Segmental patterning of heart precursors in *Drosophila*

Peter A. Lawrence[1],*, Rolf Bodmer[2] and Jean-Paul Vincent[1],*,†

[1]Medical Research Council, Laboratory of Molecular Biology, Hills Rd, Cambridge CB2 2QH, UK
[2]Department of Biology, University of Michigan, Ann Arbor, MI 48109-1048, USA

*These two authors contributed equally to the work
†Author for correspondence (email: jpv@mrc-lmb.cam.ac.uk)

SUMMARY

The mesoderm of *Drosophila* embryos is segmented; for instance there are segmentally arranged clusters of cells (some of which are heart precursors) that express *even-skipped*. Expression of *even-skipped* depends on Wingless, a secreted molecule. In principle, Wingless could act directly in the mesoderm or it could induce the pattern after crossing from ectoderm to mesoderm. Using mosaic embryos, we show that Wingless produced in the mesoderm is sufficient for *even-skipped* expression. This proves that induction is not essential. However, induction can occur: when patches of *wingless* mutant mesoderm are overlaid by wild-type ectoderm, they do express *even-skipped*. We therefore believe that Wingless from both the ectoderm and mesoderm may contribute to patterning the mesoderm. Using the UAS/Gal4 system, we made embryos in which the Wingless protein is uniformly expressed. This is sufficient to rescue the repeated clusters of *even-skipped* expressing cells, although they are enlarged. We conclude that the mesoderm is segmented in some way not dependent on the distribution of Wingless, suggesting a more per-missive and less instructive role for the protein in this instance.

Key words: mesoderm segmentation, *wingless*, *even-skipped*, heart precursors, embryonic induction

INTRODUCTION

Segmentation has been most extensively studied in the ectoderm of insect embryos. There, the founding unit of seg-mentation is the parasegment (Martinez Arias and Lawrence, 1985) and, because all the parasegments are homologous one to another, structures and patterns reiterate with variations on a common theme. Parasegments consist of sets of cells that develop somewhat independently; cells do not mix across the borders between them and are subject to independent genetic control, for example by selector genes which are expressed in specific parasegments (Garcia-Bellido, 1975; Lawrence, 1992). In the ectoderm of *Drosophila*, maintenance of borders between parasegments depends on the difference between confronting anterior and posterior cells, a difference that is determined by the *engrailed* gene (Morata and Lawrence, 1975; Kornberg, 1981; Basler and Struhl, 1994; Capdevila et al., 1994).

Is the mesoderm segmented in the same way? The adult muscle precursors are divided into distinct segmental sets at the blastoderm stage (Lawrence, 1982), but it is not yet clear whether the larval muscle precursors are similarly divided (see Bate, 1993 for discussion). Still, the serial repetition of structures and gene expression (e.g. Frasch et al., 1987; Dohrmann et al., 1990) indicates that the somatic muscles and the heart are segmented. Also, the limits of expression, in the mesoderm, of selector genes such as *Ultrabithorax* and *abdominal-A* are approximately collinear with the paraseg-mental boundaries in the ectoderm (reviewed in Bate, 1993) and these genes determine the pattern of muscle precursors, rather as they help determine pattern in the ectoderm (Greig and Akam, 1993; Michelson, 1994). In the visceral mesoderm, the modular expression of selector genes such as *Sex combs reduced* and *Ultrabithorax* also suggests a subdivision into parasegmental units (Tremml and Bienz, 1989). These lines of evidence establish that mechanisms of segmentation are shared between the mesoderm and the ectoderm. However, there may be one important difference: unlike the ectoderm, there is evidence from mosaic animals that *engrailed* has no role in the mesoderm (Lawrence and Johnston, 1984).

Segmentation of the mesoderm could arise in different ways: it could be induced by the overlying ectoderm, meaning that a segmental pattern within the ectoderm could be imprinted onto the underlying mesoderm. Or, the mesoderm could segment inde-pendently of the ectoderm. Alternatively, segmentation could begin prior to specification of these two germ layers and divide the whole embryo. Later, interactions between the two layers could keep the segments in registration and define details of pattern. This paper asks how far the mesoderm segments itself.

To investigate this we have used a small, but clearly differ-entiated, group of mesoderm cells that are segmentally repeated. These cells are dorsal and become distinct soon after comple-tion of the dorsalward migration of the mesoderm from its ventral site of invagination (Frasch et al., 1987). They express the Even-skipped (Eve) protein and we call them the 'eve cells'. These cells contribute to the larval heart as well as to dorsal somatic muscles. In the absence of segment polarity genes such as *wingless*, these cells do not appear, or at least they do not express *eve* and heart formation is abolished (Wu et al., 1995).

