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Induction of identified mesodermal cells by CNS midline progenitors in *Drosophila*

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SUMMARY

The *Drosophila* ventral midline cells generate a discrete set of CNS lineages, required for proper patterning of the ventral ectoderm. Here we provide the first evidence that the CNS midline cells also exert inductive effects on the mesoderm. Mesodermal progenitors adjacent to the midline progenitor cells give rise to ventral somatic mucles and a pair of unique cells that come to lie dorsomedially on top of the ventral nerve cord, the so-called DM cells. Cell ablation as well as cell transplantation experiments indicate that formation of the DM cells is induced by midline progenitors in the early embryo. These results are corroborated by genetic analyses. Mutant *single minded* embryos lack the CNS midline as well as the DM cells. Embryos mutant for any of the spitz group genes, which primarily express defects in the midline glial cell lineages, show reduced formation of the DM cells. Conversely, directed overexpression of secreted SPITZ by some or all CNS midline cells leads to the formation of additional DM cells. Furthermore we show that DM cell development does not depend on the absolute concentration of a local inductor but appears to require a graded source of an inducing signal. Thus, the *Drosophila* CNS midline cells play a central inductive role in patterning the mesoderm as well as the underlying ectoderm.

Key words: *Drosophila*, mesoderm, cell fate, CNS midline, induction, DER signaling pathway

INTRODUCTION

The *Drosophila* embryo is initially subdivided by dorsal/ventral patterning genes into several discrete domains (e.g. reviewed in Anderson, 1987; Roth et al., 1989; Govind and Steward, 1991; St Johnston and Nüsslein-Volhard, 1992). The ventral domain of the blastoderm stage embryo represents the mesodermal anlagen. The ventrolateral domain on either side forms the neuroectoderm, which is separated from the mesoderm by a single row of cells, called mesectoderm (Poulson, 1950; Thomas et al., 1988; Crews et al., 1988). The early specification of both the mesectodermal and the mesodermal domains requires the transcriptional regulators TWIST and SNAIL (Rushlow and Arora, 1990; Kosman et al., 1991). Upon gastrulation, *twist* expression is confined to the mesoderm. It is required for the expression of other mesodermal genes (Thisse et al., 1987; Bodmer et al., 1990; Leptin, 1991) and probably acts as a key factor in initiating subsequent mesodermal differentiation (for reviews see Bate, 1993; Baylies and Bate, 1996).

First patterning of the mesoderm, establishing the primordia of the somatic and visceral muscles, the heart and the fat body, is mediated by genes like *tinman*, *bagpipe* and *serpent*. Their restricted expression in the mesoderm appears to involve the function of segmentation genes (Azpiazu et al., 1996). During early stages at the onset of gastrulation, however, mesodermal cells invaginating through the ventral furrow do not yet seem to be committed to form particular mesodermal derivatives (Beer et al., 1987). Thus, the specification of the various mesodermal cell fates could result from an autonomous self-organizing capacity of the mesoderm itself and/or it could be non-autonomously provided by other tissues during early stages.

The latter is supported by transplantation experiments, which suggest that the positions occupied by the mesodermal cells following invagination and subsequent dorsal movement across the inner surface of the ectoderm are critical for their determination (Beer et al., 1987). Position-dependent differentiation of mesodermal cells could be evoked by inductive signals from the ectoderm. Indeed, recent data have provided evidence for ectodermal signals controling various aspects of mesodermal patterning (Staehling-Hampton et al., 1994; Baylies et al., 1995; Frasch, 1995; Baker and Schubiger, 1995; Bate and Baylies, 1996; Ranganayakulu et al., 1996).

An alternative source for inductive signals patterning the mesoderm could be the mesectodermal cells, which give rise to the CNS midline cells (Klämbt et al., 1991; Bossing and Technau, 1994). During gastrulation the two rows of mesectodermal cells become juxtaposed along the ventral midline while still being in direct contact with cells of the mesodermal anlage. The gene *single minded* (*sim*) acts as a master regulatory gene of CNS midline development, and in *sim* mutant embryos midline cells fail to differentiate (Thomas et al., 1988;

Crews et al., 1988; Nambu et al., 1991). In addition *sim* is required for proper patterning of the ventral ectoderm. Since *sim* is not expressed in this tissue the establishment of ventral ectodermal cell fates appears to require an inductive signal originating from the CNS midline cells (Kim and Crews, 1993). Based on its characteristic ventral cuticle phenotype, *sim* has been placed together with *sichel*, *rhomboid*, *Star*, *pointed* and *spitz* in the so-called spitz group (Mayer and Nüsslein-Volhard, 1988). Mutations in all spitz group genes also lead to defects in CNS midline development (Klämbt et al., 1991; Sonnenfeld and Jacobs, 1994). Ventral ectodermal cell fates are specified by differential activation of the Drosophila EGF receptor homologue (DER) through specific ligands (Raz and Shilo, 1993; Schweitzer et al., 1995a). *spitz* encodes such a DER-activating ligand, and recent experiments suggest that the midline cells can provide SPITZ protein required for the correct patterning of the ventral ectoderm (Golembo et al., 1996b).

To further investigate the question of whether the ventral CNS midline cells also induce aspects of mesodermal patterning, we have employed a combination of experimental and genetic tools. We have focused our analyses on the formation of a special set of mesodermal cells, the dorsal median (DM) cells. The DM cells (described as transverse nerve exit glia by Gorczyca et al., 1994) are arranged as a pair in each of the three thoracic and in the first seven abdominal segments medially on the dorsal surface of the ventral nerve cord and close to the segmental boundaries (Fig. 3A). They send cell processes laterally to muscle attachment sites, which pioneer and enwrap a set of motoneuronal axons exiting from the dorsal midline of the CNS to form the transverse nerve on either side (Osborne, 1964; Beer et al., 1987; Chiang et al., 1994; Gorczyca et al., 1994; Dunin Borkowski et al., 1995). In the thoracic segments, during larval development, the DM cells lose their lateral extensions to form one dorsal neurohemal organ per thoracic segment (Gorczyca et al., 1994; R. Cantera, personal communication), which expresses the hormone allatotropin (Zitman et al., 1993) and becomes innervated by fibers containing FMRFamide-like peptide (White et al., 1986; Schneider et al., 1991).

We show that in the early embryos a single mesodermal progenitor cell adjacent to the CNS midline progenitors gives rise to a segmental pair of DM cells. The formation of these mesodermal cells is controled by inductive signals from the CNS midline cells. Overexpression of *spitz* in all midline cells induces the formation of supernumerary DM cells by triggering additional mitoses of the DM progenitor cells. Transplantation of midline cells, which express high levels of SPITZ into the midline of wild-type embryos, results in an even more prominent induction of extra DM cells. Our experiments suggest that patterning of the mesoderm requires the presence of gradients, and the steepness of the gradient eventually regulates the formation of the correct number of DM cells.

MATERIALS AND METHODS

Drosophila strains

A reporter strain harbouring the P[*btn* 1.85-*lacZ*] constuct was used to trace the fate of the DM cells (Chiang et al., 1994; kindly provided by P. Beachy). The following mutant lines have been used: sim^{H9} (Hilliker et al., 1980; kindly provided by S. Crews); btn^{r3} (Chiang et al., 1994; kindly provided by P. Beachy); flb^{IK35} (Schejter and Shilo, 1989); $spitz^{IIA14}$ (Nüsslein-Volhard et al., 1984), $Star^{126}$ (Kolodkin et al., 1994); pnt^{rM254} ; $pnt^{\Delta 114}$ (Klämbt, 1993); rho^{del1} (Sturtevant et al., 1996; kindly provided by E. Bier); Df(1)KA14 uncovering *otd* (Finkelstein and Perrimon, 1990); $cycA^{3}$ (Lehner and O'Farrell,1989; kindly provided by C. Lehner). Homozygous mutant embryos were identified by the absence of 'blue balancer' chromosomes. For ectopic expression of *sspi* we employed the GAL4 system (Brand and Perrimon, 1993). The following GAL4 inducer and UAS lines were used: *sim*-GAL4 (Scholz *et al*. 1997); *rho*-GAL4 (kindly provided by M. Levine); UAS-*sspi*4a (Schweitzer et al., 1995a; kindly provided by B. Shilo).

