

Autonomy and non-autonomy in *Drosophila* mesoderm determination and morphogenesis

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SUMMARY

The mesoderm in *Drosophila* invaginates by a series of characteristic cell shape changes. Mosaics of wild-type cells in an environment of mutant cells incapable of making mesodermal invaginations show that this morphogenetic behaviour does not require interactions between large numbers of cells but that small patches of cells can invaginate independent of their neighbours' behaviour. While the initiation of cell shape change is locally autonomous, the shapes the cells assume are partly determined by the individual cell's environment. Cytoplasmic transplantation

experiments show that areas of cells expressing mesodermal genes ectopically at any position in the egg form an invagination. We propose that ventral furrow formation is the consequence of all prospective mesodermal cells independently following their developmental program. Gene expression at the border of the mesoderm is induced by the apposition of mesodermal and non-mesodermal cells.

Key words: gastrulation, *twist*, *snail*, mosaic analysis, germ layers, pattern formation, induction

INTRODUCTION

The invagination of the mesoderm in *Drosophila* begins with the formation of the ventral furrow, which is driven by a series of cell shape changes. During furrow formation the most ventral subpopulation of prospective mesoderm cells constrict their apical sides while the remaining mesodermal cells follow into the furrow without these shape changes (Leptin and Grunewald, 1990; Sweeton et al., 1991). The shape changes in the individual cells of the ventral furrow (or of any other epithelial invagination) could either occur in a coordinated fashion, directed by cell interactions, or by the independent activities of single cells which each follow their own developmental program. The latter mechanism implies cell autonomy in the behaviour of the invaginating cells. The ventral cells in the *Drosophila* embryo do not constrict simultaneously but in an apparently stochastic order (Kam et al., 1991; Sweeton et al., 1991) which is more consistent with cell autonomy than with coordination by cell interactions. In contrast, the phenotypes of mutants in two genes affecting gastrulation, *folded gastrulation* (*fog*) and *concertina* (*cta*) (which code for a secreted protein and a G-protein subunit, respectively), suggest a degree of cell interactions of at least certain aspects of ventral furrow formation (Parks and Wieschaus, 1991; Costa et al., 1994). In mutant embryos, the cell shape changes leading to ventral furrow formation occur more slowly and in a less orderly fashion than in wild-type embryos. However, the ventral cells do have the capacity to constrict apically, a furrow is eventually formed and the mesoderm develops normally (Parks and Wieschaus, 1991; Sweeton et al., 1991; Costa et al., 1994).

Whether ventral cells change their shapes and invaginate autonomously, i.e. independently of the activities of their neighbours, or whether interactions between neighbouring cells are required can be distinguished by making mosaics of cells that are able to invaginate in an environment that is incapable of invaginating. Mesoderm development and ventral cell shape changes require the activity of the zygotic genes *twist* and *snail*. These genes are expressed ventrally, in the prospective mesoderm, under the control of maternal positional information (St Johnston and Nüsslein-Volhard, 1992). In embryos mutant for both *twist* and *snail* no furrow forms, no mesoderm develops and the region normally occupied by the prospective mesoderm is indistinguishable from the ventrolateral ectoderm (Simpson, 1983; Leptin and Grunewald, 1990). The phenotype of mutant embryos also indicates that parts of the embryo outside the prospective mesoderm make no major contribution to ventral furrow formation (Leptin and Grunewald, 1990). *snail twist* double mutant embryos are therefore suitable for making mosaics to test the extent of cell autonomy in early mesoderm development.

Cell behaviour can be influenced not only by communication of cells with their neighbours, but also by the mechanical properties of their environment (Hardin and Keller, 1988). Thus the formation of a tube-like invagination on the ventral side of the embryo might require the particular geometry of that part of the egg. Mutant eggs can also be used to study this question. Embryos from mothers mutant for any of the maternal effect genes of the *dorsal* group have no cells with ventral fates and form no ventral furrow. Ventral fates can be re-established in such embryos by the injection of wild-type cytoplasm and, in one case (embryos from mothers lacking the

function of the gene *Toll*), ventral fates, gene expression and morphogenetic behaviour are induced ectopically at the site of injection (Anderson et al., 1985; Roth, 1993). We report here experiments using both nuclear transplantations and cytoplasmic transplantations to study the autonomy of cell shape changes and gene expression during early mesoderm development.

MATERIALS AND METHODS

Flies

We used *white*⁻ or OregonR flies as wild-type flies. Flies carrying the *Toll*^{5BRE}, *spz*⁶⁷ and *spz*¹⁹⁷ mutations, the *Df(3R)ro^{XB3}* chromosome and the *snailIG Df(2R)S60 (snail twist)* double mutant chromosome (Arora and Nüsslein-Volhard, 1992) were from the Tübingen stock collection (Tearle and Nüsslein-Volhard, 1987).