(Another marker of mesoderm segmentation, *S59*, also depends on *wingless* (Bate and Rushton, 1993).) *wingless* is required for *eve* expression early, before any overt patterning of the mesoderm (as shown with a temperature-sensitive allele; Wu et al., 1995). Since segment polarity genes (Nüsslein-Volhard and Wieschaus, 1980) are primarily known for their effects on the ectoderm and since *wingless* is expressed most strongly in that germ layer, there might be a tendency to favour the induction model. However, it is equally possible that Wingless acts within the mesoderm (it is expressed and can have effects there: Lawrence et al., 1994) and therefore, that segmentation of the mesoderm is largely or partly autonomous. If so, it seems likely that many of the segmentation genes will act independently but similarly within the mesoderm and ectoderm.

We have made embryos that are mosaic for *wingless*⁺ and *wingless*⁻ cells and asked the following question: Does development of the eve cells depend on *wingless* function in the overlying ectoderm or within the mesoderm itself? We find that it is sufficient for Wingless to be provided by **either** the ectoderm or the mesoderm. Further, if the only Wingless protein present in the embryo is evenly distributed, the eve cells appear in enlarged clusters. These clusters are found in a restricted part of each segment, showing that the segmental pattern of the mesoderm does not simply follow from segmentally repeated stripes of expression of *wingless*. Both these results suggest that there is autonomous patterning within the mesoderm. Even so we find indications that the ectoderm may influence mesodermal patterning.

MATERIALS AND METHODS

Nuclear transplantation and preparations of embryos were as before (Vincent and Lawrence, 1994). Donors were marked with *armadillo-lacZ* and hosts were a stock in which one quarter of the embryos were homozygous *wingless*⁻; these could be distinguished as they did not carry a balancer chromosome that expressed *hunchback-lacZ*. Apart from Fig. 2, embryos were double stained with anti-β-galactosidase (monoclonal, Promega) in brown, and for Eve in black, using a polyclonal rabbit anti-serum (Frasch et al., 1987). *UAS-Wingless* was made as follows: A *Bam*HI fragment containing the *wingless* ORF was obtained from Neil Parkin (Aviron Corp, Redwood City, CA). It extends to the *Afl*II site just downstream of the stop codon. This fragment was inserted in the *Bgl*II site of pUAST (Brand and Perrimon, 1993). The *e22c-Gal4* driver was made by Ken Yoffe (we obtained it from Andrea Brand). We tested it by crossing it to *UAS-lacZ* (gift of Andrea Brand). Staining the embryos for β-galactosidase indicates that the *e22c-Gal4* directs expression in the ectoderm only (as far as we could determine by rotating whole mounts under high magnification) although we cannot exclude low level expression in the mesoderm. β-galactosidase expression begins at late stage 9 with some cells expressing much more than others but, by stage 10, all cells stain fairly heavily. The *twist-Gal4*; *24B-Gal4* stock was a gift of Mary Baylies (see Staehling-Hampton et al., 1994). Both *Gal-4* stocks are viable as homozygotes and we crossed them to homozygous *UAS-Wingless* flies.

RESULTS

The eve cells

These cells (Frasch et al., 1987; Azpiazu and Frasch, 1993; Bodmer, 1993) are first detectable at stage 10 and consist of a small cluster of 3 or 4 cells located in the most dorsal mesoderm of 11 segments (Fig. 1). Dissected preparations as well as sections of embryos at stage 11 show that the cells are

divisible into two types; in each cluster there is one large nucleus in a cell that is somewhat indented into the overlying ectoderm, and smaller nuclei which lie more within the mesodermal sheet. There is no doubt that all these cells are mesodermal in provenance: in those control mosaics (that is mosaics in which both donor and host cells are *wingless*⁺), where the donor cells are confined to mesoderm and extend dorsally into the cluster, all the eve cells belong to the donor while, in control mosaics in which the donor cells are confined to the ectoderm, none of the eve cells come from the donor. The eve cells are not a clone: in some control mosaics, the border between marked and unmarked cells cuts through a cluster.

We wished to map the eve cells within the segmental pattern. The parasegment boundaries are defined before gastrulation by the anterior bounds of *eve* and *fushi tarazu* (*ftz*) expression. In the ectoderm, these boundaries coincide with the anterior edges of *engrailed* stripes (Lawrence and Johnston, 1989). A long-lasting marker of the parasegment boundaries in both ectoderm and mesoderm is provided by *eve-lacZ* and *ftz-lacZ* transgenes and they show the boundaries to be in almost exact registration in the two germ layers (Lawrence, unpublished data). This implies that the mesodermal cells migrate in under the epidermis in an orderly fashion with little mingling, at least across parasegment border. Therefore, one can locate cells in the mesoderm relative to segmentation markers in the ectoderm. We have mapped the eve cells within the parasegment, by finding out their registration with respect to *wingless-lacZ* and Engrailed. It is clear that the eve cells underlie the *wingless* stripes in the ectoderm (Fig. 1) and therefore originate from the most posterior dorsal cells of the parasegment. Knowing this, one can allocate the clusters to parasegments 2-12 and 14 (Fig. 1). The cluster in parasegment 14 is somewhat more ventral than the others.
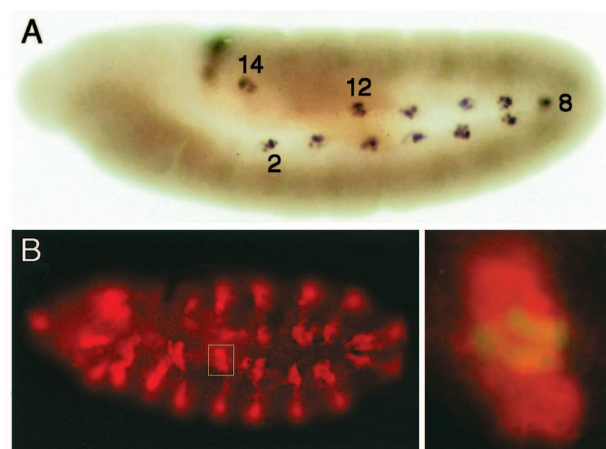