DiI labelling

Embryos were mounted ventral side down on coverslips, dessicated, covered with fluorocarbon oil and placed on an inverted microscope equipped with a micromanipulator (Leica). DiI labelling as well as processing and photoconversion of flat preparations were performed as previously described (Bossing and Technau, 1994; Bossing et al., 1996). Individual mesodermal primordial cells were labelled with DiI during gastrulation, shortly before the mesectodermal cells meet at the ventral midline (stage 6). Mesodermal cells that were among the latest to invaginate into the ventral furrow were labelled. Subsequently, upon completion of gastrulation (stage 7/8), their position relative to the midline progenitors (which are clearly identifiable at this stage, see below) was determined under the fluorescent microscope. Only those labelled cells that were clearly located adjacent to the midline cells (see Fig. 1D) were further analyzed.

Cell ablations

Embryos were mounted ventral side down, as described for DiI labelling. A capillary (tip diameter 6-8 μm) was moved along the surface of the coverslip towards and in line with the ventral furrow of an embryo. The capillary was introduced through the vitelline membrane midventrally from the posterior side at the place where the vitelline membrane is attached to the coverslip. 15-25 minutes after the onset of gastrulation (stage 7/early stage 8; stages according to Campos-Ortega and Hartenstein, 1985) the two rows of mesectodermal midline cells are juxtaposed at the ventral midline, and their cell diameters are significantly increased, as they will divide shortly afterwards (see Bossing and Technau, 1994). Thus cell ablations were performed at this stage, when the midline progenitor cells were clearly distinguishable *in vivo* from neighbouring neuroectodermal or mesodermal cells by their position, size and shape (magnification used: 50×10). The capillary was carefully moved along the ventral midline and all midline progenitors within 1-2 segmental anlagen were selectively removed, leaving their neuroectodermal and mesodermal neighbours in place. Upon the removal of midline cells it was verified that the neighbouring cells remained intact. At late embryonic stages (stage 16) specimens were stained for various markers and processed as described below.

Cell transplantations

Donor and host embryos were mounted ventral side down. Donors were labelled with HRP, as previously described (Prokop and Technau, 1993). Midline cells were selectively removed with a capillary (tip diameter 12 μm) from donors at stage 7, as described above. Depending on the type of experiment one or several cells were transplanted into the midline of hosts at the same stage. In one experiment hosts were at a later stage, as indicated in the text. For implantation the capillary was oriented perpendicular to the midline of host embryos to minimize spatial distortions. Precise position and number of implanted cells were determined under direct optic control. At late embryonic stages (stage 16) hosts were stained and processed as described below.

Antibodies and staining

In addition to the P[*btn 1.85 lacZ*] transformant strain (Chiang et al., 1994; kindly provided by P. Beachy), antibodies against glutactin (Olson et al., 1990) or collagen IV (Murray et al., 1995; both kindly provided by L. Fessler and J. Fessler) were used for tracing DM cells, and BP102 antibody (kindly provided by C. S. Goodman) for staining CNS fiber tracts. Anti-β-galactosidase antibodies (Dianova) or X-Gal were utilized for staining embryos of the P[*btn 1.85 lacZ*] strain or to detect blue balancer chromosomes. Antibody, HRP and X-Gal single or double stainings were performed on flat preparations as previously described (Schmidt-Ott and Technau, 1992: Bossing et al., 1996).

RESULTS

The pattern of the somatic muscles and other mesodermal derivatives has been described in detail (for a review, see Bate, 1993). However, except for an assignment of mesodermal cells to a metamerically repeated anterior-posterior subdivision (Dunin Borkowski et al., 1995), a fate map of the mesodermal anlage is still lacking. Assuming that possible inductive signals from the mesectoderm would most significantly affect mesodermal cells close to the midline, we attempted to trace the fate of mesodermal cells that are in direct contact with the midline cells.

Mesodermal progenitors adjacent to the ventral midline give rise to dorsal median (DM) cells and ventral somatic muscles

Single mesodermal primordial cells were labelled with DiI during gastrulation, just before they move into the ventral furrow. Labellings were performed within the region of segment anlagen T2 to A3. Upon completion of ventral furrow formation, when the mesectodermal cells can be clearly identified at the ventral midline, the position of the labelled cell with respect to the midline cells was checked (Fig. 1D). The progeny of mesodermal cells that were located adjacent to the midline were further traced into late embryonic stages (stage 16) and subjected to photoconversion to analyze the structures formed by these cell clones in fixed preparations (n=29). These ‘first row’ mesodermal cells gave rise to three types of structures (Fig. 1A-C): ventral somatic muscles (n=16), cells associated with the salivary glands (presumably fat cells, n=8) and the dorsal median (DM) cells (n=5). Most of the muscle clones obtained from the ‘first row’ mesodermal progenitors belonged to the ventral longitudinal group and ventral oblique group (nomenclature according to Bate, 1993). Clonal progeny cells participated in the formation of up to five muscles in different combinations.

Each pair of DM cells derives from one mesodermal progenitor

DiI labellings indicate that each segmental pair of DM cells derives from one mesodermal progenitor cell. This agrees with previous data obtained from lineage analysis on mesodermal cells using HRP as a lineage tracer (Beer et al., 1987). Furthermore, the clonal analysis reveals that, in addition to the DM cells, the progenitor cell gives rise to further progeny, which participate in the formation of ventral somatic muscles.

In one case, in which we simultaneously labelled a midline progenitor and an adjacent mesodermal progenitor cell, we obtained a clone of ventral unpaired median (VUM) neurons (an interneuron and a motoneuron; see also Bossing and Technau, 1994) in the CNS midline, and a mesodermal clone consisting of DM cells and a group of ventral somatic muscles (Fig. 1C). This shows that the DM cell progenitor is adjacent to the midline and suggests that it maps near the VUM progenitors (for the arrangement of midline progenitors, see Klämbt et al., 1991; Bossing and Technau, 1994).

Spatiotemporal aspects of DM cell development

In embryos that lack the function of *cyclin A* mitosis is arrested during the 16th cell cycle (Lehner and O’Farell,