Transplantations and processing of embryos

The use of nuclear transplantations for making embryonic mosaics was first used by Lawrence and Johnson (1984) and we used a protocol similar to theirs. Recipient embryos were collected for 30 minute periods, dechorionated with bleach, mounted on glue-coated coverslips, dried for 10-15 minutes and covered with 10S Voltalef oil. They were injected before reaching nuclear cleavage cycle 9. Donor embryos were treated similarly, but they were collected for 2 hours, aged for one hour and not dried. A donor embryo of the appropriate age (late cellularisation; cleavage furrows approx. 60-70% of full depth) was selected under the microscope and its nuclei were aspirated into a microinjection needle (approx. 13 µm inner diameter at the tip). Aliquots of nuclei were injected from the dorsal side into the recipient embryos. The embryos were left to develop until gastrulation, the oil was rinsed off the slide with heptane and the slide inserted into a glass vial filled with a 1:1 mixture of heptane and fixative (4% formaldehyde in PBS). The embryos were fixed for 20 minutes, transferred to methanol for 2 minutes and then into a dish with PBS, and peeled out of their vitelline membranes by hand. The fixed embryos were processed for staining with antibodies or in situ hybridisation and then dehydrated, embedded in Araldite and sectioned (10 µm sections) as described (Leptin and Grunewald, 1990).

25% of the recipient embryos are homozygous mutant. These can be recognized by their phenotype and by the absence of twist staining, and cells of donor origin can be identified by twist expression. Of the *snail twist* mutant embryos, approximately one third contained patches of wild-type cells, ranging in size from 6 to more than 100 cells.

Cytoplasmic transplantations were performed as described previously (Roth, 1993).

RESULTS

Nuclear transplantation chimeras

We made mosaics of wild-type cells in a mutant, non-invaginating background by transplanting wild-type nuclei into *snail twist* double mutant embryos. Irrespective of which region of the donor embryo the nuclei are taken from, they always develop according to the position they occupy in the host embryo. The donor nuclei migrate to the egg cortex together with the host nuclei, and the progeny of those nuclei that populate the ventral region of the host respond to the host's maternal positional information and express mesodermal genes (like *twist*, which we used as a marker for prospective

mesoderm). The injected nuclei participate in the host developmental program and proliferate, generating small clones. Since we did not monitor the number of nuclei injected into each embryo, we cannot be sure whether groups of donor cells are clones and we will therefore refer to them as patches. The smallest patches that we found contained 6-8 cells. The smallest clones expected (if a single nucleus transplanted into a late stage (cycle 9) immediately started to divide) would consist of 16 cells. The fact that our smallest patches consist of fewer cells suggests that the implanted nuclei do not immediately recover and enter the hosts cell cycles.

The injected embryos were left to develop until early gastrulation and were then fixed and stained with antibodies against the twist protein to identify wild-type donor cells in the mutant host embryos. To test the validity of twist as a marker for donor cells in the ventral region, we also stained embryos for an independent marker present in the donor but not in host cells (a β-galactosidase construct expressed in all nuclei at this stage). β-galactosidase in this construct is driven by a ftz-promoter from which certain upstream sequences have been removed (Brown et al., 1991). β-galactosidase is expressed around nuclei before blastoderm formation and continues to be ubiquitously expressed throughout gastrulation. All ventral cells that expressed twist also expressed β-galactosidase (not shown).

Fig. 1 shows examples of invaginating mesoderm patches in mosaic embryos. The twist-stained cells undergo typical ventral cell shape changes including nuclear movement and apical constriction (Fig. 1L). This was true for all cells, as long as they were in the region that would normally be occupied by prospective mesoderm and in which the donor cells therefore expressed twist. Thus, cells constricted even when located in the region that would normally give rise to the peripheral, non-constricting cells (compare Fig. 1F with Fig. 1H,J). We saw apical constrictions, nuclear movement and invaginations even in the smallest patches that we found. In addition, in other situations where the size of the mesoderm primordium is drastically reduced, a small furrow can still be formed. For example,