Fig. 1. The normal pattern of *wingless* and *even-skipped*. (A) Stage 10 embryo stained with an antibody to Eve (Frasch et al., 1987). (B) Stage 11 embryo carrying *wingless-lacZ* (Kassis et al., 1992) double stained with anti-β-galactosidase in red (rhodamine) and anti-Eve in green (fluorescein). Only the red channel is shown in the main panel. The small panel is a double exposed detailed view of the boxed area. It appears in the detail that the eve cells in the dorsal mesoderm underlie the *wingless* ectodermal stripe. Numbers indicate the parasegments. Note that the period of Wingless requirement for eve cells is earlier than stage 11 (around stage 9; see Wu et al., 1995). Thus, at the stage shown (when eve cells are clearly visible in the wild type) eve cells no longer require Wingless.

As for the fate of the eve cells, we presume that, at least in parasegments 4-12, the larger cell is the founder cell of somatic muscle number 1 (see Bour et al., 1995) and the other smaller ones contribute to the heart. The heart arises from cells expressing *tinman*, a gene required for its formation (Bodmer, 1993). These cells include both prospective contractile and pericardial cells. Eve cells give rise to pericardial cells only; there are 6-8 pericardial cells per hemisegment, two of which arise from the small eve cells (Bodmer, 1995).

The mosaics

Cells that are wild type and donor-derived are marked with β-galactosidase to distinguish them from host cells that are mutant for *wingless*. The mosaics of interest are those in which, in specific parasegments, the dorsal ectoderm and the underlying mesoderm are of different genotypes. There are 14 cases where *wingless*+ mesoderm underlies *wingless*− ectoderm and where the mesoderm appears to extend sufficiently far dorsally, and in the right place, for eve cells to stain and in 9 of these they do (Figs 2, 3). In 8/9 of these cases, all of the eve cells stain for Eve as they do in the wild type but, in 1 case, only a single cell expresses Eve, showing that the cells do not have to be identified in an all-or-none fashion. It may be that the 5 cases of absent eve cells are significant; in control mosaics, although damage consequent on the transplantation sometimes eliminates the eve cells, this occurs rarely. Nevertheless, the important finding is that *wingless*+ in the mesoderm alone is sufficient to generate the eve cells. In some of these
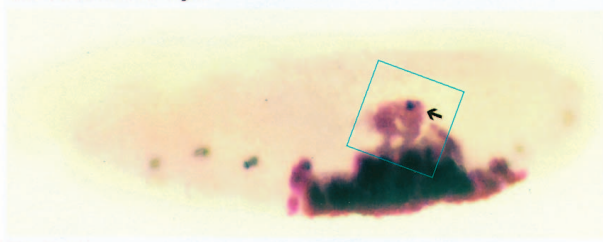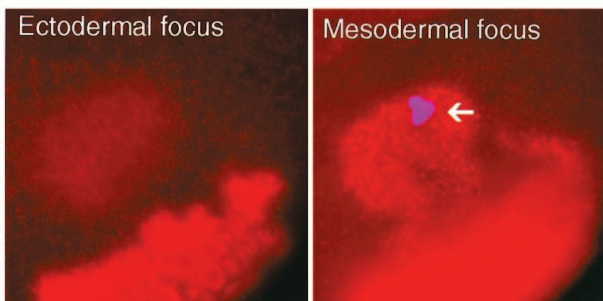