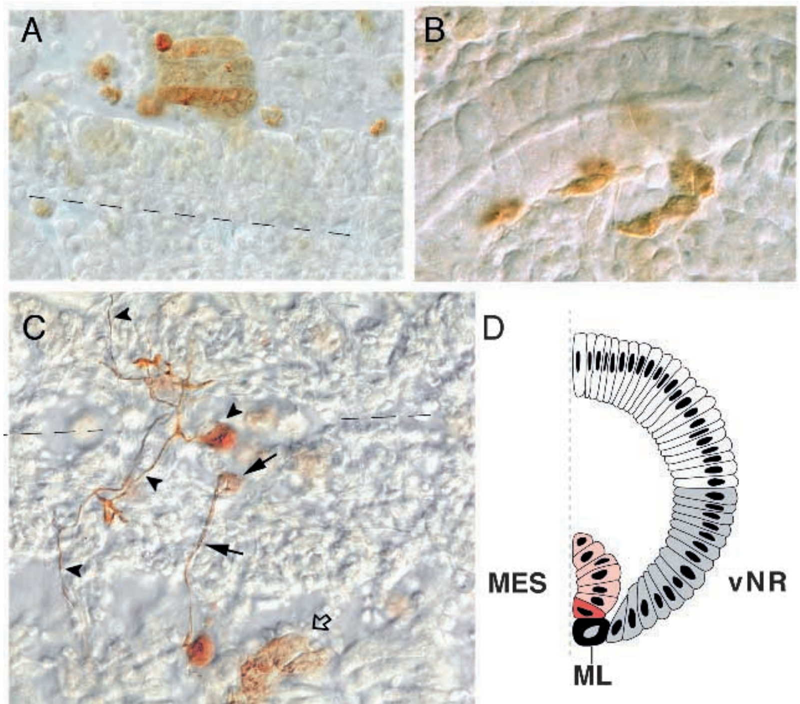

Fig. 1. The fate of mesodermal progenitor cells adjacent to the ventral midline. Progenitor cells were individually labelled with DiI during stage 6 and their position adjacent to the midline was confirmed at stage 7/8 (see below). Examples of the three main types of clones obtained from these cells are shown in flat preparations of embryos at stage 16 upon photoconversion of the dye. The clone shown in (A) participated in the formation of somatic muscles belonging to the ventral longitudinal group. The clone shown in (B) is located close to the salivary gland and presumably consists of fat cells. Cells in (C) derive from two simultaneously labelled progenitor cells: a CNS midline progenitor, which gave rise to a VUM clone consisting of a motoneuron and an interneuron with bilateral projections (arrowheads; see also Bossing and Technau, 1994); an adjacent mesodermal progenitor cell, which gave rise to DM cells (arrows point to one DM cell on top of the ventral cord; the second DM cell is probably dislocated), as well as progeny cells participating in the formation of somatic muscles belonging to the ventral oblique group (open arrow). Different focal planes were digitally montaged with Adobe Photoshop. Broken line in (A,C) marks the CNS midline. (D) Diagram of a half cross-section through the gastrula embryo at stage 7, demonstrating the spatial arrangement of the mesoderm (MES, red), midline cells (ML, black) and ventral neuroectoderm (vNR, grey). Mesodermal progenitor adjacent to the midline is marked (dark red).

1989). Nevertheless, cells in this mutant are able to differentiate and DM cells carrying bilateral cytoplasmic extensions are clearly identifiable in late embryos (Fig. 5C). In *cyclin A* mutants there is only one DM cell per segment (Figs 4, 5C) suggesting that in the wild type the DM cells appear in the lineage after the second postblastodermal mitosis. To reveal the spatiotemporal pattern of DM cell development in some more detail we used a reporter strain harbouring the P[*btn 1.85 lacZ*] construct, which confers β-galactosidase expression specifically in the DM cells (Fig. 2; Chiang et al., 1994). Expression of the marker is first detectable at early stage 11 in a cluster of 2-4 cells at the midline on top of the CNS (Fig. 2A; see also Chiang et al., 1994). One of these cells expresses the marker significantly more strongly than the others. A short time later (stage 11) this cell appears to divide (Fig. 2B), and the strongly labelled progeny subsequently differentiate into the DM cells (Fig. 2C,D). During their entire development these cells stay close to the CNS midline. In contrast, the weakly labelled mesodermal cells migrate laterally during stage 13 (Fig. 2C) and subsequently participate in the formation of ventral somatic muscles (Fig. 2D). These cells probably correpond to those sharing the same lineage with the DM cells (see above).

DM cell formation requires the function of *single minded*

The *single minded* gene encodes a basic HLH transcription factor and acts as a master regulator of CNS midline development (Nambu et al., 1991). In *sim* mutant embryos the midline progenitor cells fail to divide and do not migrate in the CNS layer where they normally differentiate into neurons and glia (Nambu et al., 1991). Furthermore, tracing their fate with cell-specific markers suggested that the midline cells do not survive in *single minded* mutant embryos (Sonnenfeld and Jacobs, 1994).

To see whether the DM cells are affected in *sim* mutants we used the P[*btn 1.85 lacZ*] reporter element. In sim^{H9} mutant embryos (sim^{H9} represents a null *sim* allele, Nambu et al., 1991), DM cells are generally missing (except in 8 out of 130 segments; Fig. 3A,B). This was confirmed by staining mutant *single minded* embryos with antibodies against glutactin and collagen IV (Olson et al., 1990; Murray et al., 1995) as independent markers for the DM cells. Thus, expression of *sim* is required for the formation of the DM cells.

DM cells do not form in the absence of midline progenitor cells

sim expression is not strictly confined to the midline cells but is also found in ventral mesodermal progenitor cells (Lewis and Crews, 1994). Therefore, failure of DM cell formation in *sim* mutants could be a non-autonomous as well as an autonomous effect. To distinguish between these possibilities we selectively removed midline progenitors from embryos carrying the P[*btn 1.85 lacZ*] transgene (*n*=22). Midline cells were removed under direct optic control with a capillary shortly after gastrulation, when they are clearly identifiable and distinguishable from neighbouring cells in the living embryo by their position, shape and size (see Materials and Methods). Up to 18 consecutive midline cells were removed, corresponding to the entire population of 2-3 segmental anlagen (number of midline progenitors per segment: 6-9; Bossing and Technau, 1994). In addition to producing a local phenocopy of the CNS defects described for *sim* mutants (absence of commissural axon tracts, fusion of the longitudinal axon tracts and reduced nerve cord condensation; Thomas et al., 1988), we find a clear effect on the formation of the DM cells (Fig. 3C). This effect depends on the number of consecutive midline cells removed from the embryos. Removal of fewer than ten cells had no effect on the formation of DM cells and resulted in only a weak local phenocopy of the *sim* mutant CNS phenotype (*n*=15; Fig. 3D). Upon removal of 12-18 midline progenitor cells one pair of DM cells was lacking (*n*=6), and in one case two pairs of DM cells in consecutive segments were lacking. These data indicate that the midline cells are required for the formation of the DM cells.

Formation of the DM cells is induced by midline cells

To test whether the midline cells are sufficient for the induction of the mesodermal DM cells we performed a series of transplantation experiments. In the first experiment, 3-6 midline progenitors were transplanted from wild-type donors into the midline region of *sim* mutant hosts carrying the P[*btn 1.85 lacZ*] construct (stage 7). Following further development (stage 16) the hosts were stained for β-galactosidase expression. In all cases the implanted wild-type cells led to a local rescue of the *sim* phenotype in that they induced the formation of 2-6 DM cells (*n*=11). These are located dorsally on the ventral cord, although their paired arrangement and lateral processes are disturbed (Fig. 3F). Furthermore, the implanted cells also had rescuing effects on CNS fiber tract formation in otherwise mutant *single minded* embryos (data not shown).

In a second experiment we implanted about ten wild-type midline cells in the CNS midline of wild-type hosts carrying the P[*btn 1.85 lacZ*] construct. These additional midline cells led to the formation of supernumerary DM cells (*n*=13; Fig. 3E). Since transplantation of fewer than five cells was insufficient to induce the recruitment of additional DM cells (*n*=30), we conclude that a certain threshold in the level of a midline-derived inductor is required.

The spitz group genes control DM cell formation

The CNS midline cells induce the formation of the DM cells. Do all midline cells share similar inductive capabilities? To address this question we took advantage of mutations only affecting specific cell types within the developing CNS midline. In contrast to *sim,* mutations in other spitz group genes most prominently affect the midline glial cell lineage. This is most clearly seen in mutant *pointed* embryos. *pointed* encodes an ETS transcription factor, which in the CNS is expressed only in glial cells (Klämbt, 1993). In *pointed* mutant embryos, these glial cells fail to differentiate properly (Klaes et al., 1994).

DM cell formation is affected by all spitz group mutations (Fig. 4). In mutant *pointed* embryos, reduced numbers of DM cells (2-12; compared to 20 DM cells in wild-type embryos) form. In *spi* mutant embryos either no DM cells (in about 30% of the cases) or reduced numbers of 4-12 DM cells per embryo are formed, as revealed with an anti-glutactin or anti-collagen IV antibody. In *Star* mutant embryos DM cell numbers varied between 2 and 6 per embryo, and in *rhomboid* mutant embryos between 6 and 10. These data suggest that the midline glial

cells perform an important function during the induction of the DM cells.