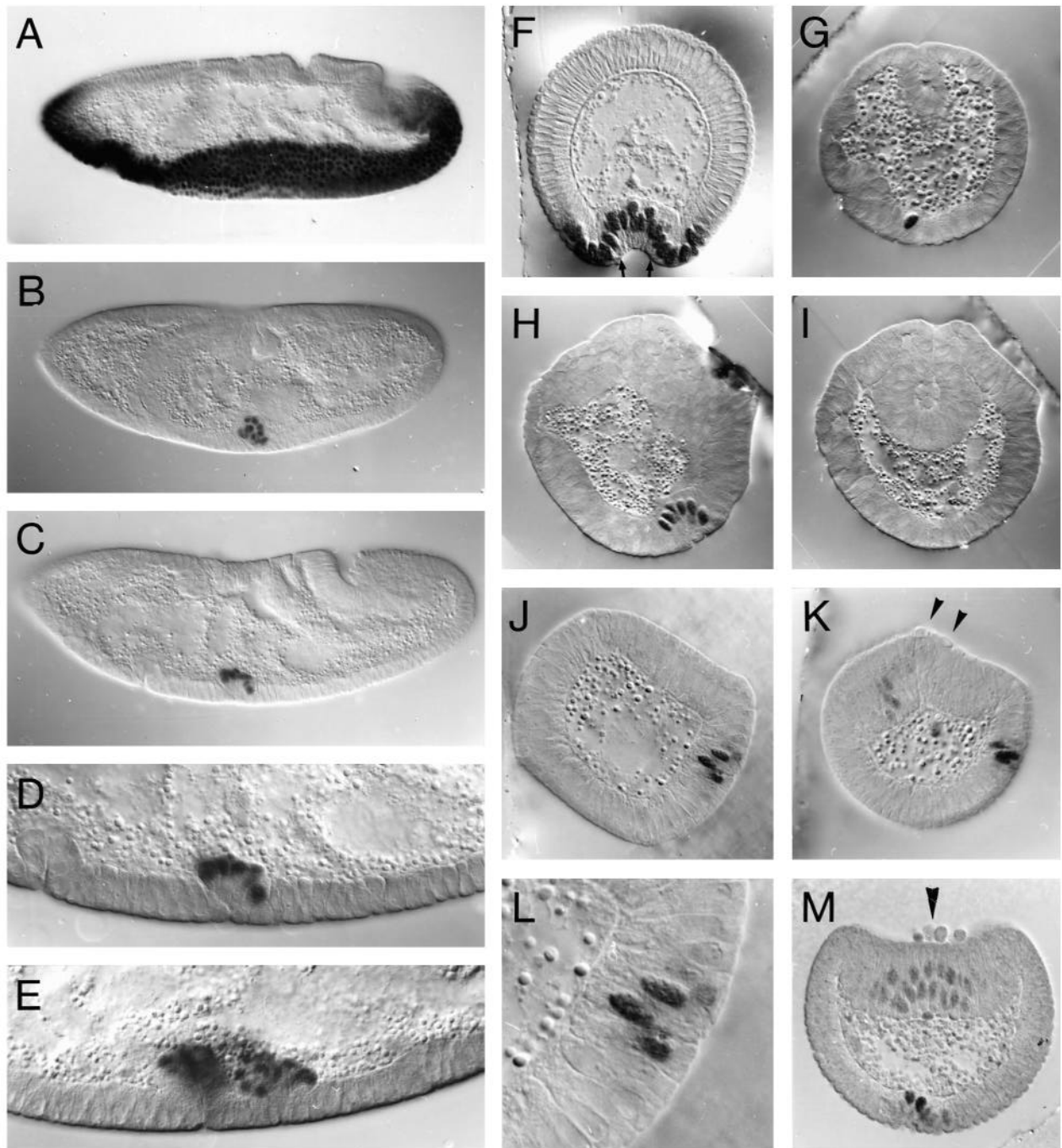
Fig. 1. Invaginations of small patches of prospective mesoderm. (B-E,G-L) Mosaic embryos created by the injection of wild-type blastoderm nuclei into *snail twist* mutant hosts; (M) embryo from mutant *spz*⁶⁷/*spz*¹⁹⁷ mother. (A) Gastrulating wild-type embryo stained with anti-twist antibodies. The mesoderm has invaginated on the ventral side. (B,C) *twist snail* double mutant embryos with patches of wild-type cells forming invaginations. (D,E) Higher magnifications of patches of wild-type cells in mutant hosts. (F) Transverse section of a gastrulating wild-type embryo. The central population of prospective mesoderm cells (between arrows) has constricted apically and their nuclei have moved basally, while the 4-5 more peripheral cells on each side have not undergone these changes. (G-L) Sections of mosaics. The section in H is of the same embryo as that in I, and sections J and K also show sections of the same embryo. In each case, one section (I and K, respectively) shows the orientation of the dorsoventral axis of the embryo by the position of the pole cells (arrow heads). (L) Higher magnification of the patch of invaginating cells in J. Cells in this patch constrict apically, although the patch is located in the region normally occupied by the peripheral, non-constricting prospective mesoderm cells. (M) Section of a gastrulating embryo (non-mosaic) in which the mesoderm has been reduced by genetically altering the *dorsal* gradient (eggs laid by mutant *spz*⁶⁷/*spz*¹⁹⁷ mothers).

if the width of the prospective mesoderm is decreased to one or two cells by alterations in the maternal positional information (Fig. 1M shows a section through an embryo derived from a mother carrying weak mutant alleles of the dorsal group gene *spätzle*), these cells still constrict apically and their nuclei migrate basally. Thus, no large-scale cooperation of mesoderm cells is required to allow invagination, but small foci of cells anywhere in the mesodermal region can initiate invagination by themselves.