Fig. 2. An example of a *wingless*− embryo with a patch of *wingless*+ cells in the mesoderm and ectoderm. The mesodermal patch extends far more dorsally than the ectodermal one and, at its most dorsal extent, a single cluster of 3 eve cells is found. The pictures below, in two planes of focus, show that the *wingless*+ patch is confined to the mesoderm. Anti-β-galactosidase was revealed with an alkaline phosphatase-labeled secondary and Vector Red (Vector labs). The product of the Vector Red reaction is visible both in bright field (top panel) and fluorescence (bottom panels). Eve was stained using the traditional biotin/streptavidin/HRP system (see for example, Lawrence and Johnston, 1989).
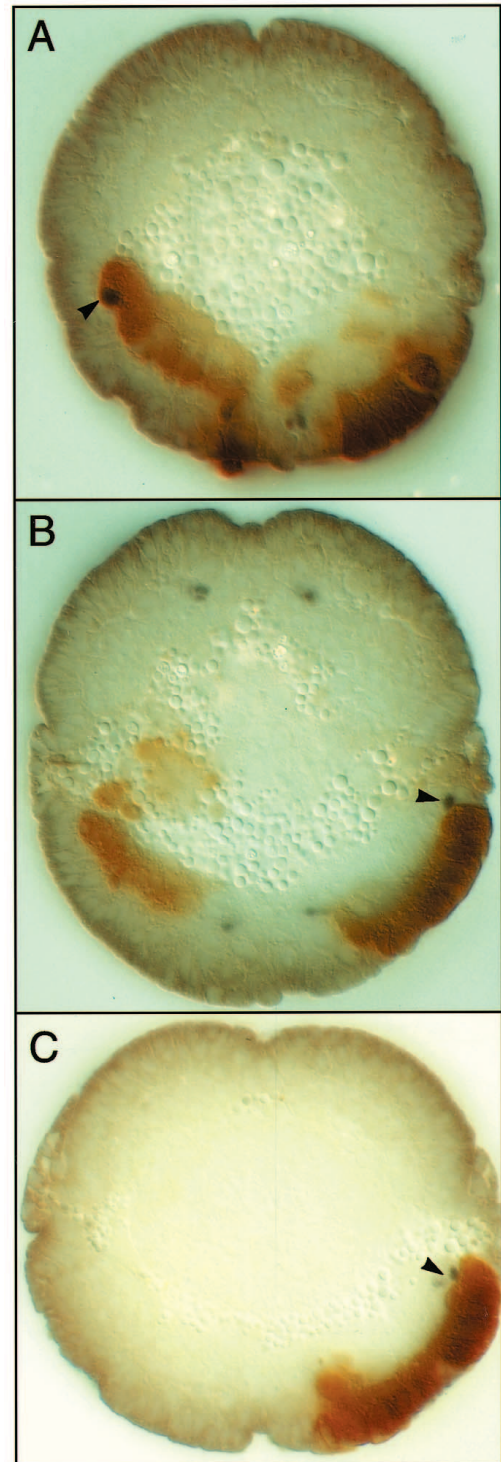

Fig. 3. Sections through three mosaics. These are durcupan sections at about 5 μm stained for donor tissue (β-galactosidase) in brown and Eve in black. (A) On the left, the mesoderm is wild type and shows an Eve-positive cell (arrow). The overlying ectoderm on this side is *wingless*−. The contralateral side contains wild-type tissue in both the ectoderm and mesoderm. (B) An embryo showing induction of *eve* expression in the mesoderm (right side) by wild-type ectoderm (the mesoderm is *wingless*− on this side). On the left, the *wingless*+ mesoderm does not extend far enough dorsally to include the eve cells. Eve-positive cells in the midline are neurons which are independent of *wingless*. (C) Another example of induction from the ectoderm.

mosaics, there is no marked ectoderm at all on the side where the eve cells develop, so we can be quite sure of this.

We turn now to the opposite class of mosaics, in which, in the relevant area, the ectoderm is *wingless*⁺ and the mesoderm *wingless*⁻. We analysed 26 examples of single parasegments where there is an induction from the *wingless*⁺ ectoderm to the underlying *wingless*⁻ mesoderm, i.e. where eve cells are found in *wingless*⁻ mesoderm that is overlaid by wild-type ectoderm (Fig. 3). Amongst these cases are examples where, on the whole of the relevant side, only ectoderm is marked, showing that eve cells can form without any Wingless contribution from the mesoderm.

We have attempted to compare the two classes of mosaics with respect to how often eve cells develop when only one of the germ layers is *wingless*⁺. This is not easy because, for each individual mosaic, a judgement has to be made as to whether the wild-type patch falls within (or covers) the area where eve cells would normally form. While we did see clear cases of missing eve cells in mesodermal wild-type patches (see above), we saw no obvious example of the converse (missing eve cells underneath *wingless*⁺ ectoderm). This suggests that induction from the ectoderm is more reliable than the mesoderm itself in specifying eve cells. A simple explanation for this is the amount of Wingless being expressed. Staining for Wingless shows that there is more protein in the ectoderm than in the mesoderm (van den Heuvel et al., 1989).

These two classes of mosaics show that it is sufficient to produce the Wingless protein in either germ layer for development of the eve cells in the mesoderm. Our experiments also give information on the range of the *wingless*-dependent signal. In most cases the induced eve cells are immediately underneath the *wingless*⁺ ectoderm but, in a few examples the cells are offset slightly. In no case are the induced eve cells more than one or two cell diameters from the source of the induction, suggesting that the *wingless*-dependent effect does not have a long range. Conceivably, the effect could spread a little from its source, but one should remember that the mesoderm is migrating on the inner face of the ectoderm. If migration were to continue after the interaction is completed this alone would produce some offset. In those cases where the *wingless*⁺ tissue is confined to the mesoderm, the eve cells are, in all the 9 examples, included within the wild-type patch.

