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Control of cell fates and segmentation in the *Drosophila* mesoderm

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SUMMARY

The primordia for heart, fat body, and visceral and somatic muscles arise in specific areas of each segment in the *Drosophila* mesoderm. We show that the primordium of the somatic muscles, which expresses high levels of *twist*, a crucial factor of somatic muscle determination, is lost in *sloppy-paired* mutants. Simultaneously, the primordium of the visceral muscles is expanded. The visceral muscle and fat body primordia require *even-skipped* for their development and the mesoderm is thought to be unsegmented in *even-skipped* mutants. However, we find that *even-skipped* mutants retain the segmental modulation of the expression of *twist*. Both the domain of *even-skipped* function and the level of *twist* expression are regulated by *sloppy-paired*. *sloppy-paired* thus controls segmental allocation of mesodermal cells to different fates.

Key words: *Drosophila*, mesoderm, muscle development, fat body, segmentation, *twist*, *slp*, *bap*

INTRODUCTION

The *Drosophila* mesoderm gives rise to a number of tissues whose primordia become determined soon after the mesoderm has spread out on the ectoderm. Most of these primordia cover the whole length of the segmented trunk region of the embryo, consisting of groups of cells at specific positions repeated in each parasegment, as indicated in Fig. 1. Boundaries between repeated units in the embryo can be drawn in different ways. The boundaries used for the subdivision of the ectoderm do not map unambiguously onto the borders of developmental units or domains defined by gene expression in the mesoderm (for a detailed discussion cf. appendix). We will therefore refer to two alternating mesodermal domains by the names of the two genes whose functions are essential for the development of these regions, *even-skipped* (*eve*) (Azpiazu et al., 1996) and *sloppy-paired* (*slp*) (Fig. 1; Grossniklaus et al., 1992; Cadigan et al., 1994 and the work described here). The *slp* domain gives rise to the dorsally located heart precursors and to the majority of somatic muscles that develop from the more ventrally located cells (Dunin Borkowski et al., 1995). In the *eve* domain, the most dorsally located group of cells gives rise to the visceral mesoderm that forms the muscles surrounding the gut (Azpiazu et al., 1996). The fat body arises from a group of cells in a more external and slightly more ventral location characterised by the expression of the gene *serpent* (*srp*). The most ventral cells in this domain probably develop into somatic musculature and a few mesodermal glia cells (Gorczyca et al., 1994; Dunin Borkowski et al., 1995).

The subpopulations of mesodermal cells can be distinguished by gene expression patterns at early stages of development. The first sign of the subdivision of the mesoderm into segmentally specified groups of cells is the expression of the gene *bagpipe* (*bap*) at late stage 9. *bap* is a homeobox gene expressed in the primordium of the visceral muscles and is required for their development (Azpiazu and Frasch, 1993). A slightly later marker for this cell population is the cell surface protein Fasciclin III (FasIII). A further important manifestation of segmentation is the differential decay of the HLH-protein Twist, which is initially expressed homogeneously throughout the whole mesoderm. Cells in the *eve* domain show reduced levels of Twist by late stage 10. The maintenance of high levels of Twist in the *slp* domain is required for somatic myogenesis and blocks the formation of other mesodermal derivatives (Baylies and Bate, 1996). This striped pattern of *twist* expression is further refined by differential maintenance along the dorsoventral axis resulting in a ventrolateral patch of high *twist* expression in each segment. Later, a number of proteins, such as Krüppel and S59, mark specific groups of muscle founder cells (for review see Bate, 1993). The fat body primordium expresses the GATA factor Srp, which is required for proper fat body development (Abel et al., 1993; Rehorn et al., 1996). A cluster of three to four mesodermal cells in each segment express the homeobox gene *eve* (Frasch et al., 1987) marking the region of the heart primordium. These cells are the precursors of the pericardial cells and of one dorsal muscle (Dunin Borkowski et al., 1995).

