiz-Gómez *et al.,* 1997). *Kr* is expressed in the mesoderm in the progenitors of a subset of muscles and is differentially maintained in one of the two sibling founder cells resulting from their division. Thus, one progenitor expressing *Kr* generates two founders: one maintaining *Kr* expression, the other not, and they give rise to muscles that differ in patterns of gene expression and morphology. Loss of *Kr* leads to a premature loss of expression of other genes, such as *S59,* in those muscle precursors where *Kr* is normally maintained. This is accompanied by muscle transformations: two muscles with the morphology characteristic of the sibling muscle that normally looses *Kr* expression develop in these positions. On the other hand, the ectopic expression of *Kr* can maintain the expression of *S59* and of other genes in the

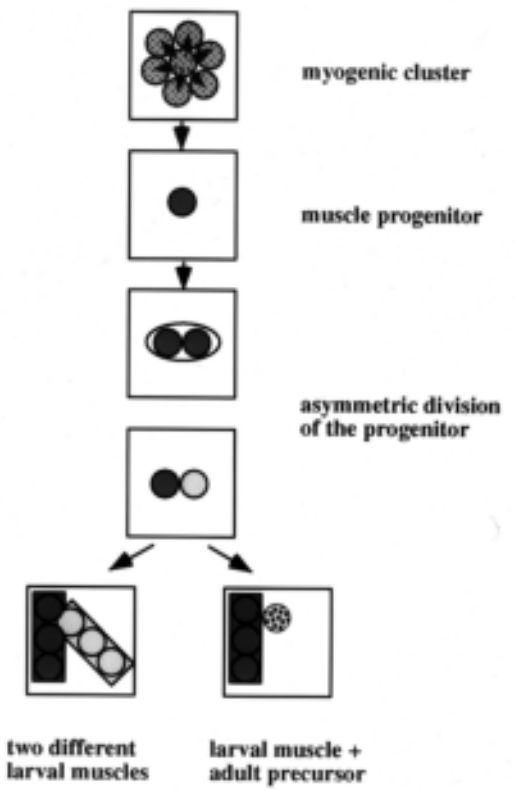

Fig. 5. Schematic representation of the generation of myogenic lineages. *In Drosophila muscle progenitors are selected from groups of cells that express lethal of scute (myogenic clusters), and divide asymmetrically to produce a (+) founder and either a (-) founder or the precursor of an adult muscle. Adult muscle precursors do not fuse with myoblasts as their sibling founders and they maintain Twi expression (indicated by a dotted circle in the Figure).*

precursors from which they are normally lost and induce the opposite transformation (Fig. 4).

These results show that local expression of some transcription factors in the myogenic lineage regulates individual characteristics of the muscles that express them, without affecting myogenesis in general. Transcription factors such as *Kr* could regulate muscle identity by modulating the expression of downstream genes, that are responsible of controlling specific muscle characteristics such as insertion sites and innervation. The fact that the loss of *Kr* produces complete a muscle transformations and can modulate the expression of several other genes such as *S59* (Ruiz-Gómez *et al.,* 1997) and *knockout (ko)* (Hartmann *et al.,* 1997), suggests that *Kr* is very high in the hierarchy of genes controlling muscle specificity. Given that *Kr* is only expressed in a subset of muscles, there must be additional genes that act as determinant of muscle identity, and whose expression is probably also differentially regulated between sibling founders.

Lineages in the somatic mesoderm

A general property of the muscle progenitors is that they divide asymmetrically and in every case give rise to two cells that follow alternative fates: either the founders of two distinct muscles or a larval founder and an adult muscle precursor. These alternative fates represent two alternative states: one in which the genes expressed in the progenitor cell are maintained in the "plus" (+) founders, and the other in which their expression is repressed, the "minus" (-) founders. In the particular case of progenitors that produce a larval and an adult precursor, the generation of the adult precursor is associated with the repression of the progenitor marker gene expression and the maintenance of *twi* expression. This is equivalent to the (-) founder fate (Fig. 5 and Ruiz-Gómez and Bate, 1997).

Recent results have shown that the gene *numb,* which encodes a membrane associated protein, acts as an intrinsic determinant of the asymmetric division of the progenitors (Ruiz-Gómez and Bate, 1997; Carmena *et al.,* 1998). Thus, Numb is asymmetrically distributed in the progenitors and differentially segregated to the two daughter cells. It is the presence or absence of Numb in those cells that determines which of the two alternative fates will be taken on. Loss of function of *numb* duplicates the fates associated with the repression of progenitor marker gene expression, and results in the formation of two (-) founders or two adult precursors are produced by the division of the progenitors. On the contrary, ectopic expression of *numb* duplicates the alternative fates, generating pairs of (+) founder cells.