Uniform expression of wingless

We asked whether the segmental stripes of *wingless* expression are responsible for the metameric arrangement of the eve cells. Our approach was to provide uniformly distributed Wingless protein to *wingless* mutant embryos using the Gal4/UAS method of Brand and Perrimon (1993). We analysed embryos and larvae arising from a cross between flies bearing a *UAS-Wingless* transgene and flies bearing a Gal4 driver called e22c. We believe that this driver expresses Gal4 uniformly in the ectoderm (see methods). Both parents were also heterozygous for *wingless*^CX4, a null allele. Cuticle of embryos arising from this cross (they all die before hatching) provide additional evidence that *wingless* expression is uniform because they show the phenotype obtained with Wingless overexpression using a *hs-Wingless* transgene (Noordermeer et al., 1992; Sampedro et al., 1993). (The phenotype obtained with the Gal4/UAS system is strong and, unlike the one arising from heat-shocked *hs-wingless* embryos, uniform.) This phenotype is also shown by *naked* null mutants, which lack ventral

denticle belts, apart from the 'beard' in the first thoracic segment (Nüsslein-Volhard and Wieschaus, 1980; Sampedro et al., 1993). All individuals expressing *UAS-Wingless* under the control of *e22c-Gal4*, independently of whether they were *wingless*⁺ or *wingless*⁻, showed this 'naked' phenotype.

In the mesoderm of *e22c-Gal4/UAS-Wingless* embryos (uniform ectodermal Wingless), the clusters of eve cells were enlarged to a mean of 8 cells (ranging from 4 to 14 cells; Fig. 4). Expansion of the clusters appeared to occur anteriorly (using *engrailed* expression and parasegment grooves as landmarks; data not shown). Thus, the enlarged clusters occupy the posterior halves of parasegments and, again, *wingless*⁺ and *wingless*⁻ embryos were identical with respect to enlarged eve clusters. Soon after their formation, these enlarged clusters join together to form a continuous line during stage 11. (Transformation into a line, a normal step towards formation of the heart tube, also occurs in the wild type but, there, it takes place around stage 12-13.) In *naked*⁻ embryos, enlarged eve clusters also form (Fig. 4). Thus, a *naked* mutation or uniform Wingless expression lead to the same phenotype, whether one looks at the ectoderm or the mesoderm. In e22c-Gal4/UAS-Wingless, *wingless*⁻ embryos, the only Wingless produced is present uniformly. Since eve clusters form in only part of the segment, we conclude that there is metameric patterning in the mesoderm that is not determined by stripes of Wingless.

Uniform expression of wingless in the mesoderm only

We sought additional evidence for our finding that Wingless supplied in only the mesoderm is sufficient to sponsor development of eve cells. We expressed Wingless constitutively in the mesoderm using a combination of *twist-Gal4*, expressed early in the mesoderm (Staehling-Hampton et al., 1994) and *24B-Gal4* which provides persistent Gal4 activity at a later stage in the mesoderm (Michelson, 1994). The ectoderm of these embryos is affected by expression of Wingless in the underlying mesoderm. The effect is strongest in the mid-ventral ectoderm, which now makes naked cuticle everywhere — except in the T1 segment where there is an enlarged beard. By contrast, the dorsolateral parts of the denticle belts and the dorsal cuticle are relatively normal (Fig. 5).