Induction of ectopic mesoderm by transplantation of cytoplasm

The results described so far show that individual cells or small

patches of cells undergo the shape changes normally seen during ventral furrow formation and invaginate, even in the absence of a complete ventral furrow. However, these cells were always situated in the region of the egg where the furrow is normally formed. Therefore, they do not preclude the possibility that other parameters of the ventral region of the egg, like its shape, or the properties of the ventral cytoplasm or the underlying yolk, are partly responsible for the invagination of ventral cells (as in frogs, where the yolk in the vegetal hemisphere influences the cell shapes at the invaginating dorsal lip (Gerhart and Keller, 1986; Hardin and Keller, 1988)). The following experiments show that this is not the case. Embryos derived from mothers mutant for the gene *Toll* are dorsalized



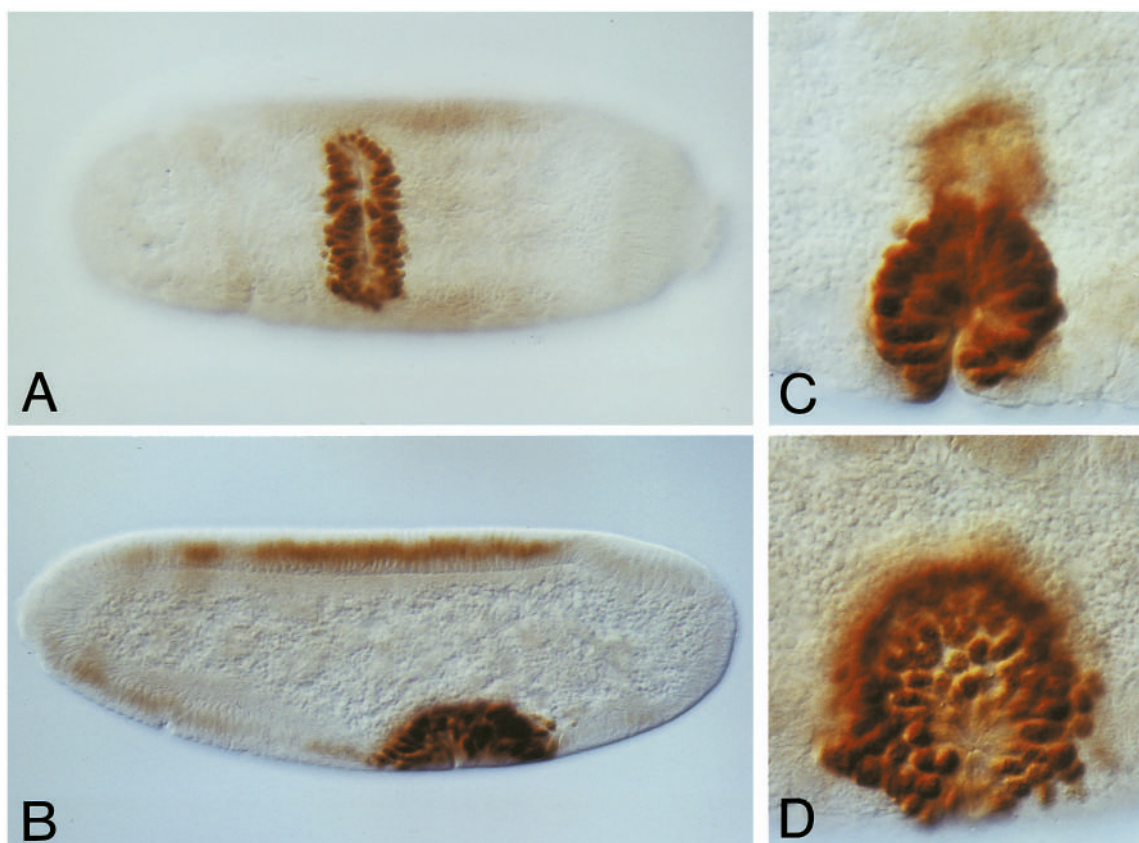


Fig. 2. Mesoderm induced in maternally dorsalized embryos by injection of wild-type cytoplasm. Eggs from mothers mutant for the gene *Toll* ($Tl^{5BRE}/Df(3R)ro^{XB3}$) were injected with cytoplasm from wild-type eggs. The cytoplasm was deposited in various locations and shapes, the embryos were left to develop until the beginning of gastrulation and were then fixed and stained with anti-*twist* antibodies. (A) Dorsal view of an embryo with a furrow along the left-right axis across the dorsal side of the embryo. (B) Lateral view of an embryo with a round invagination on the ventral side, seen in optical cross section. (C,D) Details of two other embryos: a furrow in optical cross section and a pit-shaped invagination.

and have no cells with ventral fates. Ventral fates can be re-established in such embryos by the injection of wild-type cytoplasm, and ventral fates and gene expression are induced ectopically at the site of injection (Anderson et al., 1985; Roth, 1993). Fig. 2 shows such embryos at the stage of ventral furrow formation. We deposited the injected cytoplasm in different shapes and in different places in the embryo. This results in the expression of zygotic ventral genes in the region where the cytoplasm was placed, and the repression of dorsal genes at and around that site (Roth, 1993). The areas expressing ventral genes