To assay whether the CNS midline neurons contribute to the inductive signals governing the formation of the DM cells, we analysed mutant *orthodenticle* (*otd*) embryos. *otd* encodes a homeodomain protein that is expressed in midline neurones. In *otd* mutant embryos most of the VUM neurons and probably also the median neuroblast (MNB) degenerate (Finkelstein et al., 1990). In addition, the midline glial cells fail to migrate posteriorly, but this may be an indirect effect due to the absence of the VUM neurons (Klämbt et al., 1991). In all *otd* embryos inspected, DM cell numbers corresponded to wild type (*n*=18; Fig. 4). Thus, midline neurones appear to be dispensable with regard to DM cell formation. However, in *otd* mutants not all neuronal midline lineages degenerate. It is thus possible that the midline neurons contribute to the inductive signal required for DM cell formation at earlier developmental stages.

Overexpression of secreted SPITZ by single midline cells leads to the production of additional DM cells

Cell-fate specification in the ventral ectoderm requires the graded activation of DER (*flb*) by secreted SPITZ. The RHOMBOID and STAR proteins may be required for the production or processing of the SPITZ precursor to generate the active, secreted, form (Schweitzer et al., 1995a). It has been recently shown that the midline can be the source of active SPITZ protein required for patterning in the entire ventral ectoderm (Golembo et al., 1996b). The phenotypes described above for *spi*, *S*, *rho* and *pnt* mutant embryos as well as for DER/*flb* mutants (see Fig. 4) are compatible with the hypothesis that the DER signalling pathway may also be involved in the induction of ventral mesodermal cell fates, like the DM cells.

We thus asked whether overexpression of secreted SPITZ in the midline would result in a recruitment of additional DM cells. Towards this goal we have employed the GAL4 system (Brand and Perrimon, 1993) in combination with cell transplantations. In embryos expressing secreted SPITZ in all cells of the ventral midline (*sim-GAL4*/*UAS-spitz*), the number of DM cells is increased to about the same extent as upon implantation of additional wild-type midline cells in the wild type (see above; Fig. 5A).

The increase in DM cell numbers was much more dramatic when we isotopically transplanted midline cells from *sim-GAL4*/*UAS-spitz* donors (stage 7) into the midline of wild-type hosts (same stage; *n*=17). The implantation of only one or two midline cells from these donors is sufficient for the induction of a large number of additional DM cells (Fig. 5B,D).

The supernumerary DM cells found upon transplantation of individual SPITZ-expressing midline cells could result either from conferring the DM fate onto a larger number of progenitor cells within the mesodermal primordium and/or from inducing individual DM cell progenitors to perform additional mitoses. To distinguish between these possibilities we transplanted midline cells overexpressing *spitz* into the midline of embryos that lack the function of *cyclin A*. In such mutant hosts the *spitz*-overexpressing midline cells were not able to induce additional DM cells (*n*=13; Fig. 5C). This suggests that in the wild-type background overexpression of *spitz* in the midline cells or implantation of supernumerary midline cells leads to an increase in DM cell numbers by triggering additional mitoses and not by recruiting additional DM cell progenitors.

No additional DM cells are induced when *spitz*-overexpressing midline progenitors from stage 7 donors (about 20 minutes after onset of gastrulation) are heterochronously transplanted into wild-type hosts in which the midline cells have already divided (about 50 minutes after onset of gastrulation; stage 8). At this stage rearrangement of mesodermal cells has not yet taken place and they still contact the midline (Hartenstein, 1993). Therefore, the ability of mesodermal progenitors to respond to the SPITZ signal appears to have already ceased after the early gastrula stage.

Induction of DM cells depends on local differences in the amount of secreted SPITZ

In the above experiments we noted a difference in the number of DM cells formed upon overexpression of secreted SPITZ in all midline cells versus overexpression of secreted SPITZ in only a single midline cell. The generation of a local source of secreted SPITZ protein within the midline by cell transplantation appeared to be most efficient in inducing DM cell formation. To address the question of whether general expression of secreted SPITZ in the midline as well as in the mesoderm might level out inductive gradients and subsequently block the formation of extra DM cells, we used a modified *rho*-promotor-GAL4 line (kindly provided by M. Levine; Klaes et al., 1994). The expression pattern conferred by this GAL4 line mimics the *rhomboid* expression pattern, i.e. expression in the CNS midline and the ventralmost neuroectodermal region (Bier et al., 1990). However, since SNAIL binding sites have been destroyed in the *rhomboid* promotor fragment used, GAL4 expression is also found in the mesodermal domain (data not shown).

In contrast to the DM cell phenotype of *sim-GAL4/UAS-spitz* embryos, we do not find an increase in DM cell numbers in embryos in which expression of secreted SPITZ is driven by *rho-GAL4*. This result is in line with the above-mentioned model of DM cell formation. To control for secreted SPITZ expression in *rho-GAL4/UAS-spitz* embryos we transplanted one or two midline cells from *rho-GAL4/UAS-spitz* donors into the midline of wild type (*n*=18). In these experiments we observed the same DM cell phenotypes as upon transplantation of midline cells taken from *sim-GAL4/UAS-spitz* donors (see above). Thus we conclude that DM cell formation indeed does not depend on the absolute amount of the SPITZ signal, but rather on local differences in its concentration.

In summary we have presented evidence that the midline cells activate DM cell formation via the generation of local inductive gradient(s). Beside providing instructive signals to the mesoderm, the midline cells appear to be capable of influencing cell divisions in the mesoderm.

DISCUSSION

We have studied mechanisms underlying the formation of a pair of identified mesodermal cells, the so-called DM cells. Genetic and experimental data provide evidence that their formation requires inductive signals from the CNS midline cells. A combination of cell transplantation experiments and missexpression

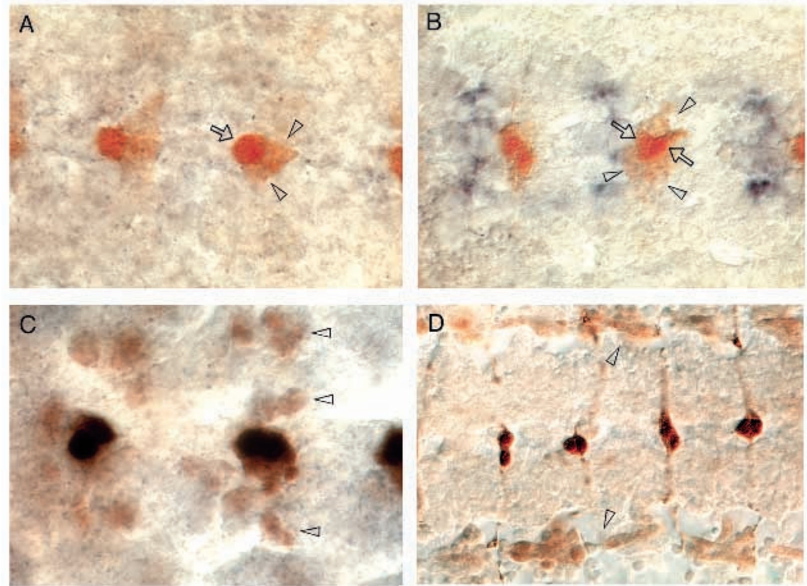

Fig. 2. Sequence of DM cell development. Flat preparations of embryos (anterior to the left) stained for β-galactosidase expression directed by the P[*btn 1.85 lacZ*] reporter construct (Chiang et al., 1994). (A) Expression is first detectable at early stage 11 in a cluster of 2-4 mesodermal cells at the midline on top of the CNS (see also Chiang et al., 1994). One of these cells (arrow) expresses the marker significantly more strongly than the others (arrowheads). (B) By the end of stage 11 this cell appears to divide as two strongly labelled cells are detected (arrows). The preparation is double-stained with BP102 antibody (dark grey), which reveals neuronal fiber formation in the underlying CNS. (C) At stage 13 the weakly labelled cells migrate laterally (arrowheads), whereas the pair of strongly labelled cells remains at the CNS midline. (D) Stage 16 embryo showing strong *btn-lacZ* expression in fully differentiated DM cells and weak expression in a group of ventral somatic muscles (ventral longitudinal and ventral oblique types; arrowheads).

studies suggest that the DER signalling pathway is required at two steps of DM cell formation: firstly it is required to recruit DM progenitor cells from the pool of mesodermal cells, and secondly DER signalling appears to control the subsequent cell divisions of the DM cell progenitor.