Extrinsic signals mediated by the neurogenic gene Notch (N) also play a role in determining cell fates in the mesoderm (Ruiz-Gómez and Bate, 1997). *Notch* activation is required to turn off marker gene expression in one of the sibling cells, and thus to produce (-) founder cells and adult precursors. The fact that loss of function for *Notch* and *numb* have opposite phenotypes and the evidence in favor of a physical interaction between Numb and the cytoplasmic domain of Notch (Guo *et al.,* 1996), strongly suggests that the differential distribution of Numb between the two sibling

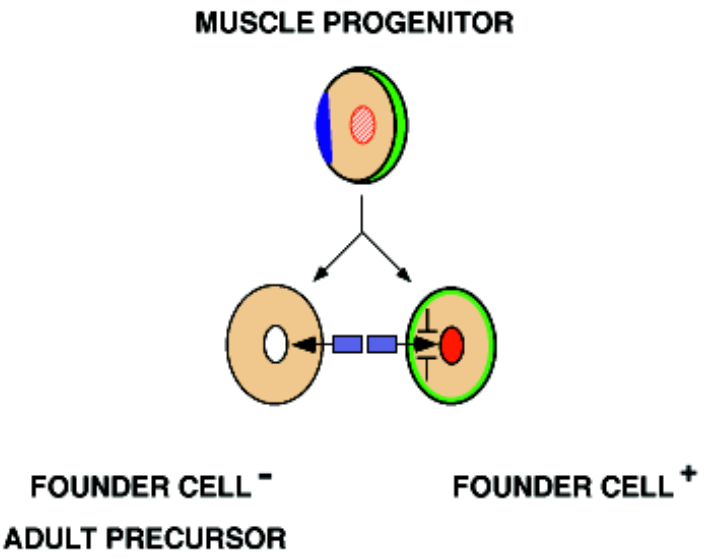

Fig. 6. Asymmetric division of muscle progenitors requires Numb and Notch. *Founders originate from the asymmetric division of muscle progenitors. Alternative fates adopted by the sibling cells depend on the unequal distribution of Numb in the progenitors (green sector) that results in a differential segregation of Numb to only one founder. Patterns of gene expression characteristic of the progenitors are maintained in the sibling cell that receives Numb (red nucleus). The presence of Numb in these cells acts to block the Notch signaling pathway that results in the repression of marker gene expression in the sibling cell that does not receive Numb.*

cells determines the selective inactivation of the N signaling pathway in the daughter cell that receives Numb (Fig. 6).

The requirements for Numb and Notch in making the choice between alternative fates are common for neuronal and myogenic lineages (Guo *et al.,* 1996; Spana and Doe, 1996; Ruiz-Gómez and Bate, 1997). PNS precursors divide asymmetrically to produce the four cells that compose the final sense organ, and at each of these divisions the choice between fates depends on the implementation of N signaling in only one of the daughter cells: the one that does not receive Numb. Therefore, there are two requirements for Notch in neurogenesis and in myogenesis alike: first in the process of lateral inhibition that ensures the restriction of the competence to single precursors (Corbin *et al.,* 1991; Bate *et al.,* 1993; Campos-Ortega, 1993; Carmena *et al.,* 1995), and later for the implementation of one of the two alternative fates to be adopted by the daughter cells resulting from asymmetric divisions (Guo *et al.,* 1996; Spana and Doe; 1996, Ruiz-Gómez and Bate, 1997).

Concluding remarks

The same genetic networks appear to be used during the early steps of both neurogenesis and myogenesis, when precursor cells are specified at particular positions within a cell layer. However, at latter stages the formation of muscles requires additional mechanisms that are not necessary in the development of the PNS. Specific for muscle development is the process of fusion between individual founder cells and "fusion competent" cells. The existence of fusions between cells belonging to these two myogenic populations raise a number of questions that are specific for muscle development and for which we do not have yet answers. For instance, how founder and fusion competent cells are specified so that they can recognize each other and fuse together, or how the number of fusions, and consequently muscle size, is controlled. It is expected that the identification of mutations affecting these processes will help to characterize the genetic and cellular bases of muscle morphogenesis.