form invaginations, with the shape of the invagination depending on the shape of ectopic ventral gene expression. Long patches form furrows, which can lie perpendicular to the normal direction of the ventral furrow, and even on the dorsal side of the embryo. Round patches form pit-like invaginations. The cell shapes in these invaginations resemble the shapes in normal ventral furrows. However, unlike most normal ventral furrow cells, or cells in oblong patches, which can begin their apical constriction isometrically but end up wedge-shaped (Sweeton et al., 1991), cells in round invaginations end up cone-shaped. This shows that the final cell shape depends on the shape of the primordium and not on the shape of the embryo. The size and shape of the furrow is only determined by the size and shape of the patch of cells expressing ventral-specific genes. These findings, together with the apparently sto-

chastic behaviour of prospective mesodermal cells (Kam et al., 1991), suggests that each cell can undergo its developmental program, including shape changes, autonomously, but that the final shape of each cell is partly influenced by the cell's neighbours.

Gene expression at the border of the mesoderm

In wild-type embryos, the prospective mesoderm is bordered on each side by a line of mesectodermal cells which express the gene *single-minded* (*sim*; Fig. 3) (Crews et al., 1988). The development of these cells and the expression of *sim* depends on both *twist* and *snail*. No mesectodermal cells are found in *twist snail* double mutants, and *sim* and other mesectodermal genes and enhancer traps are not expressed (Rushlow and Arora, 1990; Kosman et al., 1991; Leptin, 1991). However, patches of wild-type prospective mesoderm cells in *twist snail* mutants are surrounded by a ring of *sim*-expression (Fig. 3). Thus the patch of donor mesodermal cells organizes gene expression patterns resembling the wild-type situation.

We wanted to know whether, like in the wild type, *sim* was expressed in the cells adjacent to the mesoderm (in this case, in the mutant host cells), or in the mesodermal cells (the transplanted wild-type cells). To test this, we used hosts containing a *lacZ* gene controlled by *sim* regulatory elements (Nambu et al., 1990). β -galactosidase from this *sim-lacZ* construct is

