**INTRODUCTION**

The somatic muscles of the *Drosophila* larva are arranged as a precise pattern of syncytial fibres. There are 30 fibres in each abdominal hemisegment and each muscle is a unique element of the pattern. Muscle identity is reflected in position, size, attachment site in the epidermis and innervation (Bate, 1993). There is good evidence that during myogenesis the muscle pattern is seeded by the specification of muscle founder cells which are endowed with the information required for the assembly of individual muscles (Bate, 1990; Rushton et al., 1995). Individual founders express marker genes such as **S59** (Dohrmann et al., 1990) and fuse with neighbouring myoblasts to form the syncytial precursors of mature muscles. Thus the formation of the muscle pattern depends critically on mechanisms that lead to the segregation of muscle founder cells at precise locations in the somatic mesoderm.

In a previous study we showed that **S59**-expressing founder cells are absent in **wg** mutant embryos, and the corresponding muscles fail to form (Bate and Rushton 1993). This striking requirement for **wg** in the formation of a subset of muscle founder cells suggests that **wg** may form part of the mechanism that leads to the definition of founder cells in the somatic mesoderm. **Wingless**, a secreted glycoprotein and well characterized signalling molecule, acts directly on the mesoderm to ensure the formation of **S59**-expressing founder cells. Moreover, we present evidence that **Wg** can signal across germ layers and that, in the wild-type embryo, **Wg** from the ectoderm could constitute an inductive signal for the initiation of the development of a subset of somatic muscles.

**SUMMARY**

The final pattern of the *Drosophila* larval body wall muscles depends critically on the prior segregation of muscle founder cells. We would like to understand the underlying molecular mechanisms which ensure the precise allocation and placement of these muscle founder cells. We have begun our analysis by examining the role of the segment polarity genes, known to be involved in the patterning of the ectoderm. Mutations in only one member of this class, **wingless (wg)**, lead to the complete loss of a subset of muscle founder cells characterised by the expression of **S59**. Using the GAL4-targetted expression system, we find that **Wingless**, a secreted glycoprotein and well characterized signalling molecule, acts directly on the mesoderm to ensure the formation of **S59**-expressing founder cells. Moreover, we present evidence that **Wg** can signal across germ layers and that, in the wild-type embryo, **Wg** from the ectoderm could constitute an inductive signal for the initiation of the development of a subset of somatic muscles.

Key words: *Drosophila*, mesoderm, myogenesis, founder cells, **wingless**, cell-cell communication

**MATERIALS AND METHODS**

**Fly stocks**

The segment polarity mutants (Lindsley and Zimm, 1992) used in this study are: 

- **wg**^CX4^/*CyOftzlacZ;
- **hh**^IJ35^ / **Ubx**^1^ / TM3;
- **ptc**^IN108^ / *CyOftzlacZ;
- **nkd**^7E^ / TM3;
- **Df(en)**^E^ / SM6a-TM6b (a generous gift from A. Hildalgo). 

Where applicable, mutant embryos were distinguished from wild-type
embryos using the anti-β-galactosidase antibody which detects the ftz driven β-galactosidase from the balancer.

**GAL 4/UAS stocks**

For expression of wg in the mesoderm we used a twist-GAL4 construct, the construction of which is to be described in detail elsewhere (M. K. B. and M. B., manuscript in preparation). Expression of wg in the ectoderm was accomplished by using the 69B GAL4 line generously provided by A. Brand (Brand and Perrimon, 1993) and described by Staehling-Hampton et al. (1994) and Castelli-Gair et al. (1994). 69B GAL4 is reported to promote expression solely in the ectoderm. We have cut serial cross sections through embryos carrying 69B GAL4; UAS-lacZ and monitored the expression of β-galactosidase at early stages (up to and including stage 11, when we think wg is acting on the mesoderm). We find prominent expression in the ectoderm and its derivatives (CNS and tracheal pits) but no expression in the mesoderm (data not shown). We do not claim that our sections can exclude the possibility of expression in occasional mesodermal cells. However, they demonstrate that expression is predominantly ectodermal. The UAS-wg construct was described by Wilder and Perrimon (1995). This construct was prepared using the cDNA from the wg Gal4 allele and is therefore temperature sensitive. At 18°C, the wg protein derived from this construct has wild-type function whereas at 25°C, this wg protein is null.

Embryos carrying the GAL4/UAS-wg constructs were prepared as follows.