Some of the genes that initiate the regional specification of the mesoderm are known. Dpp-signalling from the ectoderm controls the subdivision along the dorsoventral axis, with cells receiving the Dpp signal developing as visceral muscles, heart and dorsal somatic muscles (Staehling-Hampton et al., 1994; Frasch, 1995; Bate and Baylies, 1996). The homeobox gene *tinman* (*tin*) is initially expressed in all mesodermal cells but its expression is only maintained in dorsal mesodermal cells receiving the Dpp signal (Frasch, 1995). In *tin* mutant embryos, *bap* is not activated and, as a consequence, no visceral mesoderm develops (Azpiazu and Frasch, 1993). *tin* is also

essential for the formation of the heart and dorsal somatic muscles (Azpiazu and Frasch, 1993; Bodmer, 1993).

Along the anterior-posterior axis, the subdivision into the primordia of heart, fat body and visceral mesoderm, as well as the differentiation of the somatic mesoderm is regulated by segment polarity genes, especially *wingless* (*wg*) and *hedgehog* (*hh*), with cues coming from both mesoderm and ectoderm. The primordia of the visceral mesoderm and the fat body overlap the *engrailed* (*en*) and *hh* expression domain in the ectoderm, and these genes are necessary (but not sufficient) for their spatial determination (Azpiazu et al., 1996). The heart primordium and the high Twist domain overlap the *wg* expression domain and require *wg* for their proper development (Bate and Rushton, 1993; Lawrence et al., 1995; Wu et al., 1995; Azpiazu et al., 1996; Park et al., 1996; Ranganayakulu et al., 1996). The determination of the heart primordium also depends on *slp* (Park et al., 1996). These genes' activities are partly controlled by the segmentation gene *eve*, and both mesoderm and ectoderm are thought to be unsegmented in *eve* mutants (Nüsslein-Volhard et al., 1985; Azpiazu et al., 1996). For the mesoderm, this hypothesis is based on the finding that *bap*, the earliest marker for mesoderm segmentation, is not expressed in *eve* mutants. Indeed all mesodermal derivatives from the *eve* domain, in which *eve* is expressed after the refinement of its initial pair-rule pattern, fail to develop (Azpiazu et al., 1996). However, the loss of cell fates in this domain is not accompanied by a transformation to fates typical for the neighbouring domain, since we find that Twist modulation still occurs in *eve* mutants. Thus, mesodermal segmentation is not completely lost in *eve* mutants and another segmental regulator must exist. We show that *slp* controls all aspects of differentiation in the *slp* domain, including segmental repression of *bap*, maintenance of high levels of Twist and hence, myogenesis.

MATERIALS AND METHODS

Fly stocks

We used two *sloppy-paired* (*slp*) deficiencies on the CyO chromosome. On CyOΔ34B P^{lArB}A208.1M2 both *slp* loci are deleted, on CyOΔ46G P^{lArB}A208.1M2 *slp1* is deleted (Cadigan et al., 1994). These stocks were obtained from the Gehring laboratory in Basel, Switzerland. We used the *eve* null allele eve^{r13}, which, like $fushi\text{-}tarazu^{7B}$ and wg^{IIID23}, was from the Tübingen stock collection, the *eve*, *slp* double mutant (Df(2L)edSZ1, eve^{r13}) was a gift from Ken Cadigan.

Antibody stainings and in situ hybridisation of embryos

The following primary antibodies were used: monoclonal mouse-anti En (4D9, provided by C. Klämbt (Patel et al., 1989)), rabbit anti-Eve (provided by M. Frasch (Frasch et al., 1987)), monoclonal anti-FasIII (provided by R. Smith (Brower et al., 1980)), rabbit anti-MHC (provided by D. Kiehart (Kiehart and Feghali, 1986)), rabbit anti-Pox-meso (provided by M. Noll), rabbit anti-Krüppel (provided by C. Rushlow), rabbit anti-Srp (provided by M. Brennan), rabbit anti-S59 (provided by M. Bate), rabbit anti-Twi (provided by S. Roth (Roth et al., 1989)).

