Muscle patterning and specification in *Drosophila*

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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 "neurontype 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...
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 (Thissell 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
Muscles are syncytial and very similar to each other in terms of their physiological and structural characteristics (Bate and Rushton, 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.

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 prevented 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.

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 lateral-ventral high Twist domain.
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; Ruíz-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

<table>
<thead>
<tr>
<th>GENE</th>
<th>PROTEIN MOTIF</th>
<th>MUSCLE EXPRESSION</th>
<th>DIFFERENTIALLY EXPRESSED IN FOUNDERS</th>
</tr>
</thead>
<tbody>
<tr>
<td>even-skipped</td>
<td>homeodomain</td>
<td>DA1</td>
<td>yes</td>
</tr>
<tr>
<td>S9</td>
<td>homeodomain</td>
<td>DT1, VA2, VT1</td>
<td>yes</td>
</tr>
<tr>
<td>Krüppel</td>
<td>Zinc finger</td>
<td>DA1, DO1, LL1, LT2, 4</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>VL3, VA2, VO2, 4</td>
<td>yes</td>
</tr>
<tr>
<td>apterous</td>
<td>LIM +</td>
<td>LT1-4, VA2, 3</td>
<td>no</td>
</tr>
<tr>
<td>vestigial</td>
<td>homeodomain</td>
<td>DA1-3, LL1, VL1-4</td>
<td>no</td>
</tr>
<tr>
<td>ladybird</td>
<td>homeodomain</td>
<td>SBM</td>
<td>no</td>
</tr>
<tr>
<td>Toll</td>
<td>transmembrane</td>
<td>LRR repeats</td>
<td>no</td>
</tr>
<tr>
<td>connectin</td>
<td>transmembrane</td>
<td>LRR repeats</td>
<td>no</td>
</tr>
</tbody>
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and myogenic potentiality, thus behaving as a proneural and a
promyogenic gene (Jiménez and Campos-Ortega, 1987; Martin-
Bermudo et al., 1993; Carmona et al., 1995). A possible explana-
tion 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 medi-
ated by the neurogenic genes (Corbin et al., 1991; Bat et al., 1993;
Campos-Ortega, 1993; Carmona et al., 1995).

Muscle specification

Proneural gene expression is general to all mesodermal clus-
ters that give rise to progenitor cells, and probably reflects the
acquisition by those cells of the competence to initiate the myo-
genic pathway (Carmona 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 β-
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 transcrip-
tion 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 to 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 morphol-
ogy. 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 transforma-
tions: 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
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 muscle transformations and can modulate the expression of several other genes such as SS9 (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 founders.

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**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).

**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 Martinez-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.

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