For mesodermal expression: yw; w<sup>CM4</sup>/twist-GAL4/CyO;ftz-lacZ crossed to yw; w<sup>CM4</sup>/CyO;ftz-lacZ; UAS-wg generated embryos which when homozygous for wg null allele carried one copy of both the twist-GAL4; UAS-wg constructs.

For ectodermal expression: w; w<sup>CM4</sup>/CyO;ftz-lacZ; 69B GAL4 crossed to yw; w<sup>CM4</sup>/CyO;ftz-lacZ; UAS-wg generated embryos which when homozygous for wg null allele carried one copy of both the 69B GAL4/UAS-wg constructs.

We made use of the UAS-wg temperature sensitivity to generate embryos in which the level of functional wg protein was altered. At 18°C we could induce the maximal amount of functional wg protein, be it in the mesoderm (driven with twist-GAL4) or ectoderm (driven with 69B GAL4). By increasing the temperature in steps of 1-2°C and then analysing the embryos derived from the above crosses at that temperature, we were able to find optimal conditions (22-23°C) in which wg expressed in the mesoderm or ectoderm was sufficient to rescue the wg null phenotype only in that germ layer.

**Antibody staining**

Anti-S59 antibody was prepared in rabbits by M. K. B., E. Rushton, and F. Jimenez using a portion of a S59 cDNA, kindly provided by M. Frasch. The anti-sera recognized the same pattern of expression described by Dohrmann et al. (1990) and was used at a concentration of 1/1000 in a manner described previously (Bate and Rushton, 1993). The anti-En and anti-Wg were used according to established protocols (Asburner, 1989). Anti-β-galactosidase was purchased from Cappel and used at a concentration of 1/10,000. The anti-muscle myosin antibody was a kind gift from Dr D. Kiehart and was used as previously described (Bate and Rushton, 1993). Double labelling experiments with anti-S59 and anti-En were performed by incubating blocked embryos with anti-S59 (overnight, 4°C), followed by washing and blocking, and then incubating with the rabbit secondary antibody (1 hour, room temperature; Jackson Labs). After further washing and blocking, we incubated the embryos with the anti-En antibody (2-3 hours, room temperature). After washing, the anti-S59 antibody-secondary complex was developed with DAB (Sigma) and NiCl, which results in the black stain. Following extensive washing, the anti-En secondary antibody (Jackson Labs) was added for 1 hour at room temperature. After washing, the anti-En-secondary antibody complex was developed using DAB alone which results in brown stain.

**RESULTS**

**wg mutant phenotype in the embryonic mesoderm**

Mutations in wg cause drastic defects in the normal differentiation of the mesoderm. These are very simply demonstrated by staining late embryos with an antibody against muscle myosin (Fig. 1). This reveals that in wg mutant embryos there is a severe derangement in somatic myogenesis (Fig. 1A,B) and the heart (Fig. 1C,D) fails to form (also reported by Wu et al., 1995). However, in these embryos the progenitors of the visceral mesoderm segregate relatively normally (anti-fasciclin III staining, data not shown), although, as previously reported, the morphogenesis of the midgut and midgut visceral mesoderm is abnormal (Fig. 1E,F; Immergöllück et al., 1990). Here we focus on the patterning of the somatic muscles. Myosin staining shows that in wg mutant embryos virtually all the muscles of the lateral and dorsolateral sectors of the pattern are missing. Mutations in other segment polarity genes also produce deranged late muscle patterns (data not shown; Volk and Vijayaraghavan, 1994).

**Wild-type expression of S59**

The final muscle pattern depends critically on two processes. (1) The segregation of muscle founder cells which are endowed with all the information to build individual muscles. (2) Properly patterned epidermis, which provides the substrate on which the differentiating muscles extend and form their attachments. Thus, mutations in genes such as those of the segment polarity class, which lead to alterations in the epidermal pattern, necessarily also cause secondary defects in the arrangement of the muscles, independently of any direct effects on the patterning of founder cells and the formation of the muscle pattern. For this reason we chose to study the initial pattern of S59-expressing cell clusters as an index of the segregation of the muscle founder cells in the mesoderm in wild-type embryos and in different segment polarity mutant backgrounds. In wild-type embryos, the initial pattern of S59 emerges in the mesoderm during midstage 11 to stage 12. Three clusters of S59 expression appear sequentially in the somatic mesoderm of each abdominal hemisegment (Fig. 2A). Each cluster arises in a stereotyped position relative to ectodermal engrailed expression, with one cluster lying just on the posterior edge of the stripe and the other two clusters lying on the anterior edge (figure 6 in Dohrmann et al., 1990). These three clusters of S59-expressing cells give rise to the cells which found several different muscles in each segment (Dohrmann et al., 1990; Carmena et al., unpublished data). S59 also marks a distinct population of mesodermal cells in the thoracic segments which we have not considered in this study.