Embryos were fixed and stained following standard protocols. For double labelling, both primary antibodies were applied together and incubated overnight at 4°C. The secondary antibody (biotinylated goat anti-rabbit IgG from Jackson, Bar Harbor, USA), was applied for 1 hour at room temperature and the Vectastain ABC kit was used for detection. The embryos were then incubated in biotinylated goat anti-mouse IgG from Jackson (Bar Harbor, USA) and stained using the same procedure except that $CoCl_2$ and $NiSO_4$ (0.03% each) were added to the DAB substrate to create the blue stain.

mRNA was detected in situ as described by Tautz and Pfeifle (1989).

Microscopy

Embryos were mounted individually in Araldite. Pictures were taken on a Zeiss Axiophot on Kodak Ektachrome 64T slide film.

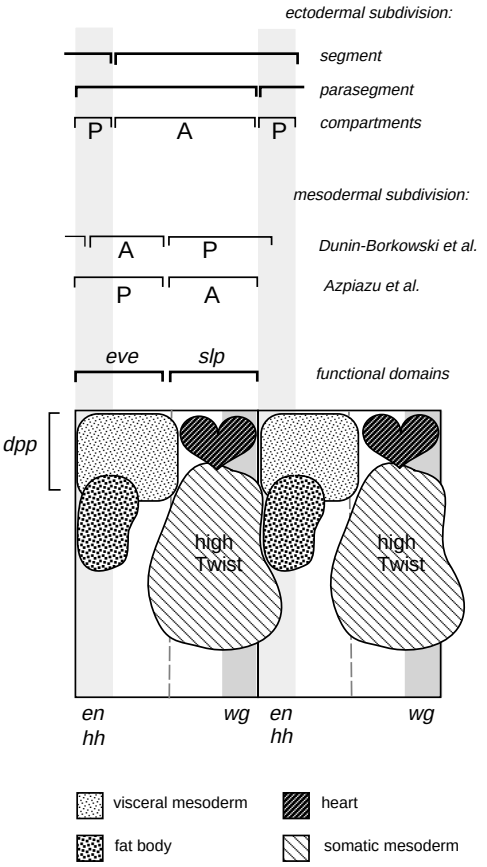

Fig. 1. Positions of mesodermal subpopulations. The diagram shows the locations of mesodermal subpopulations relative to the units of subdivision used for the ectoderm (compartments and parasegments), the early mesoderm (A and P according to Azpiazu et al., 1996), later mesoderm (A and P according to Dunin Borkowski et al., 1995) and the domains of *eve* and *slp* function in the mesoderm as used in this paper. The *slp* domain gives rise to the dorsally located heart precursors and to the majority of somatic muscles. Development of somatic muscles requires the maintenance of high levels of *twist* expression (Baylies and Bate, 1996). In the *eve* domain of each segment, the most dorsally located group of cells gives rise to the visceral musculature. The fat body primordium lies slightly more ventrally. The primordia of the visceral muscles and the fat body overlap the *en* and *hh* expression domain, and these genes are necessary for their spatial determination (Azpiazu et al., 1996). The heart primordium and the high Twist domain overlap the *wg* expression domain and require *wg* for their proper development (Bate and Rushton, 1993; Baylies et al., 1995; Lawrence et al., 1995; Wu et al., 1995; Azpiazu et al., 1996; Park et al., 1996; Ranganayakulu et al., 1996). Dpp signalling from the ectoderm induces development of visceral mesoderm, heart and dorsal somatic mesoderm (Staehling-Hampton et al., 1994; Frasch, 1995; Bate and Baylies, 1996).