Acknowledgments

I am grateful to José de Celis, Alfonso Martínez-Arias and Beatriz San Martín for comments on the manuscript, to Michael Bate for many stimulating discussions and to Alain Ghysen for his invitation to contribute to this homage to Antonio García-Bellido as well as for his patience. Research support was provided by the Wellcome Trust.

References

ABMAYR, S.M., ERICKSON, M.S. and BOUR, B.A. (1995). Embryonic development of the larval body wall musculature of *Drosophila melanogaster. Trends Genet. 11*: 153-159.

AZPIAZU, N. and FRASCH, M. (1993). *tinman* and *bagpipe*: two homeo box genes that determine cell fate in the dorsal mesoderm of *Drosophila*. *Genes Dev. 7*: 1325-1340.

AZPIAZU, N., LAWRENCE, P.A., VINCENT, J-P. and FRASCH, M. (1996). Segmentation and specification of the *Drosophila* mesoderm. *Genes Dev. 10*: 3183-3194.

BATE, M. (1990). The embryonic development of larval muscles in *Drosophila*. *Development 110*: 791-804.

BATE, M. (1993). The mesoderm and its derivatives. In *The development of Drosophila melanogaster.* (Eds. M. Bate and A. Martínez-Arias). Cold Spring Harbor, New York, CSH Laboratory Press, pp. 1013-1090.

BATE, M. and BAYLIES, M. (1996). Intrinsic and extrinsic determinants of mesodermal differentiation in *Drosophila. Semin. Cell Dev. Biol. 7: 103-111.*

BATE, M. and RUSHTON, E. (1993). Myogenesis and muscle patterning in *Drosophila. C. R. Acad. Sci. Paris 316*: 1055-1061.

BATE, M., RUSHTON, E. and FRASCH, M. (1993). A dual requirement for neurogenic genes in *Drosophila* myogenesis. *Development (Suppl.) :* 149-161

BAYLIES, M.K. and BATE, M. (1996). *twist*: A myogenic Switch in *Drosophila. Science 272*: 1481-1484.

BAYLIES, M.K., MARTINEZ-ARIAS, A. and BATE, M. (1995). *wingless* is required for the formation of a subset of muscle founder cells during *Drosophila* embryogenesis. *Development 121*: 3829-3837.

BEER, J., TECHNAU, G. and CAMPOS-ORTEGA, J.A. (1987). Lineage analysis of transplanted individual cells in embryos of *Drosophila melanogaster*. IV. Commitment and proliferative capabilities of individual mesodermal cells. *Roux Arch. Dev. Biol. 196*: 222-230.

BERNSTEIN, S.I., O'DONELL, P.T. and CRIPPS, R.M. (1993). Molecular genetic analysis of muscle development, structure and function in *Drosophila. Int. Rev. Cytol. 143*: 63-144.

BODMER, R., BARBEL, S., SHEPHERD, S., JACK, J.W., JAN, L.Y. and JAN, Y.N. (1987). Transformation of sensory organs by mutations of the *cut loc*us of *D. melanogaster. Cell 51*: 293-307.

BODMER, R., CARETTO, R. and JAN, Y.N. (1989). Neurogenesis of the peripheral nervous system in *Drosophila* embryos: DNA replication patterns and cell lineages. *Neuron 3*: 21-32.

BOURGOUIN, C., LUNDGREN, S.E. and THOMAS, J.B. (1992). *apterous* is a *Drosophila* LIM domain gene required for the development of a subset of embryonic muscles. *Neuron 9*: 549-561.

BROADIE, K.S. and BATE, M. (1993). Development of the embryonic neuromuscular synapse of *Drosophila melanogaster. J. Neurosci. 13*: 144-166.

CABRERA, C.V. and ALONSO, M.C. (1991). Transcriptional activation by heterodimers of the a*chaete-scute* and *daughterless* gene products of *Drosophila. EMBO J. 10*: 2965-2973.

CABRERA, C.V., MARTINEZ-ARIAS, A. and BATE, M. (1987). The expression of three members of the *achaete-scute* gene complex correlates with neuroblast segregation in *Drosophila. Cell 50*: 425-433.

CAMPOS-ORTEGA, J.A. (1993). Early neurogenesis in *Drosophila melanogaster*. In *The development of Drosophila melanogaster.* (Eds. M. Bate and A. Martínez-Arias). Cold Spring Harbor, New York, CSH Laboratory Press, pp. 1091-1129.