**Expression of S59 is absent from the mesoderm in wg mutants but is present in naked, patched, engrailed, and hedgehog mutants**

To evaluate the role of the segment polarity genes in the patterning of the muscles, we analyzed the mesodermal
expression of S59 in a number of segment polarity gene mutants. In each case, we used a null allele (see Materials and Methods) and in all cases (with the exception of wg), we found that S59 is expressed in muscle forming mesoderm, although, in some mutants there may be abnormalities in the precise pattern of expression. In nkd, ptc and Df(en)E mutant embryos (Fig. 2B,C,D respectively), the mesodermal expression of S59 appears in three clusters in the mesoderm. In hh mutants (Fig. 2E), single clusters of S59 expression may be missing. However, the location and identity of the missing cluster varies from embryo to embryo. In striking contrast to these relatively mild phenotypes, in wg mutant embryos (Fig. 2F) S59 expression is completely absent from the mesoderm. Thus, despite the fact that the embryonic ectoderm in nkd, ptc, en, and hh mutants is deranged, the expression of S59 in the mesoderm is relatively normal and it is only mutations in wg which lead to a complete loss of mesodermal S59 expression.

Expression of wg
Because wg is unique in its dramatic effects on the mesodermal expression of S59, we analysed the distribution and timing of wg expression in the mesoderm and compared this with wg expression in the ectoderm. wg is conspicuously expressed in the embryonic ectoderm, beginning at the blastoderm stage and evolving into 14 stripes of expression which further develop into dorsal and ventral sectors during late stage 10. This pattern of expression is maintained virtually unchanged until the end of embryogenesis (Baker, 1988). In the mesoderm however, after the blastoderm stage, wg is undetectable except for a spotty and irregular pattern of expression with a pair rule component (compare Fig. 3A,B). This expression occurs during gastrulation and disappears completely during stage 9 (data not shown). The only exception to this rule is the visceral mesoderm of parasegment 7 which acquires wg expression during late stage 11 (Immerglück et al., 1990).

While we do not exclude the possibility that the early, patchy expression of wg may have some function in the mesoderm (see below) it is also possible that the far higher levels of wg in the ectoderm could act directly to influence mesodermal expression of S59. To investigate this, we decided to make use of a mosaic approach by using the GAL4 targetted expression system (Brand and Perrimon, 1993) to promote wg expression in one or the other of the two germ layers. In the first set of experiments, we tested the idea that ectodermal wg could influence S59 expression in the mesoderm. To do this, we expressed wg throughout the ectoderm of wg null mutants and used S59 expression to assay the effects of this ectodermal expression on the development of the muscle-forming mesoderm.

Ectodermal expression of wg can rescue mesodermal defects in wg mutant embryos
We expressed wg under the control of the GAL4 driver, 69B (Brand and Perrimon, 1993; Staehling-Hampton et al., 1994;
Castelli-Gair et al., 1994), which is expressed generally in the ectoderm from late stage 9, in a \( wg \) null mutant background and monitored mesodermal expression of \( S59 \). The loss of \( S59 \) expression normally seen in \( wg \) null mutants is rescued. The rescued pattern of \( S59 \) expression consists of appropriately positioned clusters each consisting of the normal number of cells (Fig. 4B). In addition, the pattern of the epidermis in these embryos measured both by the expression of a \( wg \)-dependent gene, \( en \) (reviewed by Martinez Arias, 1993), and by the cuticle phenotype is substantially rescued (Fig. 5G-I). These experiments show that some aspects of mesodermal development in \( Drosophila \) can be influenced by ectodermally derived cues. It is not clear, however, whether this is a direct effect of \( wg \) on the mesoderm or an indirect effect of the improved patterning of the ectoderm. We addressed these issues in the following experiments.

**Mesodermal GAL4 driven expression of \( wg \) can rescue mesodermal defects in \( wg \) mutants**

We used a \( twist \)-GAL4 construct to promote \( wg \) expression in the mesoderm in wild-type and \( wg \) null mutant embryos. This construct (M. K. B. and M. B., unpublished data) drives expression of \( wg \) throughout the mesodermal primordium starting at stage 7 as the embryo gastrulates and continuing at least until stage 12 when the germ band retracts (data not shown). In addition, we detect expression of \( wg \) in the mesoderm when using this GAL4 driver.