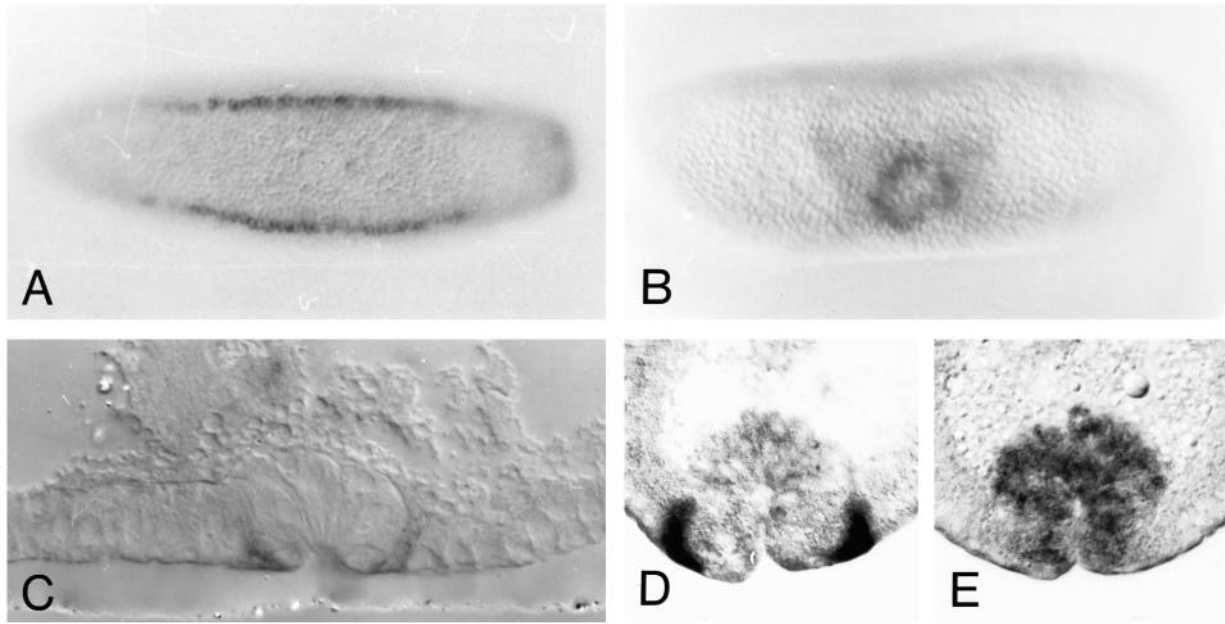


Fig. 3. Mesectodermal gene expression in wild-type and mosaic embryos. (A) *sim* transcripts in a wild-type embryo (ventral view). *sim* is expressed in two lines bordering the prospective mesoderm. (B) *sim* transcripts in a mosaic embryo (ventral view). *sim* is expressed in a ring around the invaginating wild-type donor cells. (C) Sagittal section through an embryo similar to the one shown in B. The region between the *sim*-expressing cells has begun to invaginate. Note that the cells in the center of the invaginating patch have constricted at their apical ends, while those at the edges (next to the *sim*-expressing cells) have not. Although this invagination is sectioned along the anterior-posterior axis of the embryo, it resembles the situation normally seen along the left-right axis. (D,E) Comparison of the distribution of *sim* and *twist* transcripts in a wild-type embryo. Alternating frozen sections of a gastrulating embryo were hybridized with *sim* and *twist* probes. *twist* transcripts are found in the region between the *sim*-expressing cells.

detectable in mesectodermal cells during late germ band extension (and like *sim* itself, it is not expressed in *snail twist* mutant embryos). Mosaics of wild-type cells in mutant hosts containing the β -galactosidase gene should distinguish whether the *sim* expression at the edge of the patch of transplanted cells is within host or donor cells. If it is in donor cells, then these mosaics should not express β -galactosidase. If, however, it is induced by the donor cells in the surrounding host cells, then the mosaic should express β -galactosidase next to the patch of mesoderm. Mosaic embryos doubly stained for *twist* and for β -galactosidase show that the latter is the case. Thus, the wild-type donor cells cause *sim* expression in neighbouring *twist snail* mutant ectodermal cells.

DISCUSSION

Cell shape changes in the central population of prospective mesoderm cells

Our results show that autonomous and non-autonomous processes determine different aspects of cell shape changes during ventral furrow formation. Small patches of cells anywhere in the mesodermal region can constrict apically even when surrounded by non-constricting mutant cells. This autonomous behaviour of small groups of cells argues against the cell shape changes being induced by a trigger (either mechanical, as proposed by Odell et al. (1981), or chemical, in the form of signalling molecules) from a single source within the mesoderm. This might appear to be inconsistent with an

apparent, if very slight, temporal sequence of initiation of apical constrictions in mesoderm cells from the midline of the prospective mesoderm outwards (Costa et al., 1994). Furthermore, it raises the question of how the independently occurring constrictions are timed such that they all happen within a very narrow time window and thus cause an invagination to form. These two problems can both be solved by proposing that the cell shape changes begin as soon as a critical level of some zygotic gene product (*twist* or *snail*, and products of the genes that they control) has accumulated in a cell. Since the expression of ventral genes like *twist* and *snail* is first seen in a much narrower domain than they eventually occupy, with the highest levels nearest the ventral midline (Leptin, 1991), this level would be reached first in the most ventral cells and then in progressively more lateral ones. The hypothesis of such a dose dependence is supported by the phenotype of embryos with only half the wild-type amount of *twist* protein, which begin to form a ventral furrow several minutes later than wild-type embryos (unpublished observations). It does not touch upon the role of the gene *folded gastrulation* (*fog*) in regulating the timing of apical constrictions, nor is the autonomy of cell behaviour suggested by our experiments incompatible with the non-autonomy suggested by the *cta* and *fog* mutant phenotypes and protein products. We propose that each mesodermal cell is able to constrict autonomously, while *fog* functions in a non-autonomous way after the first cells have begun their apical constrictions to accelerate apical constrictions in the remaining cells (Costa et al., 1994).