CAMPUZANO, S. and MODOLELL, J. (1992). Patterning of the *Drosophila* nervous system: the *achaete-scute* gene complex. *Trends Genet. 8*: 204-208.

CARMENA, A., BATE, M. and JIMENEZ, F. (1995). *lethal of scute*, a proneural gene, participates in the specification of muscle progenitors during *Drosophila* embryogenesis. *Genes Dev. 9*: 2373-2383.

CARMENA, A., MURUGASU-OEI, B., MENON, D., JIMENEZ, F. and CHIA, W. (1998). *Inscuteable* and *numb* mediate asymetric muscle progenitor cell divisions during *Drosophila* myogenesis. *Genes Dev. 12:* 304-315.

CORBIN, V., MICHELSON, A.M., ABMAYR, S.M., NEEL, V., ALCAMO, E., MANIATIS, T. and YOUNG, M. (1991). A role for the *Drosophila* neurogenic genes in mesoderm differentiation. *Cell 67*: 311-323.

DAMBLY-CHAUDIERE, C., JAMET, E., BURRI, M., BOPP, D., BASLER, K., HAFEN, E., DUMONT, N., SPIELMANN, P., GHYSEN, A. and NOLL, M. (1992). The paired box gene *pox neuro* : a determinant of poly-innervated sense organs in *Drosophila. Cell 69*: 159-172.

DOHRMANN, C., AZPIAZU, N. and FRASCH, M. (1990). A new *Drosophila* homeobox gene is expressed in mesodermal precursor cells of distinct muscles during embryogenesis. *Genes Dev. 4*: 2098-2111.

DUNIN BORKOWSKI, O. M., BROWN, N. H. and BATE, M. (1995). Anterior-posterior subdivision and the diversification of the mesoderm in *Drosophila. Development 121*: 4183-4193.

FRASCH, M. (1995). Induction of visceral and cardiac mesoderm by ectodermal Dpp in the early *Drosophila* embryo. *Nature 374*: 464-467.

FRASCH, M., HOEY, T., RUSHLOW, C., DOYLE, H.J. and LEVINE, M. (1987). Characterization and localization of the *even-skipped* protein of *Drosophila. EMBO J. 6*: 749-759.

GAUL, U., SEIFERT, E., SCHUH, R. and JÄCKLE, H. (1987). Analysis of *Krüppel* protein distribution during early *Drosophila* development reveals posttrancriptional regulation. *Cell 50*: 639-647.

GHYSEN, A. and DAMBLY-CHAUDIERE, C. (1989). genesis of the *Drosophila* peripheral nervous system. *Trends Genet. 5*: 251-255.

GHYSEN, A., DAMBLY-CHAUDIERE, C., ACEVES, E., JAN, L.Y. and JAN, Y.N. (1986). Sensory neurons and peripheral pathways in *Drosophila* embryos. *Roux' Arch. Dev. Biol. 195*: 281-289.

GUO, M., JAN, L.Y. and JAN, Y.N. (1996). Control of daughter cell fates during asymmetric division: interaction of Numb and Notch. *Neuron 17*: 27-41.

HALFON, M.S., HASHIMOTO, C. and KESHISHIAN, H. (1995). The *Drosophila Toll* gene functions zygotically and is necessary for proper motoneuron and muscle development. *Dev. Biol. 169*: 151-167.

HARTENSTEIN, V. and JAN, Y.N. (1992). Studying *Drosophila* embryogenesis with P-*lacZ* lines. *Roux Ach. Dev. Biol. 201*: 194-220.

HARTMANN, C., LANDGRAF, M., BATE, M. and JÄCKLE, H. (1997). *Kruppel* target gene *knockout* participates in a proper innervation of a specific set of *Drosophila* larval muscles. *EMBO J. 16:* 5299-5309.

JAGLA, K., JAGLA, T., HEITZLER, P., DRETZEN, G., BELLARD, F. and BELLARD, M. (1997). *ladybird*, a tandem of homeobox genes that maintain late *wingless* expression in terminal and dorsal epidermis of the *Drosophila* embryo. *Development 124*: 91-100.

JAN, Y.N. and JAN, L.Y. (1992). Neuronal specification. *Curr. Opin. Genet. Dev. 2:* 608-613.