UAS/GAL4 strains

Slp1 and Slp2 cDNAs cloned into pBluescript were kindly provided by W. Gehring. The construct pUAS:Slp2 was built by linearizing Slp2 with *Nde*I and converting the *Nde*I site to an *Xba*I site using the large (Klenow) fragment of DNA polymerase I and (TGCTCTAGAGCA) linkers. Following digestion of the resulting clone with *Xba*I and *Eco*RI, a 1.5Kb fragment containing the entire open reading frame was inserted into pUAST. To create the construct pUAS:Slp1, the *Nde*I site within the Slp1 cDNA was converted to an *Xba*I site as described above. The *Eco*RV site of the clone containing the new *Xba*I site was converted to a *Bgl*II site by addition of (GGAAGATCTTCC) linkers and subsequently a 1.3 kb *Bgl*II/*Xba*I fragment encoding the open reading frame of Slp1 was ligated into pUAST. For ectopic expression in the mesoderm, we used *twi-GAL4* (*twi-GAL4* insertion on the second chromosome which was kindly provided by B. Giebel). Ectopic expression in the ectoderm was achieved with the driver line *69B* (Brand and Perrimon, 1993).

RESULTS AND DISCUSSION

Loss of cell fates in the mesoderm of *slp* mutants

In *eve* mutants, the primordia of the fat body and visceral mesoderm fail to develop (Azpiazu et al., 1996). However, the loss of these cell fates in the *eve* domain does not appear to be accompanied by a transformation to fates typical of the complementary domain, since the subdivision into alternating domains of cells expressing high and low levels of Twist still takes place in *eve* mutants, although the segments are twice as large, similar to the situation in other pair-rule mutants (Fig. 2). Thus, while the differentiation of specific fates in the mesoderm is abolished in *eve* mutants, the underlying segmentation of the mesoderm cannot be controlled by *eve* alone.

We find that the determination of those primordia not affected by *eve* depend on *slp*. We will therefore refer to the domain containing these primordia (the heart and the somatic muscle precursors expressing high levels of Twist) as the *slp* domain. Two *slp* transcripts, *slp1* and *slp2*, are encoded by two separate, neighbouring genes, which have been shown to have largely overlapping functions (Grossniklaus et al., 1992; Cadigan et al., 1994). Both transcripts encode forkhead domain proteins and are expressed in the ectoderm as well as in the mesoderm (Fig. 4A,C). The two genes are expressed in almost identical patterns. Deletion of both genes causes a much stronger segmentation phenotype than mutations in either gene alone. In embryos lacking the function of both *slp1* and *slp2* (which we will call *slp* mutants in this paper), no heart precursors (Fig. 3O,P and Park et al., 1996) or other mesodermal fates of the *slp* domain develop and cells with fates typical of the *eve* domain occupy the full length of each segment (Fig. 3). *slp* mutant embryos show no segmental patterning of *twist* into high and low expression domains and the whole mesoderm is transformed into a continuous low-*twist* domain (Fig. 3A,B). Slightly later the expression in the trunk mesoderm is completely lost while the expression in the hindgut visceral primordium is not affected (Fig. 3C,D). In these mutant embryos, expression of *pox meso*, normally seen in the ventral mesodermal cells in the *slp* domain (Bopp et al., 1989), is absent (Fig. 3E-H). In addition, later muscle markers, such as *S59* (Dohrmann et al., 1990) and *Krüppel* (Gaul et al., 1987; Gisselbrecht et al., 1996), which are normally observed in cells arising from the high *twist* domain, are not expressed (Fig. 3I-L). (Some *S59*-expressing cells are still present, which may originate from the ventral region in the *eve* domain). In older mutant embryos, muscles fail to form and only groups of largely unfused cells expressing Myosin heavy chain are observed (Fig. 3M,N).