While the process of shape change seems to be autonomous

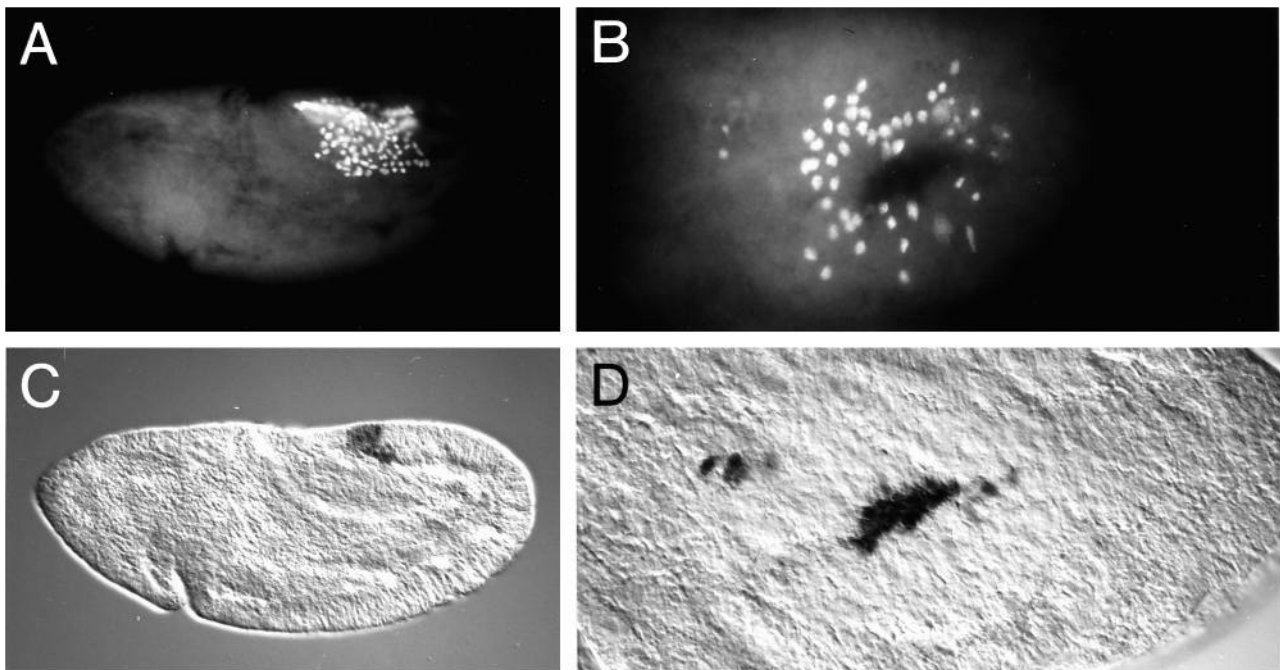


Fig. 4. Expression of β -galactosidase as a marker for mesectodermal cells in mosaics: mesectodermal cell fates are induced in the host by adjacent donor cells. Wild-type nuclei were injected into *snail twist* mutant hosts carrying a construct that directs β -galactosidase expression in mesectodermal cells (under the control of the *sim* promoter). Since β -galactosidase under this promoter can only be detected during late germ band extension, the embryos shown here had to be left to develop much longer than those shown in the previous figures. At the stages shown, the mesoderm has already invaginated completely, and the mesectodermal cells have coalesced at the site of invagination. The mosaics were stained with antibodies against twist protein (fluorescent staining) and against β -galactosidase (brown staining). Two examples of mosaic embryos are shown. (A,B) Expression of *twist*. (C,D) Expression of β -galactosidase. (A,C) A whole embryo. The patch of wild-type mesodermal cells has invaginated (A) while the mesectodermal cells expressing β -galactosidase (C) have moved together at the site of invagination and remain at the surface of the embryo. (B,D) Ventral view of another mosaic at higher magnification. The invaginated twist stained cells have spread away from the site of invagination at which the β -galactosidase-stained cells have coalesced. We do not know whether this embryo contained two independent patches of donor cells, or whether one large patch was separated by cell intercalation during germ band extension.