Whatever the reason for the dorsoventral difference in the ectodermal pattern (see Fig. 5 legend), these embryos gave us an experimental opportunity: the dorsal ectoderm is near wild type, and the mesoderm is expressing *wingless* uniformly and would be expected to have a 'naked' pattern (enlarged eve clusters). We therefore thought the two germ layers would pull the pattern in opposite ways and we could see which was paramount. For example, we might have found that repatterning of the mesoderm by Wingless overexpression could occur independently of the ectoderm. But the result was not so simple — embryos overexpressing Wingless in the mesoderm activated *eve* prematurely at stage 9 (in the wild type, eve cells appear at late stage 10) and in enlarged clusters (Fig. 5). But, by stage 11, the cluster size had decreased to near normal, unlike in *naked*⁻ or *e22c-Gal4/UAS-Wingless* embryos. Thus, it seems that Wingless expression within the mesoderm sponsors a 'naked' phenotype there during stages 9 and 10 but that later, the ectoderm, which is wild type in pattern, may force the underlying mesoderm back towards the wild-type state. So it may be that stable patterning of the mesoderm does require input from the ectoderm.
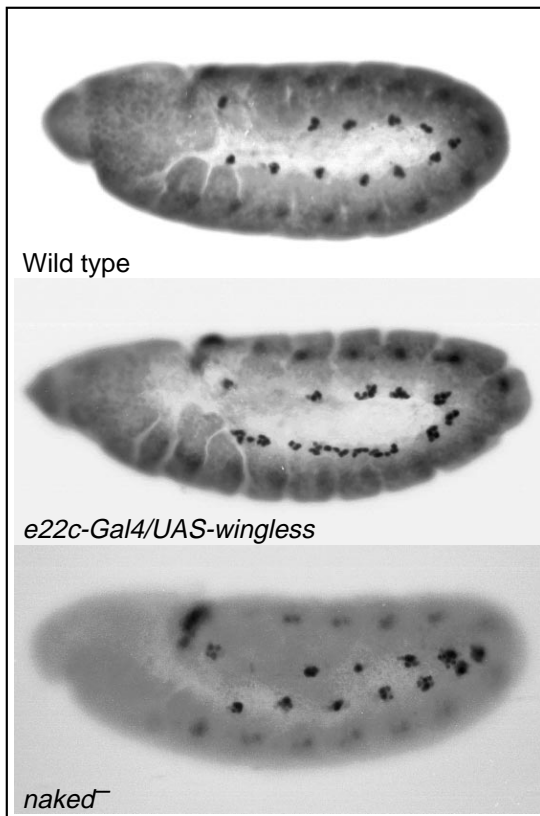
Fig. 4. Top two panels: embryos at stage 11, from a cross between *wg^{CX4} e22c-Gal4/CyO hunchback-lacZ* and *wg^{CX4}/CyO hunchback-lacZ; UAS-Wingless*. The upper panel shows a wild-type sibling, the eve cell clusters are 2-4 cells and the middle one shows the 'naked' phenotype induced by ubiquitous Wingless, clusters are 4-14 cells. Note that in the anterior part of the embryo, the clusters are beginning to fuse. For comparison, a *naked^{7E89}* homozygous embryo at stage 10, is shown in the lower panel.

DISCUSSION

In the mesoderm, in a specific set of metameres, there are a few cells that express *eve*. These cells are formed with a segmental periodicity, so they are an early indication of segmentation within the mesoderm. The development of eve cells depends on at least two segment polarity genes, *wingless* (Wu et al., 1995) and *hedgehog* (M. Park, X. Wu, K. Golden and R. Bodmer, unpublished), which are known to be active in segmentation of the ectoderm. Here, we focus on the action of *wingless* in eve expression — others have recently studied the effect of wingless on *S59*, another marker of mesoderm segmentation (Baylies et al., 1995). Since Wingless is secreted, it could mediate an induction from the ectoderm to the mesoderm. However, when we use mosaics to analyse where *wingless* is active, we find that the eve cells are locally rescued in regions where only the mesoderm carries the *wingless^+* gene. We also find that the action of *wingless^+* in the ectoderm can restore the eve cells in the underlying *wingless^-* mesoderm, showing that induction can occur and could be important in the wild type. Our data suggests that the contribution from the ectoderm may be predominant but this is hard to prove. We suspect that, in the wild type, specification of eve cells and, therefore, the pattern of the mesoderm, may depend on
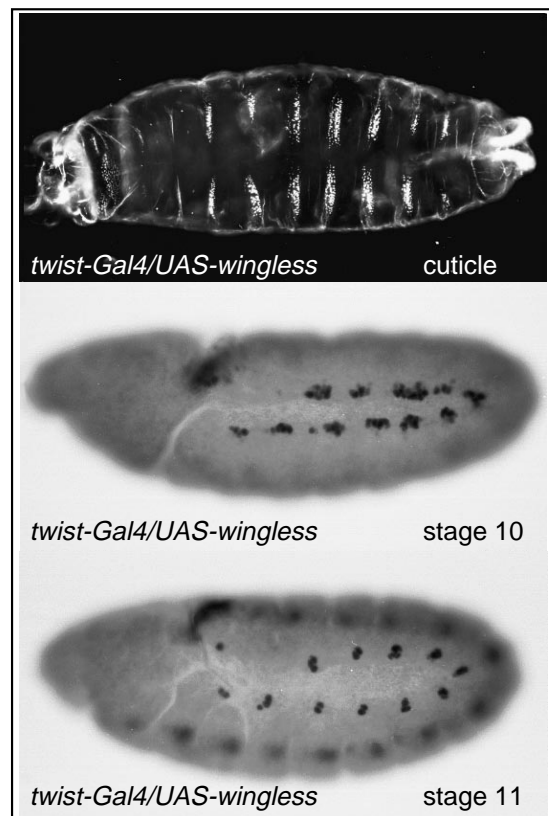

Fig. 5. A 1st stage larval cuticle to show the phenotype induced when *twist-Gal4 + 24B-Gal4* drive *UAS-Wingless*. Note that only the mid-ventral portion shows the 'naked' phenotype, which reverts towards the normal more laterally. The cause of this difference between dorsal and ventral is not known: maybe it is because dorsal ectoderm is not exposed to ectopic Wingless until stage 9, when *wingless*-expressing mesodermal cells complete their dorsalward migration. By contrast, ventral ectoderm contacts mesodermal cells as soon as migration begins (stage 7). Alternatively, the uneven action of the *twist-Gal4* driver may be responsible as it is expressed in the mesectoderm (Staehling-Hampton et al., 1994) and might therefore affect nearby ventral ectoderm more strongly. Below is shown a stage 10 embryo showing the enlarged eve clusters at that stage. However, later, by stage 11, the embryo shows a return to eve clusters that are similar to the wild-type ones (see bottom).