In wild-type or \( wg \) mutant embryos carrying the \( twist\-
Expression of embryo in which can be detected in the thorax. (B) Homozygous to specific muscles. In addition, large clusters of S59 expression is also rescued (Fig. 4C). Interestingly, at the onset of S59 expression in these mutant embryos carrying the constructs, the size of the clusters is often increased; however, in general, later in development the correct number of cells segregates in each cluster. Thus wg appears to act directly on the mesoderm to influence patterns of gene expression.

We also find that wg expression driven in the mesoderm of wg null embryos is capable of restoring some ectodermal en expression as well as partially rescuing the mutant phenotype of the cuticle (Fig. 5J-L). The most conspicuous ectodermal rescue is ventral: a prominent stripe of en expression spanning the ventral midline of every segment. In addition, there are segmentally arranged clusters of en-expressing cells in dorsolateral regions of the developing epidermis (Fig. 5K). While the ventral stripes probably depend on the provision of wg from the cells of the mesectoderm rather than the underlying mesoderm, the appearance of dorsolateral clusters of en-expressing cells can only depend on wg derived from the mesoderm. Thus while wg in these experiments appears to act directly on the mesoderm to influence patterns of gene expression, it could be argued that the mesodermal rescue that we observe is simply a consequence of some wg-dependent signal coming from the partially rescued ectoderm and not a response to wg itself. To exclude this possibility we performed a series of experiments in which we progressively reduced the amount of wg activity in the experimental embryos.

**Rescue of S59 expression in wg mutants by mesodermally driven expression of wg can occur without rescue of the ectoderm**

To promote the expression of different levels of wg in the mesoderm, we raised embryos at temperatures which would progressively affect the efficiency of the wg construct which is inherently temperature sensitive (see Methods). In this way we found a level of activity at which wg expressed in the mesoderm of a wg mutant embryo can rescue S59 expression without rescuing the pattern of the ectoderm. Fig. 6 documents this effect: the low levels of wg expressed under these conditions are insufficient to maintain en expression in the mesoderm but do rescue S59 expression. Dorso lateral en expression in the ectoderm is not rescued, and the cuticle is typical of a wg mutant embryo (Fig. 6A,B, compare with 5K,L). Despite the derangement of the ectoderm, the onset and development of the mesodermal S59 pattern is good, with all three clusters appearing at the appropriate time and place (Fig. 6A). This result strongly suggests that the rescue we observe in our experiments is caused by wg acting directly in the mesoderm.

In addition we went on to exclude the possibility that the rescue we observe in this experiment results from a wg-dependent, en-independent signal emanating from the rather disturbed ectoderm which we find in these embryos. We performed the converse experiment where we expressed varying amounts of wg in the ectoderm of a series of wg mutant embryos. Weak wg expression can rescue the ectoderm and...
Fig. 5. Expression of *engrailed* (*en*) and cuticle pattern of wild type (A-C), *wg*<sup>Cx4</sup> mutants (D-F) and *wg*<sup>Cx4</sup> mutants in which *wg* is ectopically expressed in the ectoderm (G-I) or in the mesoderm (J-L). In wild-type embryos (A-C) *en* is expressed in the ectoderm in a pattern of stripes that is propagated during development (A, B). The wild-type cuticle contains a distinct pattern of denticles in the anterior part of every segment and naked cuticle in the posterior part (C). The extent of the naked cuticle depends on the activity of *wg* (reviewed by Martinez Arias, 1993). In *wg* mutant embryos (D-F) *en* expression is initiated normally but fades in the ectoderm and the mesoderm during stages 9 and 10 (D). By stage 12, *en* is only expressed in groups of neurons in the developing central nervous system (E). These embryos secrete a cuticle that is characterized by a lawn of denticles ventrally (F). This lawn reflects segmental fusions and the absence of naked cuticle. In *wg* mutant embryos to which *wg* function has been supplied from the ectoderm (*wg*<sup>Cx4</sup>; 69BGAL4; UAS-*wg*) (G-I), *en* expression is partially rescued in the ectoderm (late stage 10) (G). By stage 12, *en* is clearly expressed in stripes in the ectoderm ventrally and in clusters of cells laterally and dorsally (H). These embryos secrete a cuticle that is rescued in two respects: first the metameric pattern is visible, although not the wild-type extent and in addition, they have a strip of naked cuticle running through the middle (I). This is caused by high levels of *wg* expressed in these embryos and the relationship that exists between the activity of *wg* and the extent of naked cuticle (compare I with F). Wild-type siblings carrying the 69BGAL4; UAS-*wg* constructs from this cross also show a large strip of naked cuticle running along the midline (data not shown). In *wg* mutant embryos to which *wg* function has been supplied from the mesoderm (*wg*<sup>Cx4</sup> twist-GAL4; UAS-wg), *en* expression can be clearly detected in stripes in the ectoderm and the mesoderm (late stage 10) (J); the broad stripes that can be observed here are a combination of *en* expression in the mesoderm (3-4 cells wide) and the ectoderm. Ectodermal expression is in the dark clusters of cells preferentially at the anterior region of every broad stripe. By stage 12, although *en* has decayed from the mesoderm, there are clusters of ectodermal cells that express *en* laterally (K). These embryos secrete a cuticle that is considerably rescued compared with the *wg* mutants (L; and compare with F). The cuticle is similar to that shown in I except that there is no strip of naked cuticle ventrally, perhaps because there is not enough *wg* activity to override the normal differentiation of those cells.
this ectodermal rescue is better than that obtained when \( wg \) is expressed at high levels in the mesoderm (compare cuticles, Figs 6D, 5L). Nonetheless, under these conditions and despite good ectodermal rescue, we fail to rescue \( S59 \) expression in the mesoderm.