Expansion of cell fates of the *eve* domain

Concomitant with the loss of fates from the *slp* domain, we observe an expansion of fates from the *eve* domain throughout the segment. The expression of *bap* marks the future midgut visceral mesoderm and is the earliest indicator of mesodermal segmentation (Azpiazu and Frasch, 1993). In *slp* mutant embryos, *bap* expression is no longer confined to the *eve* domain but is expanded to cover a continuous band of mesodermal cells (Fig. 3Q,R), which resembles the expression domain of *tin* (Azpiazu and Frasch, 1993; Bodmer, 1993) at this stage of development. This transformation of cell fates is clearly seen in embryos doubly labelled with antibodies directed against Twist and FasIII. Fig. 3S shows the alternating groups of *FasIII*-expressing visceral mesoderm cells (brown) and *twist*-expressing somatic mesoderm cells (blue). In *slp* mutant embryos, *twist* expression (and ectodermal *FasIII* expression) is lost and, as a result of the transformation of somatic to visceral mesodermal fate, the visceral mesoderm contains many more cells than in the wild type. It is worth noting that neither the expression pattern of *eve* nor that of *en*, both positive regulators of *bap*, show an expansion similar to the expansion of *bap* and *FasIII* (Cadigan et al., 1994). Thus, *bap* and *FasIII* are expressed in cells that have never expressed *eve*. It follows that *eve*, at least in this mutant situation, is not an essential activator of *bap* expression. An alternative that we discuss below is that *eve* acts as a negative regulator of a repressor of *bap*.

The primordium of the fat body

Expression of *srp* in the trunk mesoderm at stage 10 is the

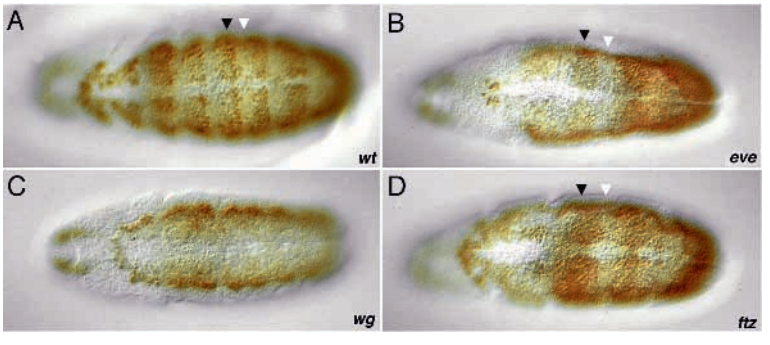

Fig. 2. Segmentation of the mesoderm as judged by segmental modulation of Twist protein. Ventral views of stage 10 embryos. High and low levels of Twist are marked by arrowheads in wild-type and *eve*, *fushi-tarazu* (*ftz*) and *wg* mutant embryos. In the wild type, *twist* begins to decay in a segmental pattern. This is also seen in *eve* and *ftz* embryos, but the segments are twice as large. In embryos mutant for *wg*, the segmental modulation is still visible, but the highest Twist levels do not correspond to the highest levels seen in the wild type.

earliest sign of fat body differentiation. The first fat body precursors arise in the dorsal part of the *eve* domain directly under the stripe of ectodermal *en* expression (Fig. 3U,W). *srp* expression unexpectedly did not expand throughout the whole segment in *slp* mutant embryos. Rather, the distribution of fat body precursors followed precisely the pattern of *en* expression, such that even-numbered segments had enlarged groups of *srp*-stained cells while odd-numbered segments had