in each cell, the final shape the cells assume is determined partly by their neighbours. When the first cells begin to constrict apically their shapes become conical. However, slightly later, when most cells are constricting and the furrow begins to invaginate, the apical cross-sections of the cells are no longer round, but oblong, so that the cells are wedge-shaped and not conical (Sweeton et al., 1991). If this shape was determined autonomously, the internal forces that constrict each cell must somehow act mainly along the right-left axis of the embryo. Constricting cells in mosaic patches should then also be wedge-shaped. However, they are not (neither in nuclear transplantation mosaics, nor in small round patches of mesoderm induced by cytoplasmic transplantation). The shape of the cells depends on the shape of the invaginating patch. In long patches, the cells are wedge-shaped with their long axis along the long axis of the patch, while in round patches and in very small patches, irrespective of the shape of the patch, they are conical. Thus, the shape of the patch of prospective mesoderm dictates the final shape of the invaginating cells, and also the shape of the invagination. It is worth mentioning that this non-autonomy is not due to the mechanism normally invoked for non-autonomy, namely cell-cell communication via secreted or cell-surface cell signalling molecules, but to the external mechanical forces acting on the constricting cells.

Cell shape changes in peripheral prospective mesoderm cells

In wild-type embryos, the more peripheral cells of the mesoderm do not constrict, but become stretched when the more central cells constrict (Fig. 1F). This could either be for purely mechanical reasons (the force exerted by the central cells might stretch the peripheral cells and not allow them to constrict), or the two subpopulations might have different morphogenetic programs (such that the genes required for these shape changes might not be active in peripheral positions). Several of our findings argue against the latter possibility. First, cells at the edge of invaginating patches sometimes behave slightly differently from cells in the middle of the patch, or from host cells not adjacent to the patch. The apical surfaces of host cells can be slightly stretched towards the invagination (e.g. the *sim*-expressing cells in Fig. 3C), and donor cells at the edge of a patch are sometimes less (or not at all) constricted (e.g. the cells just inside the *sim*-expressing cells in Fig. 3C). It is especially important to point out that these basally expanded donor cells in the sagittal section in Fig. 3C are at the anterior and posterior, and not the lateral edges of the patch, and therefore not in the position where wild-type peripheral cells would normally be. This suggests that their stretched shape might be caused by the constrictions of the neighbour-

ing cells. Second, wild-type donor patches situated in the region normally occupied by the peripheral population of mesoderm cells invaginate with the same shape changes normally seen only in the central cell population (Fig. 1H,J). Thus all mesodermal cells, in both central and peripheral mesodermal positions, seem to be capable of constricting apically and invaginating. In the wild type, the stretched shape of the peripheral cells is then probably a passive result of the constriction of central cells (these cells express most mesodermal genes earlier than the peripheral cells, and are therefore probably slightly ahead in their development).

Gene expression at the border of the mesoderm

It is interesting that host cells adjacent to furrows are never incorporated into the furrow, no matter what the size of the patch. This indicates that, even if they are initially stretched towards the invagination, non-mesodermal cells are eventually prevented from entering it. It is possible that the mesectodermal cells at the boundary between mesoderm and ectoderm have special properties (e.g. adhesive properties) that limit the invagination. This would also suggest that the borders of mosaic patches have properties similar to the borders of the normal mesoderm. Consistent with this, we find expression of the gene *sim* around the mosaic patches. It is worth emphasizing that, because of the circular shape of the *sim* line, part of it runs along the maternal *dorsal* gradient, rather than at one particular level of it, as it would in the wild type. A cell can therefore express *sim* at various different concentrations of *dorsal*, as long as it is at the edge of a patch of mesoderm cells.

We cannot say whether the expression of *sim* is caused by classical induction involving cell communication across cell membranes, or by diffusion of factors (possibly the twist protein itself) from mesodermal into ectodermal cells during precellular stages. In either case, it is the apposition of mesodermal and ectodermal cells that induces the domain of *sim* expression in these mosaics.

CONCLUSION

In summary, we have demonstrated both autonomy and non-autonomy in early mesoderm differentiation and morphogenesis. Mesodermal cells have the capacity to cause neighbouring non-mesodermal cells to express specific mesectodermal genes. What the nature of the interaction between mesoderm and mesectoderm is remains to be seen.

We propose that all mesodermal cells independently follow their mesodermal differentiation program, involving the transcription of specific genes under the control of the transcription factors *dorsal*, *twist* and *snail*. As a result, cells can independently undergo the cell shape changes characteristic of early mesoderm morphogenesis. The sum of many independent shape changes then results in the formation of the furrow. However, non-autonomous mechanisms are superimposed on this autonomous behaviour. The final shapes of central as well as peripheral mesodermal cells are partly determined by mechanical forces from neighbouring cells, and cell signalling mechanisms (as suggested from the phenotypes of mutants in the *fog* and *concertina* genes (Parks and Wieschaus, 1991;

Sweeton et al., 1991; Costa et al., 1994)) may be used to speed up the process of furrow formation and to make it less prone to errors.

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