Wingless being provided by both the ectoderm and the mesoderm. The mesoderm's sensitivity to a secreted molecule produced in the ectoderm makes it likely that the molecular mechanisms of segmentation are shared in the two germ layers.

If Wingless induces *eve* expression in dorsal mesodermal cells, one might expect that uniform Wingless expression either in the ectoderm or in the mesoderm could induce, at the outset, a continuous line of eve cells at a dorsoventral position that would be specified independently. But, we find that is not the case: uniform Wingless results in segmentally patterned eve cells, even in *wingless^-* embryos. Therefore Wingless expression is not sufficient to position eve cells along the anteroposterior axis and, moreover, some aspects of the segmental pattern of *eve* expression in the mesoderm could be autonomous to the mesoderm. Thus Wingless could be more permissive than instructive, a view that has been put forward before in the case of ectodermal patterning (Sampedro et al.,

1993). Contrary viewpoints and examples are reviewed elsewhere (Nusse and Varmus, 1992; Perrimon, 1994).

Although segmental clusters of eve cells form when the only source of Wingless is uniform, these clusters are not normal, they are enlarged and extend anteriorly. This may reflect a minor or a major repatterning of the mesoderm, we cannot tell with our limited assay. An analogy with the ectoderm may help. Uniform Wingless in the ectoderm leads to a 'naked' phenotype, a radical departure from the normal alternation of denticles and naked cuticular bands. But the effect of uniform Wingless on *engrailed* expression is relatively minor, giving only a broadening of the stripes (Noordermeer et al., 1992). Thus, it is possible that expansion of the eve clusters is analogous to broadening of *engrailed* expression and that, had we more markers of mesodermal differentiation, we might have uncovered a more profound repatterning. We favour the view that, in the ectoderm, the 'naked' phenotype is a complete repatterning (not merely a matter of substituting naked for denticulate cuticle) and is a consequence of the formation of ectopic parasegment boundaries (Sampedro et al., 1993). We wonder if the same might be occurring in the mesoderm, although we do not yet know how to assay for parasegment boundaries in the mesoderm and how to study fine patterning there.

Uniform mesodermal expression of Wingless apparently results in conflicting influences from the two germ layers. We expected continuously supplied Wingless within the mesoderm to lead to a cryptic 'naked' phenotype there (enlarged clusters of eve cells) even though the overlying ectodermal pattern is relatively normal in this region. But the behaviour of eve cells under these conditions appears indecisive. They begin by showing a response to uniform expression of Wingless (enlarged clusters) and then regulate back towards the normal, despite continuing Wingless overexpression in the mesoderm. This might suggest that anteroposterior patterning of the ectoderm does affect the mesoderm later on, as happens with the dorsoventral patterning (Staehling-Hampton et al., 1994; Frasch, 1995).

Thus, we have shown that the mesoderm can pattern itself autonomously; however it may never be free from ectodermal influences, first because it shares at least one secreted molecule with the ectoderm (Wingless) and any molecule that is secreted may well affect the adjoining cells below. Second, because there may be as yet unidentified interactions to ensure registration of segmentation in the two germ layers.

REFERENCES

Azpiazu, N. and Frasch, M. (1993). *tinman* and *bagpipe*: two homeo box genes that determine cell fates in the dorsal mesoderm of Drosophila. *Genes Dev.* **7**, 1325-40.

Basler, K. and Struhl, G. (1994). Compartment boundaries and the control of Drosophila limb pattern by hedgehog protein. *Nature* **368**, 208-14.

Bate, M. (1993). The mesoderm and its derivatives. In *The Development of Drosophila melanogaster (ed.* M. Bate and A. Martinez Arias). pp. 1013-1090. Cold Spring Harbor Laboratory Press.

Bate, M. and Rushton, E. (1993). Myogenesis and muscle patterning in Drosophila. *C. r. hebd. Acad. Sci Paris* **316**, 1055-1061

Baylies, M.K., Martinez-Arias, A. and Bate, M. *wingless* is required for the formation of a subset of muscle founder cells during Drosophila embryogenesis. *Development* **121**.

Bodmer, R. (1993). The gene tinman is required for specification of the heart and visceral muscles in Drosophila. *Development* **118**, 719-29.