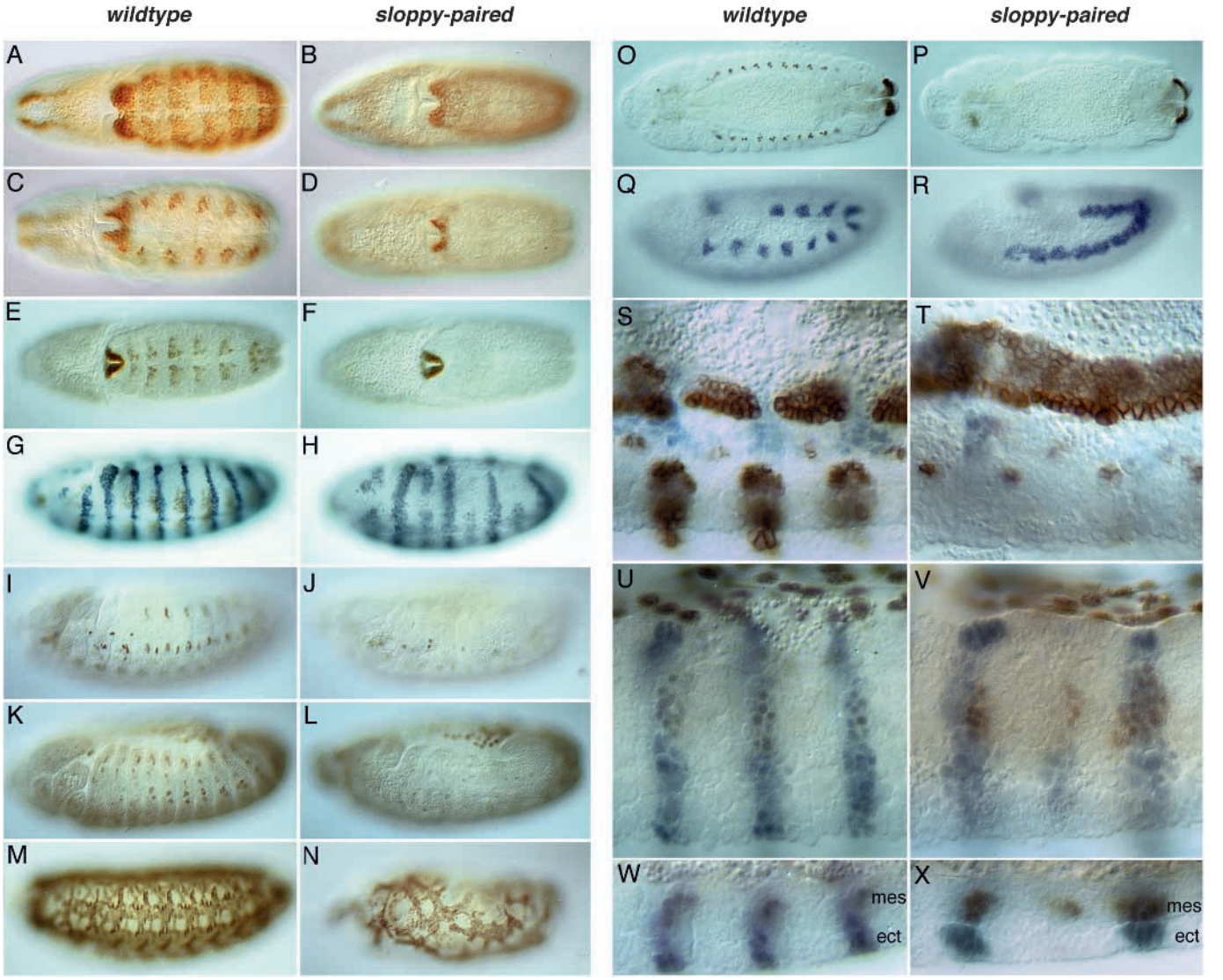

Fig. 3. Mesoderm segmentation in embryos lacking *slp1* and *slp2*. Wild-type embryos (left column) and embryos homozygous for a deficiency (Δ 34B) that uncovers both *slp* genes (right column) stained for markers expressed in subpopulations of the mesoderm. (A-D) *twist*. (A,B) Stage 10. In *slp* mutants, no segmental modulation of the level of Twist protein is visible and Twist decays at an equal rate throughout the segment. (C,D) Stage 11. Only a small group of cells in each segment continue to express *twist* in the wild type. *twist* expression is completely lost in the trunk region in *slp* mutant embryos. (E,F) *pox meso*, stage 10. *pox meso*, normally seen in the ventral mesodermal cells of the *slp* domain, is not expressed in the trunk of *slp* mutant embryos. (G,H) Stage 10 embryos stained for *pox meso* (brown) and *en* (blue) to demonstrate the location of the *pox meso* domain relative to the ectodermal parasegment boundaries. (I,J) *S59*, stage 13. The homeobox gene *S59* is expressed in a subset of muscle precursors. Expression in *slp* mutants is mostly lost; the small number of cells that stain may derive from a muscle primordium in the *eve* domain. (K,L) *Krüppel*, stage 14. *Krüppel* expression in muscle precursors is lost in *slp* mutants. (M,N) Stage 17. *slp* mutants stained with antibodies against Muscle myosin heavy chain show a severely reduced number of muscle cells that remain largely unfused. (O,P) *eve*. At stage 13, *eve* is expressed in pericardial cells, which arise from precursors located in the *slp* domain. These are not seen in *slp* mutants. (Q,R) *bap* is expressed in stage 9 embryos in a dorsal patch in the *eve* domain. In *slp* mutants its expression is no longer restricted to this domain. (S,T) *FasIII* (brown) and *twist* (blue). Segments T1-T3 are shown at high magnification. At stage 