DM cells originate from lateral regions of the mesoderm

In a series of DiI single-cell-labelling experiments we traced the fate of the mesodermal progenitors that are located adjacent to the midline. These lateral mesodermal cells were found to give rise to three cell types: ventral somatic muscles, a group of cells located close to the salivary glands (presumably fat cells) and the dorsal median cells (DM cells).

The mesodermal origin of the DM cells has been previously documented (Beer et al., 1987) and they do not express known glial or neuronal markers (e.g. *repo*, *AA142*, *pointed*, *elav*; data not shown). Beside their characteristic morphological properties, the DM cells were shown to be molecularly distinct mesodermal cells, as they express and require the homeobox gene *buttonless* (*btn*) (Chiang et al., 1994) in addition to a number of other markers (neuroglian, Bieber et al., 1989; laminin, Montell and Goodman, 1989; glutactin and collagen IV, Olson et al., 1990; Murray et al., 1995).

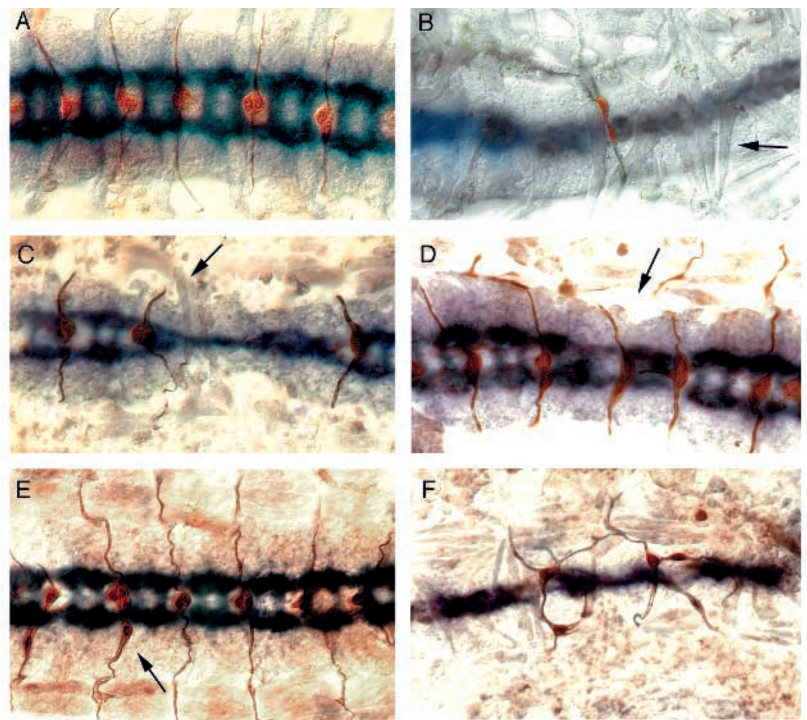

Fig. 3. Formation of the DM cells is induced by midline cells. Flat preparations of embryos at stage 16. DM cells are marked (brown colour) by β-galactosidase expression directed by the P[*btn 1.85 lacZ*] reporter construct, and fiber tracts in the underlying ventral nerve cord by BP102 antibody (dark blue). (A) In wild-type embryos a segmental pair of DM cells carrying long lateral processes is located medially on top of the ventral nerve cord. (B) In *sim* mutant embryos DM cells are generally missing. Note that CNS fiber tracts are fused and that the pattern of the ventral somatic muscles is disturbed, in that many of them cross above the ventral cord (arrow). (C) Removal of about 14 consecutive CNS midline progenitors in the wild-type gastrula leads to a local phenocopy of the *sim* mutant phenotype, with regard to the absence of DM cells as well as the pattern of ventral muscles (arrow) and CNS fiber tracts. (D) Removal of about six midline progenitors has no effect on DM cell formation. However, note the narrow CNS as well as the fusion of commissures (arrow). (E) Transplantation of about 10 wild-type midline cells in the midline of wild-type hosts leads to the formation of additional DM cells (arrow). (F) Midline progenitors transplanted from wild-type donors into the midline of *sim* mutant hosts induce the formation of DM cells.

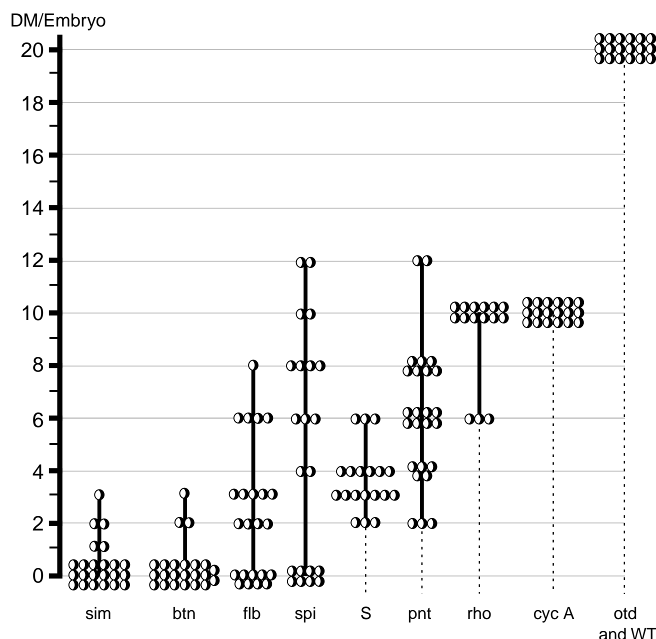

Fig. 4. Total number of DM cells in wild-type (WT) and mutant embryos. The number of DM cells was counted in embryos homozygous for the following mutations: *single minded* (*sim*), *buttonless* (*btn*; see also Chiang et al., 1994), DER/*faint little ball* (*flb*), *spitz* (*spi*), *Star* (*S*), *pointed* (*pnt*), *rhomboid* (*rho*), *cyclinA* (*cycA*), *orthodenticle* (*otd*). DM cells were marked by β-galactosidase expression (in *sim* mutants carrying the P[*btn 1.85 lacZ*] reporter construct) or by an anti-glutactin antibody (all other mutants). Each point in the diagram represents the DM cell population of one embryo. WT, wild type.

CNS midline cells flank the mesodermal anlage and induce DM cell development

The DM cells develop from the lateral mesoderm, which is in direct contact with a distinct set of neural progenitors, the later CNS midline cells. By transplantation and cell ablation experiments we showed that the CNS midline provides an inductive signal leading to DM cell formation and that this signal acts on mesodermal progenitors early in the gastrula (stage 7). Genetic data, as well as ectopic expression experiments, suggest that the inductive signal is conveyed via the DER signalling pathway. This is also corroborated by the fact that midline cells overexpressing secreted SPITZ fail to induce DM cells when isotopically transplanted into DER/*faint little ball* (*flb)* mutant hosts (*n*=9; data not shown). However, in *spitz*, *rhomboid* and *Star* mutants in which the production or processing of SPITZ is impaired (Schweitzer et al., 1995a), the effect on DM cell formation is significantly lower compared to *single minded.* Thus, the midline may contribute other signals beside SPITZ involved in DM cell formation. A candidate for such a factor could be VEIN, which has been shown to be expressed in overlapping domains with SPITZ and to act synergistically with SPITZ to achieve the required level of DER activation for normal development of ventrolateral ectodermal cells (Schnepp et al., 1996). On the other hand, since DER/*flb* mutant embryos also show a weaker DM cell phenotype compared to *sim* (Fig. 4), we cannot exclude that additional signalling pathways contribute to DM cell development.