In summary, \( wg \) expressed in the mesoderm at levels which do not rescue the ectoderm, succeeds in restoring the pattern of \( S59 \) expression. \( wg \) can be expressed in the ectoderm at levels which are sufficient to rescue the epidermis but fail to rescue \( S59 \) expression in the mesoderm. Thus ectodermal patterning itself is not a necessary or sufficient condition for rescuing \( S59 \) in the mesoderm. However, \( S59 \) expression does depend critically on available levels of \( wg \).

**DISCUSSION**

We would like to understand how patterns are formed in the mesoderm during embryogenesis. In *Drosophila*, the most conspicuous of these is that formed by the larval muscles as they assemble on the developing epidermis midway through embryogenesis. Early in development the mesoderm subdivides into the progenitors of its different derivatives, including the heart, the fat body, the visceral and somatic mesoderm. It is the cells of the somatic mesoderm that give rise to the muscle pattern: thirty individual fibers in each hemisegment of the abdomen, with other specialized patterns in the remaining segments (Bate, 1990, 1993). The minimum requirement necessary for the proper spatial organization of myogenesis is the specification of thirty distinct founder cells for the different muscles in an abdominal hemisegment. To understand how this segregation of specialized myoblasts is brought about, we need to know the source of the information which distinguishes these cells from their neighbours. This is the issue that we address in this paper.

Perhaps the simplest way of thinking about the origins of spatial organization in the mesoderm is to assume that the segment polarity gene network, responsible for the allocation of cell fates in the ectoderm, is reproduced in the mesoderm. However, there is abundant evidence that this is not the case. For example, \( hh \) is not expressed in the mesoderm (Lee et al., 1992) and the mesodermal expression of \( ptc \) is not in register with its expression in the ectoderm (Nakano et al., 1989; Hooper and Scott, 1989). Until gastrulation, elements of the segment polarity network are expressed in the domains of both the future mesoderm and ectoderm. In the ectoderm, this pair-rule-dependent pattern of gene expression is consolidated by mutually dependent patterns of segment polarity gene expression, which sustain the expression of the network until late in embryogenesis. It is this self-sustaining network which is responsible for the elaboration of ectodermal cell fates (Martinez Arias, 1993). In the mesoderm however, it is exactly at the point at which the pair-rule-dependent gene expression is superceded in the ectoderm that the expression of \( wg \) and \( en \), begins to disappear. Thus, although elements of the network are transiently expressed in the mesoderm early in development, the self-sustaining interactive network is never established.