11, *FasIII* is expressed in the midgut visceral mesoderm, in epidermal cells within the *wg* domain and in neuronal cells. The visceral mesoderm is markedly enlarged in *slp* mutants and the stripe in the *wg* domain is lost. (U-X) Fat body. Stage 10 embryos were stained with antibodies against En (blue) and Srp (brown). Segments T2-A1 are shown at high magnification in surface views (U,V) and optical cross-section (W,X) through mesoderm (mes) and ectoderm (ect). In the wild type, a cluster of fat body precursors arises in each segment, positioned underneath each *en* stripe. In *slp* mutants, the sizes of these clusters vary considerably, those in even segments being larger, and in odd segments smaller than in wild-type embryos. This size variation reflects precisely the variation in the *en* stripes.

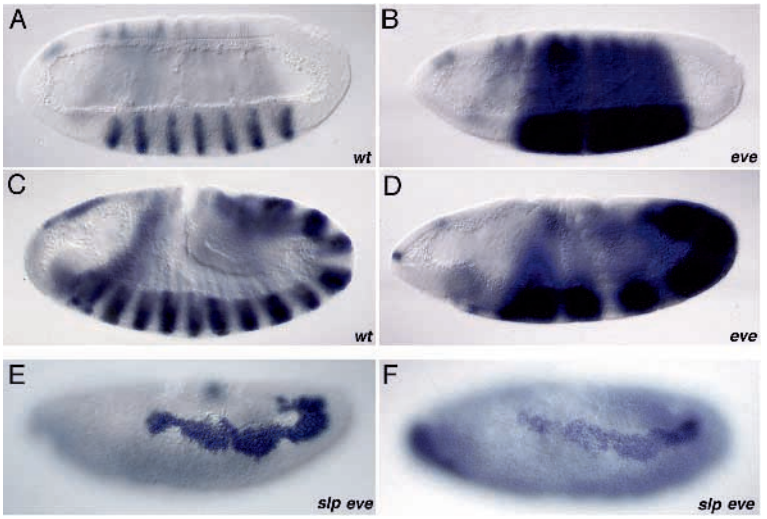

Fig. 4. Expression of *slp1* in *wt* and *eve* mutant embryos. (A-D) Stage 6 (A,B) and stage 7 embryos (C,D) hybridized with a *slp1* probe. In wild-type embryos, *slp1* first appears in a pattern of 7 stripes. A second set of stripes appears between the first 7 such that a regular 14-stripe pattern is generated by stage 7. In *eve* mutant embryos, *slp1* is first activated in a nearly homogeneous field covering the trunk region of the embryo. This field later breaks up into seven broad stripes. *slp1*-expression is clearly also seen in the mesoderm. (E,F) Midgut visceral mesoderm in *slp eve* double mutants. (E) *bap* expression, (compare to Fig. 3Q for wild type) although absent in *eve* mutants, is seen throughout the visceral mesoderm in the double mutant. Its expression domain corresponds precisely to that of its activator, *tin* (F).

reduced numbers of *srp*-stained cells (Fig. 3V,X). Thus, this population of mesodermal cells does not change in concert with the visceral mesoderm precursors and must hence be regulated differently.