JAN, Y.N. and JAN, L.Y. (1993). The peripheral nervous system. In *The development of Drosophila melanogaster.* (Eds. M. Bate and A. Martínez-Arias). Cold Spring Harbor, New York, CSH Laboratory Press, pp. 1207-1244.

JIMENEZ, F. and CAMPOS-ORTEGA, J.A. (1987). Genes of the subdivision 1B of the genome of *Drosophila melanogaster* and their participation in neural development. *J. Neurogenet. 4*: 179-200.

JIMENEZ, F. and CAMPOS-ORTEGA, J.A. (1990). Defective neuroblast commitment in mutants of the achaete-scute complex and adjacent genes of *Drosophila melanogaster. Neuron 5*: 81-89.

JOHANSEN, J., HALPERN, M.E. and KESHISHIAN, H. (1989). Axonal guidance and the development of muscle fiber-specific innervation in *Drosophila* embryos. *J. Neurosci. 9*: 4318-4332.

KELLER, C.A., ERICKSON, M.S. and ABMAYR, S.M. (1997). Misexpression of *nautilus* induces myogenesis in cardioblasts and alters the pattern of somatic muscles fibers. *Dev. Biol. 181*: 197-212.

LEPTIN, M. and GRUNEWALD, B. (1990). Cell shape changes during gastrulation in *Drosophila*. *Development 110*: 73-84

LILLY, B., ZHAO, B., RANGANAYAKULU, G., PATERSON, B.M., SCHULZ, R.A. and OLSON, E.N. (1995). Requirement of MADS domain transcription factor D-MEF2 for muscle formation in *Drosophila*. *Science 267*: 688-693.

MARTÍN-BERMUDO, M.D., GONZÁLEZ, F., DOMÍNGUEZ, M., RODRÍGUEZ, I., RUIZ-GÓMEZ, M., ROMANI, S., MODOLELL, J. and JIMENEZ, F. (1993). Molecular characterization of the *lethal of scute* genetic function. *Development 118*: 1003-1012.

MERRITT, D.J. and WHITINGTON, P.M. (1995). Central projections of sensory neurons in the *Drosophila* embryo correlate with sensory modality, soma position, and proneural gene function. *J. Neurosci. 15*: 1755-1767.

NOSE, A., MAHAJAN, V.B. and GOODMAN, C.S. (1992). Connectin: A homophilic cell adhesion molecule expressed on a subset of muscles and the motoneurons that innervate them in *Drosophila*. *Cell 70*: 553-567.

RIECHMANN, V., IRION, U., WILSON, R., GROSSKORTENHAUS, R. and LEPTIN, M. (1997). Control of cell fates and segmentation in the *Drosophila* mesoderm. *Development 124*: 2915-2922.

RUIZ-GÓMEZ, M. and BATE, M. (1997). Segregation of myogenic lineages in *Drosophila* requires Numb. *Development 124:* 4857-4866.

RUIZ-GÓMEZ, M., ROMANI, S., HARTMANN, C., JÄCKLE, H. and BATE, M. (1997). Specific muscle identities are regulated by *Krüppel* during *Drosophila* embryogenesis. *Development 124*: 3407-3414.

RUSHTON, E., DRYSDALE, R., ABMAYR, S.M., MICHELSON, A.M. and BATE, M. (1995). Mutations in a novel gene, my*oblast city*, provide evidence in support of the founder cell hypothesis for *Drosophila* muscle development. *Development 121*: 1979-1988.

SPANA, E.P. and DOE, C.Q. (1996). Numb antagonizes Notch signaling to specify sibling neuron cell fates. *Neuron 17*: 21-26.

STAEHLING-HAMPTON, K., HOFFMANN, F.M., BAYLIES, M.K., RUSHTON, E. and BATE, M. (1994). *dpp* induces mesodermal gene expression in *Drosophila*. *Nature 372*: 22-29.

TAYLOR, M.V., BEATTY, K.E., HUNTER, H.K. and BAYLIES, M.K. (1995). *Drosophila* MEF-2 is regulated by twi*st* and is expressed in both the primordia and differentiated cells of the embryonic somatic, visceral and heart musculature. *Mech. Dev. 50*: 29-41.

THISSE, B., STOETZEL, C., EL MESSAL, M. and PERRIN.-SCHMITT, F. (1987). Genes of the *Drosophila* maternal dorsal group control the specific expression of the zygotic gene *twist* in presumptive mesodermal cells. *Genes Dev. 1*: 709-715.