Bodmer, R. (1995). Heart development in Drosophila and its relationship to vertebrates. *Trends in Cardiovascular Med.* **5**, 21-28.

Bour, B. A., O'Brien, M. A., Lockwood, W. L., Goldstein, E. S., Bodmer, R., Taghert, P. H., Abmayr, S. M. and Nguyen, H. T. (1995). Drosophila MEF2, a transcription factor that is essential for myogenesis. *Genes Dev.* **9**, 730-41.

Brand, A. H. and Perrimon, N. (1993). Targeted gene expression as means of altering cell fates and generating dominant phenotypes. *Development* **118**, 401-415.

Capdevila, J., Estrada, M. P., Sanchez, H. E. and Guerrero, I. (1994). The Drosophila segment polarity gene *patched* interacts with decapentaplegic in wing development. *EMBO J.* **13**, 71-82.

Dohrmann, C., Azpiazu, N. and Frasch, M. (1990). A new Drosophila homeo box gene is expressed in mesodermal precursor cells of distinct muscles during embryogenesis. *Genes Dev.* **4**, 2098-111.

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.

Garcia-Bellido, A. (1975). Genetic control of wing disc development in Drosophila. In *Cell Patterning (CIBA Foundation Symp).* (ed. R. Porter and K. Elliott), vol. **29** pp. 161-182. Amsterdam/New York: Elsevier.

Greig, S. and Akam, M. (1993). Homeotic genes autonomously specify one aspect of pattern in the Drosophila mesoderm. *Nature* **362**, 630-2.

Kassis, J. A., Noll, E., VanSickle, E. P., Odenwald, W. F. and Perrimon, N. (1992). Altering the insertional specificity of a Drosophila transposable element. *Proc. Natl Acad. Sci. USA* **89**, 1919-1923.

Kornberg, T. (1981). Compartments in the abdomen of Drosophila and the role of the *engrailed* locus. *Dev. Biol.* **86**, 363-372.

Lawrence, P. A. (1992). *The Making of a Fly.* Oxford: Blackwell.

Lawrence, P. A. and Johnston, P. (1984). On the role of the *engrailed⁺* gene in the internal organs of Drosophila. *EMBO J.* **3**, 2839-2844.

Lawrence, P. A. and Johnston, P. (1989). Pattern formation in the *Drosophila* embryo: allocation of cells to parasegments by *even-skipped* and *fushi tarazu*. *Development* **105**, 761-767.

Lawrence, P. A., Johnston, P. and Vincent, J.-P. (1994). Wingless can bring about a mesoderm-to-ectoderm induction in Drosophila embryos. *Development* **120**, 3355-3359.

Martinez-Arias, A. and Lawrence, P. A. (1985). Parasegments and compartments in the *Drosophila* embryo. *Nature* **313**, 639-642.

Michelson, A. M. (1994). Muscle pattern diversification in Drosophila is determined by the autonomous function of homeotic genes in the embryonic mesoderm. *Development* **120**, 755-768.

Morata, G. and Lawrence, P. A. (1975). Control of compartment development by the *engrailed* gene in Drosophila. *Nature* **255**, 614-617.

Noordermeer, J., Johnston, P., Rijsewijk, F., Nusse, R. and Lawrence, P. A. (1992). The consequences of ubiquitous expression of the *wingless* gene in the Drosophila embryo. *Development* **116**, 711-9.

Nusse, R. and Varmus, H. E. (1992). *Wnt* genes. *Cell* **69**, 1073-1087.

Nüsslein-Volhard, C. and Wieschaus, E. (1980). Mutations affecting segment number and polarity in Drosophila. *Nature* **287**, 795-801.

Perrimon, N. (1994). The genetic basis of patterned baldness in Drosophila. *Cell* **76**, 781-4.

Sampedro, J., Johnston, P. and Lawrence, P. A. (1993). A role for *wingless* in the segmental gradient of Drosophila? *Development* **117**, 677-87.

Staehling-Hampton, K., Hoffmann, F. M., Baylies, M. K., Rushton, E. and Bate, M. (1994). *dpp* induces mesodermal gene expression in Drosophila. *Nature* **372**, 783-6.

Tremml, G. and Bienz, M. (1989). An essential role of *even-skipped* for homeotic gene expression in the Drosophila visceral mesoderm. *EMBO J.* **8**, 2687-2693.

Van den Heuvel, M., Nusse, R., Johnston, P. and Lawrence, P. (1989). Distribution of the w*ingless* gene product in *Drosophila* embryos: a protein involved in cell-cell comunication. *Cell* **59**, 923-931.

Vincent, J.-P. and Lawrence, P. A. (1994). Drosophila *wingless* sustains *engrailed* expression only in adjoining cells: evidence from mosaic embryos. *Cell* **77**, 909-915.

Wu, X., Golden, K. and Bodmer, R. (1995). Heart development in Drosophila requires the segment polarity gene *wingless*. *Dev. Biol.* **169**, 619-628.