The function of midline cells as inductive organizing centre

In invertebrate as well as in vertebrate development, cells located at the midline of the central nervous system often act as organizing centres. The cells located at the ventral midline of the neural tube are a set of morphological distinct cells called floor plate cells (Kingsbury, 1930). They are involved in dorsoventral patterning of the neural tube by contact-dependent

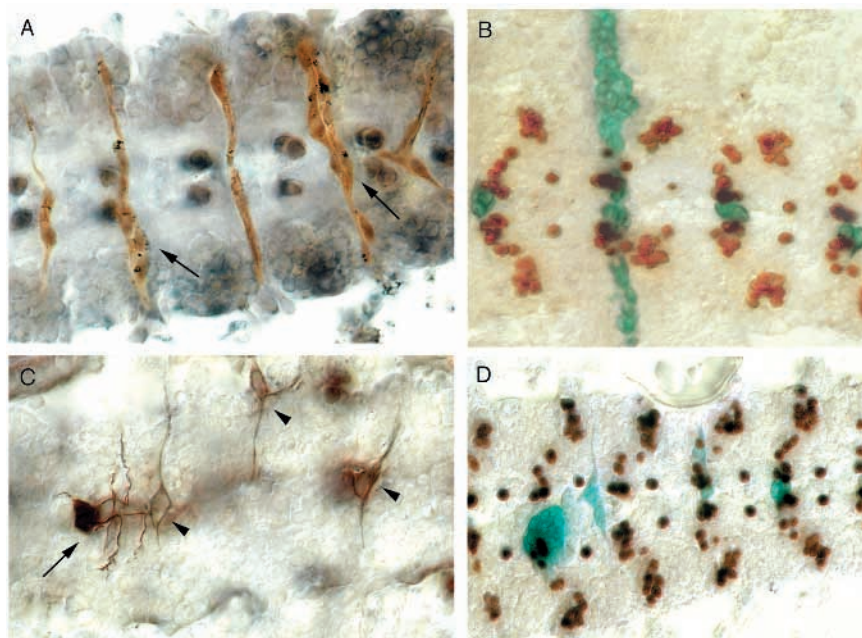

Fig. 5. Overexpression of secreted SPITZ by midline cells recruits additional DM cells. Horizontal views of flat preparations of stage 16 embryos. DM cells are marked by an anti-glutactin antibody (A,C; light brown) or by β-galactosidase expression directed by the P[*btn 1.85 lacZ*] reporter construct (B,D; blue). Specimens in A,B and D are double-stained with an antibody against EVEN-SKIPPED, which marks a population of CNS cells. (A) In embryos expressing secreted SPITZ in the entire midline (*sim-GAL4/UAS-sspitz*), extra DM cells are formed in some segments (arrows). (B,D) Transplantation of only 1 or 2 midline cells from *sim-GAL4/UAS-sspitz* donors into the midline of wild-type hosts leads to massive overproduction of DM cells in one segment. Additional DM cells express the P[*btn 1.85 lacZ*] marker; in most cases their cell bodies become distributed along the routes of the lateral processes that enwrap the transverse nerve (B, see also A). (C) Transplantation of midline cells from *sim-GAL4/UAS-sspitz* donors into the midline of embryos lacking the function of *cyclinA* does not lead to the induction of extra DM cells. In *cycA* mutants there is only one DM cell per segment (arrowheads). The arrow points to HRP-labelled VUM neurons in the CNS midline derived from the transplant.

mechanisms as well as diffusible molecules (Yamada et al., 1993; Hynes et al., 1995) and participate in controlling the patterning of somitic derivatives (e.g. Pourquie et al., 1996; Münsterberg and Lassar, 1995).

Similarly, the *Drosophila* CNS midline cells exert a number of inductive functions. The midline cells have been implicated in the patterning of the ventral epidermis (Kim and Crews, 1993) and recent experiments suggest that the CNS midline cells are able to express secreted SPITZ protein, which is required for ventral ectoderm pattern formation (Golembo et al., 1996b). Here we have shown that an inductive signal, originating from the CNS midline, governs the development of the mesodermal DM cells.

In addition, the CNS midline is also required for establishing the normal pattern of the ventral somatic muscles. In *sim* mutant embryos the pattern of the ventral somatic muscles is disturbed, in that many of them cross above the ventral cord and attach to the body wall on the opposite side of the embryo (Fig. 3B; see also Lewis and Crews, 1994). Removal of midline progenitors from the wild type leads to a phenocopy of this pattern, and implantation of wild-type midline cells into the midline of *sim* mutant embryos leads to a partial rescue of the phenotype (Fig. 3C,F), which indicates that the muscle pattern defects are non-autonomous and due to the absence of midline cells.

Thus in all systems analysed, the CNS midline cells appear to act as an important bidirectional source of inductive signals.

DER signalling during development

Inductive processes have been analysed thoroughly in many other systems, such as the induction of photoreceptor cell development in the *Drosophila* eye, where all ommatidial cells are recruited by a reiterative activation of the DER signalling pathway (Freeman, 1996). In cells competent to respond to the recruitment signal (SPITZ), DER signalling is activated and the cell adopts its appropriate fate. A fine tuning of DER activation is achieved by the interplay of two antagonising proteins, SPITZ and ARGOS. Although ARGOS antagonises SPITZ functions in many systems (Schweitzer et al., 1995b; Freeman, 1996; Golembo et al., 1996a; Okabe et al., 1996), we have found no evidence for a similar function of ARGOS in DM cell formation.

In other developmental contexts EGF-receptor signalling controls cell division (for a review, see Marshall, 1995). This is also the case in the developing eye, where DER signalling is required for apparently all cell divisions before its function is reused during ommatidial recruitment (Xu and Rubin, 1993).

Model on DM cell formation

Similar to eye development, secreted SPITZ appears to initiate DM cell formation by the recruitment of an already competent cell and to control its subsequent proliferation.

In the mesoderm the presumptive DM cell progenitors appear to be routed towards this particular developmental fate by the action of *twist,* segment polarity genes and *tinman* (Azipiazu and Frasch, 1993; Chiang et al., 1994; Gorczyca et al., 1994; Azipiazu et al., 1996; our own observations). Although only one mesodermal primordial cell gives rise to DM cells, we cannot discriminate to date whether the initial competence to develop as DM progenitor is conferred to two or only one mesodermal cell per segment. However, if two such cells are selected (flanking the mesectodermal cells, one on either side of the mesodermal anlage) a subsequent cross-talk between these cells must be postulated. *Notch* and other neurogenic genes might be candidate genes for this process and, indeed, approximately twice the number of DM cells were observed in *Notch* mutant embryos (Hartenstein et al., 1992).

Directing the competent mesodermal cell towards the DM cell fate requires signals derived from the CNS midline. Secreted SPITZ is likely to represent this midline-derived signal, since overexpression of secreted SPITZ in the midline leads to additional DM cells. We observed a less efficient induction of DM cells when secreted SPITZ was expressed in all midline cells compared to expression in only few midline cells. This could indicate that the DM progenitor cell requires graded DER activation. Once the DM cell progenitor is specified, interactions with neighboring primordial mesoderm cells might ensure that the DM cell fate is maintained and that other mesodermal cells refrain from taking over the same fate.

In summary, the midline cells of the *Drosophila* CNS, which in many respects are similar to the floorplate cells of the neural tube, act as an important signalling centre controlling ectodermal as well as mesodermal patterning. Future work is required to determine whether the underlying molecules have also been conserved.

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REFERENCES

Anderson, K.V. (1987). Dorsal-ventral embryonic pattern genes of *Drosophila*. *Trends Genet.* **3**, 91-97.