Another way of specifying a pattern of founder cells would be by a point to point imposition of ectodermal information onto the mesoderm. If this were the case, we would expect that all mutations affecting epidermal patterning would have predictable consequences for the underlying pattern of founders. No single gene involved in epidermal patterning should have more drastic effects than those predicted from its epidermal phenotype. However, this is not what we find. In our investigation of the influence of the segment polarity genes on the formation of founder cells, we have shown that only one member of the segment polarity class, \( wg \), has drastic effects on the segregation of the founder cells. It is all the more interesting that \( wg \) encodes a secreted molecule (Nusse and Varmus, 1992) which has been shown to be capable of operating across germ layers (this work; Immerglück et al., 1990; Lawrence et al., 1994). Furthermore, because sustained \( wg \) expression requires the expression of other segment polarity genes, notably \( en \) (Perrimon, 1994) the fact that mutations in these genes do not reproduce the \( wg \) phenotype in the mesoderm shows unequivocally that the requirement for \( wg \) is an early one, occurring during blastoderm to stage 10/11 and corresponding to the pair rule-dependent phase of its expression. Chu-LaGraff and Doe (1993) have shown that it is precisely during this phase of ectodermal development that \( wg \) is required for the proper segregation of a subset of neuroblasts in the developing central nervous system of the embryo. There is an obvious analogy between the segregation of a spaced pattern of muscle founder cells and the specification in the ectoderm of neuroblasts and sensory mother cells.

The evidence suggests that \( wg \) acts directly on the mesoderm. Firstly, the location of the \( S59 \)-expressing cells relative to the posterior compartment as reflected by \( en \) expression (Doehrmann et al., 1990) indicates that cells that are destined to express \( S59 \) are within the reach of \( wg \) signalling. Secondly, levels of \( wg \) in the mesoderm, which fail to rescue the ectoderm, restore \( S59 \) expression to the mesoderm in \( wg \) null mutant embryos. Interestingly, such levels of \( wg \) activity also fail to sustain the mesodermal expression of \( en \), whereas higher levels of \( wg \) activity rescue both the ectodermal and mesodermal \( en \). Thus, relatively high levels of \( wg \) activity are necessary to sustain mesodermal expression of \( en \), that is, higher levels than those that are necessary to rescue \( S59 \). This may explain why, in wild-type embryos, the ectodermal expression of \( wg \) is insufficient to maintain \( en \) expression in the mesoderm. If this is the case, it suggests that relatively low levels of \( wg \) activity are required early in embryogenesis to allow for the segregation of \( S59 \)-expressing founder cells. Given that there are endogenous low levels of \( wg \) in the early mesoderm (this work; Lawrence et al, 1994), this obviously raises the issue of the source of the \( wg \) activity required for founder cell segregation in wild-type embryos. We cannot exclude the possibility that the patchy and uneven levels of \( wg \) expression seen in the early mesoderm are sufficient to achieve this. However, it seems to us that the unevenness of \( wg \) expression in different segments makes it unreliable as the only source of this essential signal. It seems far more likely, given the evidence that we present here, that the robust \( wg \) expression in the ectoderm is the principal source of \( wg \) to which the mesodermal cells refer. However, it is almost inescapable that the cells will respond to the sum of whatever \( wg \) is presented to them.

We do not yet understand how \( wg \) acts to influence the segregation of founder cells: \( wg \) could either be required for the formation of founder cells or could act to specify particular
founder cells. This question remains open. However, we do have several lines of evidence that may provide some clues as to the mechanism of wg action. In an earlier paper, we showed that in wg mutant embryos, the population of external cells in the mesoderm which normally express twist at high levels either fail to express twist or are missing (Bate and Rushton, 1993). The S59-expressing muscle founders, which we consider in this paper, are derived from this set of cells (unpublished observations; Dohrmann et al., 1990). Thus, there may be an early function for wg in the formation of cells of the somatic muscle forming lineage.

However, although all cells destined for the somatic muscle lineage express elevated levels of twist (M. K. B. and M. B., unpublished observations) not all founder cells require Wg in order to segregate. For example, some vestigial-expressing founder cells are formed in wg mutant embryos (Bate and Rushton, 1993). In addition, when wg is expressed throughout the mesoderm or ectoderm, it does not convert all founder cells into S59-expressing cells. Together these observations show that the founder cell population is of at least two kinds, wg-dependent and wg-independent, and that only a subset of cells is competent to respond to wg by expressing S59. Thus, the somatic mesoderm seems to have intrinsic characteristics which subdivide it into distinct populations. These subdivisions constitute a framework of competence domains on which wg (and perhaps other signals) act to elicit the formation of muscle founders. It may be that this framework is established by the action of the pair rule genes which are expressed in characteristic and overlapping patterns in the antero-posterior axis of the mesoderm as it invaginates at gastrulation. We anticipate that dorsal-ventral values within this framework will be given directly or indirectly by induction from levels of dpp in the overlying ectoderm. It is likely, as we have shown here, that the ectoderm is a source of the wg-mediated signal necessary for the subsequent execution of the muscle differentiation pathway.

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