Azpiazu, N. and Frasch, M. (1993). *tinman* and *bagpipe*: two homeobox genes that determine cell fates in the dorsal mesoderm of *Drosophila*. *Genes Dev.* **7,** 1325-1340

Azpiazu, N., Lawrence, P. A., Vincent, J.-P. and Frasch, M. (1996). Segmentation and specification of *Drosophila* mesoderm. *Genes & Development* **10**, 3183-3194.

Baker, R. and Schubiger, G. (1995). Ectoderm induces muscle-specific gene expression in *Drosophila* embryos. *Development* **121**, 1387-1398.

Bate, C.M. (1993). The mesoderm and its derivatives. *In* The Development of *Drosophila melanogaster*, Vol. 2 (ed. M. Bate and A. Martinez Arias), pp. 1013-1091. CSHL Press.

Bate, M. and Baylies, M. (1996). Intrinsic and extrinsic determinants of mesodermal differentiation in *Drosophila*. *Semin. Cell .Dev. Biol.* **7**, 103-112.

Baylies, M.K. and Bate, M. (1996). *twist*: A myogenic switch in Drosophila. *Science* **272**, 1481-1484.

Baylies, M.K., Martinez Arias,A., and Bate, M. (1995). *Wingless* is required for the formation of a subset of muscle founder cells during *Drosophila* embryogenesis. *Development* **121**, 3829-3837.

Beer, J., Technau G.M. and Campos-Ortega, J.A. (1987). Lineage analysis of transplanted individual cells in embryos of *Drosophila melanogaster*. IV. Commitment and proliferative capabilities of mesodermal cells. *Roux's Arch Dev Biol* **196**, 222-230.

Bieber, A.J., Snow, P.M., Hortsch, M., Patel, N.H., Jacobs, J.R., Traquina, Z.R., Schilling, J. and Goodman, C.S. (1989). *Drosophila* neuroglian: a member of the immunoglobulin superfamily with extensive homology to the vertebrate neural adhesion molecule L1. *Cell* **59**, 447-460.

Bier, E., Jan, L.Y. and Jan, Y.N. (1990). *rhomboid*, a gene required for

dorsoventral axis establishment and peripheral nervous system development in *Drosophila melanogaster*. *Genes Dev.* **4**, 680-681.

Bodmer, R., Jan, L.Y. and Jan, Y.N. (1990). A new homeobox-containing gene, *msh*-2, is transiently expressed early during mesoderm formation of *Drosophila*. *Development* **110**, 661-669.

Bossing, T., and Technau, G. M. (1994). The fate of the CNS midline progenitors in *Drosophila* as revealed by a new method for single cell labelling. *Development* **120,** 1895-1906.

Bossing T, Udolph G, Doe CQ and Technau GM (1996). The embryonic CNS lineages of *Drosophila melanogaster*. I. Neuroblast lineages derived from the ventral half of the neuroectoderm. *Dev. Biol.* **179,** 41-61.

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

Campos-Ortega, J.A., and Hartenstein, V. (1985). *The Embryonic Development of* Drosophila melanogaster. Berlin, Heidelberg, New York: Springer Verlag.

Chiang, C., Patel, N.H., Young, K.E. and Beachy, P.A. (1994). The novel homeodomain gene *buttonless* specifies differentiation and axonal guidance functions of *Drosophila* dorsal median cells. *Development* **120**, 3581-3593.

Crews, S. T., Thomas, J. B. and Goodman, C. S. (1988). The *Drosophila single-minded* gene encodes a nuclear protein with sequence similarity to the *per* gene product. *Cell* **52**, 143-151.

Dunin-Borkowski, O.M., Brown, N.H. and Bate, M. (1995). Anterior-posterior subdivision and the diversification of the mesoderm in *Drosophila*. *Development* **121**, 4183-4193.

Finkelstein, R and Perrimon, **N.** (1990). The *orthodenticle* gene is regulated by *bicoid* and *torso* and specifies *Drosophila* head development. *Nature* **346**, 485-488.

Finkelstein, R., Smouse, D., Capaci, T.M., Spradling, A.C. and Perrimon, N. (1990). The *orthodenticle* gene encodes a novel homeodomain protein involved in the development of the *Drosophila* nervous system and ocellar visual structures. *Genes Dev.* **4**, 1516-1527.

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

Freeman, M. (1996). Reiterative use of the EGF receptor triggers differentiation of all cell types in the *Drosophila* eye. *Cell* **87**, 651-660.

Golembo, M., Schweitzer, R., Freeman, M. and Shilo, B.-Z. (1996a). *argos* transcription is induced by the *Drosophila* EGF receptor pathway to form an inhibitory feedback loop. *Development* **122**, 223-230.

Golembo, M., Raz, E. and Shilo, B.Z. (1996b). The *Drosophila* embryonic midline is the site of Spitz processing, and induces activation of the EGF receptor in the ventral ectoderm. *Development* **122**, 3363-3370.

Gorczyca, M.**, Phillis, R.W. and Budnik, V.** (1994). The role of *tinman*, a mesodermal cell fate gene, in axon pathfinding during the development of the transverse nerve in *Drosophila*. *Development* **120**, 2143-2152.

Govind, S. and Steward, R. (1991). Dorsoventral pattern formation. *Trends Genet.* **7**, 119-125.

Hartenstein, V. (1993). *Atlas of* Drosophila *Development.* Cold Spring Harbor Press.

Hartenstein, A, Rugendorf, A., Tepass, U. and Hartenstein, V. (1992). The function of the neurogenic genes during epithelial development in the *Drosophila* embryo. *Development* **116**, 1203-1220.

Hilliker, A. J., Clark, S.H., Chovnick, A. and Gelbart, W.M. (1980). Cytogenetic analysis of the chromosomal region immediately adjacent to the rosy locus in *Drosophila melanogaster*. *Genetics* **95**, 95-110.

Hynes, M., Poulsen, K., Tessier-Lavigne, M. and Rosenthal, A. (1995). Control of neuronal diversity by the floor plate: contact mediated induction of midbrain dopaminergic neurons. *Cell* **80**, 95-101.

Kim, S.H. and Crews, S. T. (1993). Influence of *Drosophila* ventral epidermal development by the CNS midline cells and spitz class genes. *Development* **118**, 893-901.

Kingsbury, B.F. (1930). The developmental significance of the floor plate of the brain and spinal cord. *J. Comp. Neurol.* **50**, 177-207.

Klaes, A., Menne, T., Stollewerk, A., Scholz, H. and Klämbt, C. (1994). The ETS transcription factors encoded by the *Drosophila* gene pointed direct glial cell differentiation in the embryonic CNS. *Cell* **78**, 149-160.

Klämbt, C. (1993). The *Drosophila* gene *pointed* encodes two ets like proteins, which are involved in the development of the midline glial cells. *Development* **117**, 163-176.

Klämbt, C., and Goodman, C.S. (1991). The diversity and pattern of glia during axon pathway formation in the *Drosophila* embryo. *Glia* **4,** 205-213.

Klämbt, C., Jacobs, J.R., and Goodman, C.S. (1991). The midline of the *Drosophila* central nervous system: a model for the genetic analysis of cell fate, cell migration, and growth cone guidance. *Cell* **64**, 801-815.

Kolodkin, A.L., Pickup, A.T., Lin, D. M., Goodman, C.S. and Banerjee, U. (1994). Characterization of *Star* and its interactions with *sevenless* and EGF receptor during photoreceptor development in *Drosophila*. *Development* **120**, 1731-1745.

Kosman, D., Ip, Y.T., Levine, M. and Arora, K. (1991). Establishment of the mesoderm-neuroectoderm boundary in the *Drosophila* embryo. *Science* **254**, 118-122.

Lehner, C. and O´Farell, P.H. (1989). Expression and function of *Drosophila* cyclin A during embryonic cell cycle progression. *Cell* **56**, 957-968.

Leptin, M. (1991). *twist* and *snail* as positive and negative regulators during *Drosophila* mesoderm development. *Genes Dev.* **5**, 1568-1576.

Lewis, J.O. and Crews, S.T. (1994). Genetic analysis of the *Drosophila single-minded* gene reveals a central nervous system influence on muscle development. *Mech. Dev.* **48**, 81-91.

Marshall, C.J. (1995). Specificity of receptor tyrosine kinase signaling: transient versus sustained extracellular signal-regulated kinase activation. *Cell* **80**, 179-185.

Mayer, U. and Nüsslein-Volhard, C. (1988). A group of genes required for pattern formation in the ventral ectoderm of the *Drosophila* embryo. *Genes Dev.* 2, 1496-1511.

Montell, D.J. and Goodman, C.S. (1989). *Drosophila* laminin: sequence of B2 subunit and expression of all three subunits during embryogenesis. *J. Cell Biol.* **109**, 2441-2453.

Münsterberg, A. E. and Lassar, A. B. (1995). Combinatorial signals from the neural tube, floor plate and notochord induce myogenic bHLH gene expression in the somite. *Development* **121**, 651-660.

Murray, M.A., Fessler, L.I. and Palka, J. (1995). Changing distributions of extracellular matrix components during early wing morphogenesis in *Drosophila*. *Dev. Biol.* **168**, 150-165.

Nambu, J. R., Lewis, J. O., Wharton, K. A. and Crews, S. T. (1991). The *Drosophila single-minded* gene encodes a helix-loop-helix protein that acts as a master regulator of midline development. *Cell* **67**, 1157-1167.

Nüsslein-Volhard, C., Wieschaus, E. and Kluding, H. (1984). Mutations affecting the pattern of the larval cuticle in *Drosophila melanogaster*. I. Zygotic loci on the second chromosome. *Roux's Arch Dev. Biol.* **193**, 267-282.

Okabe, M., Sawamoto, K. and Okano, H. (1996). The function of the *Drosophila argos* gene product in the development of embryonic chordotonal organs. *Dev. Biol.* **175**, 37-49.

Olson, P.F., Fessler, L.I., Nelson, R.E., Sterne, R., Campbell, A.G. and Fessler, J.H. (1990). Glutactin, a novel *Drosophila* basement membrane-related glycoprotein with sequence similarity to serine esterases. *EMBO J.* **9**, 1219-1227.

Osborne, M.P. (1964). The structure of the unpaired ventral nerves in the blowfly larva. *Quart. J. Micr. Sci.* **105**, 325-329.

Poulson, D.F. (1950). Histogenesis, organogenesis and differentiation in the embryo of *Drosophila melanogaster* Meigen. In *Biology of Drosophila* (ed. M. Demerec), pp. 168-274. New York: Wiley.

Pourquie, O., Fan, C. M., Coltey, M., Hirsinger, E., Watanabe, Y., Breant, C., Francis-West, P., Brickell, P., Tessier-Lavigne, M. and Le Douarin, N. M. (1996). Lateral and axial signals involved in avian somite patterning: a role for BMP4. *Cell* **84**, 461-471.

Prokop, A. and Technau, G.M. (1993). Cell transplantation. In *Cellular Interactions in Development. A Practical Approach* (ed. D.A. Hartley), pp. 33-57.

Ranganayakulu, G., Schulz, R.A. and Olson, E.N. (1996). Wingless signaling induces *nautilus* expression in the ventral mesoderm of the *Drosophila* embryo. *Dev. Biol.* **176**, 143-148.

Raz, E. and Shilo, B.-Z. (1993). Establishment of ventral fates in the *Drosophila* embryonic ectoderm requires DER, the EGF receptor homolog. *Genes Dev.* **7**, 193-1948.

Roth, S., Stein, D. and Nüsslein-Volhard, C. (1989). A gradient of nuclear localization of the dorsal protein determines dorsoventral pattern in the *Drosophila* embryo. *Cell* **59**, 1189-1202.

Rushlow, D.A. and Arora, K. (1990). Dorsal ventral polarity and pattern formation in the *Drosophila* embryo. *Semin. Cell Biol.* **1**, 137-149.

Schejter, E.D. and Shilo, B.-Z. (1989). The *Drosophila* EGF receptor homolog (DER) gene is allelic to *faint little ball*, a locus essential for embryonic development. *Cell* **56**, 1093-1104.

Schmidt-Ott, U. and Technau, G.M. (1992). Expression of *en* and *wg* in the embryonic head and brain of *Drosophila* indicates a refolded band of seven segment remnants. *Development* **116**, 111-125

Schneider, L.E., O'Brien, M.A. and Taghert, P.H. (1991). In situ hybridization analysis of the FMRFamide neuropeptide gene in *Drosophila*. Part I. Restricted expression in embryonic and larval stages. *J. Comp. Neurol.* **304**, 608-622.

Schnepp, B., Grumbling, G., Donaldson, T. and Simcox, A. (1996). Vein is a novel component in the *Drosophila* epidermal growth factor receptor pathway with similarity to the neuregulins. *Genes Dev.* **10**, 2302-2313.

Scholz, H., Sadlowski, E., Klaes, A. and Klämbt, C. (1997). Control of midline glia development in the embryonic *Drosophila* CNS. *Mech. Dev.* (in press).

Schweitzer, R., Shahrabany, M., Seger, R. and Shilo, B.-Z. (1995a). Secreted spitz triggers the DER signaling pathway and is a limiting component in the embryonic ventral ectoderm determination. *Genes Dev.* **9**, 1518-11529.

Schweitzer, R., Howes, R., Smith, R., Shilo, B.-Z. and Freeman, M. (1995b). Inhibition of Drosophila EGF receptor activation by the secreted protein Argos. *Nature* **376**, 699-702.

Sonnenfeld, M.J. and Jacobs, J.R. (1994). Mesectodermal cell fate analysis in *Drosophila* midline mutants. *Mech. Dev.* **46**, 3-13.

Staehling-Hampton, K., Hoffmann, F.M., Baylies, M.K., Rushton, E. and Bate, M. (1994). dpp induces mesodermal gene expression in *Drosophila*. *Nature* **372**, 783-786.

St. Johnston, R.D. and Nüsslein-Volhard, C. (1992). The origin of pattern and polarity in the *Drosophila* embryo. *Cell* **68**, 201-219.

Sturtevant, M.A., Roark, M., O'Neill, J.W., Biehs, B., Colley, N. and Bier, E. (1996). The *Drosophila rhomboid* protein is concentrated in patches at the apical cell surface. *Dev. Biol.* **174**, 298-309.

Thisse, B., Stoetzel, C. and Perrin-Schmidt, M. (1987). Genes of the *Drosophila* maternal dorsal group control the specific expression of the zygotic gene *twist* in presumptive mesodermal cells. *Genes Dev.* **1**, 709-715.

Thomas, J.B., Crews, S.T. and Goodman, C.S. (1988). Molecular genetics of the *single-minded* locus: a gene involved in the development of the *Drosophila* nervous system. *Cell* **52**, 133-141.

Udolph, G., Lüer, K., Bossing, T., and Technau, G.M. (1995). Commitment of CNS progenitors along the dorsoventral axis of *Drosophila* neuroectoderm. *Science* **269**, 1278-1281.

White, K., Hurteau, T. and Punsal, P. (1986). Neuropeptide-FMRFamide-like immunoreactivity in *Drosophila*: Distribution and development. *J. comp. Neurol.* **247**, 430-438.

Xu, T. and Rubin, G.M. (1993). Analysis of genetic mosaics in developing and adult *Drosophila* tissues. *Development* **117**, 1223-1237.

Yamada, T., Pfaff, S., Edlund, T. and Jessell, T.M. (1993). Control of cell pattern in the neural tube: Motorneuron induction by diffusible factors from the notochord and floor plate. *Cell* **73**, 673-686.

Zitnan, D., Sehnal, F. and Bryant, P.J. (1993). Neurons producing specific neuropeptides in the central nervous system of normal and pupariation-delayed *Drosophila*. *Dev. Biol.* **156**, 117-135.

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