The role of *eve*

Although in *eve* mutants none of the fates of the *slp* domain were expanded to cover the whole segment, we found that the expression of *slp* itself was expanded (Fig. 4A-D). Thus Eve acts as an early repressor of *slp* expression (Fujioka et al., 1995). This suggests that the loss of *bap* expression in *eve* mutant embryos may be the result of *slp* expression in the *eve* domain, with *slp* acting as a negative regulator of *bap* (Fig. 3Q,R). Thus, *eve* would not be an essential activator of *bap*, and an embryo lacking both *eve* and *slp* should express *bap*. This is indeed the case (Fig. 4E). It is not clear whether Slp acts as a direct repressor of *bap* transcription or whether it acts indirectly, for example by interfering with the function of the *bap* activator Tin.

The expression of *srp* in the *slp eve* double mutant (Fig. 5D) also shows that *eve* is not an essential activator of *srp*, although the absence of *srp* expression in *eve* mutants had suggested this as a formal possibility. As in *slp* mutants, *srp* is expressed initially in the same pattern as *en* in the ectoderm (not shown). Both *srp* expression and the seven stripes of *en* expression in the double mutant contradict the hypothesis that segments cannot be established in the absence of *eve* function. However, a reappearance of *en* expression has been observed in double mutant combinations of eve with other pair-rule mutations as well (DiNardo and O'Farrell, 1987). The findings are consistent with known regulatory inputs for *en* expression, which would allow *en* expression under positive control by *fushi-tarazu* and *paired* (Fujioka et al., 1995; Manoukian and Krause, 1993) once repression of these two genes by *eve* (Fujioka et al., 1995; Manoukian and Krause, 1992) and *slp* (Cadigan et al., 1994) is relieved. Thus, *srp* and *en* may be expressed in the absence of *eve* function because other segmentation genes, namely *fushi-tarazu* and *paired*, can exert their positive influence on *en*, and *en* in turn allows activation of *srp*.

Ectopic *slp* expression does not lead to a transformation of cell fates

slp expression in the *eve* domain cannot override the patterning systems that set up cell fates in this domain. When we expressed *slp* ectopically in the whole mesoderm or the whole ectoderm (see Material and Methods), we saw no major cell fate transformations, although the ectopic expression was at least as strong and as early as the natural expression of *slp*. We observed only a slight enlargement of the heart primordium, as has been shown previously after heat-shock expression of *slp* (Park et al., 1996), and no major change in the extent of the high Twist domain, the visceral mesoderm or any of the other mesodermal cell populations (not shown). We imagine that *slp* alone, in the absence of other segmentation gene functions is insufficient to interfere with *tin* activity on *bap* in the *eve* domain.

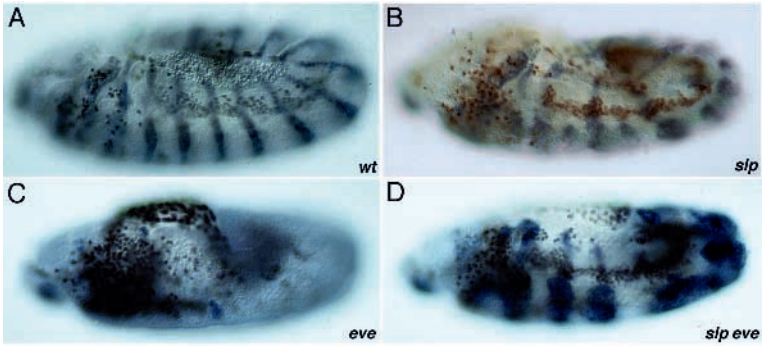

Fig. 5. Fat body development in *slp*, *eve* and double mutants. Stage 12 embryos stained with antibodies against En (blue) and Srp (brown). The clusters of fat body precursors (cf. Fig. 3U) have joined to produce a continuous fat body primordium both in the wild-type (A) and the *slp* mutant (B). Neither the fat body nor *en* stripes are seen in *eve* mutants (C), but both reappear in the double mutant (D) in a pair-rule pattern (also in this mutant the fat body arises from clusters underlying the *en* stripes; not shown). Note that *srp* is also expressed in hemocytes, developing on the ventral side of the head region and the amnioserosa. Staining in these tissues is not affected in the mutants.

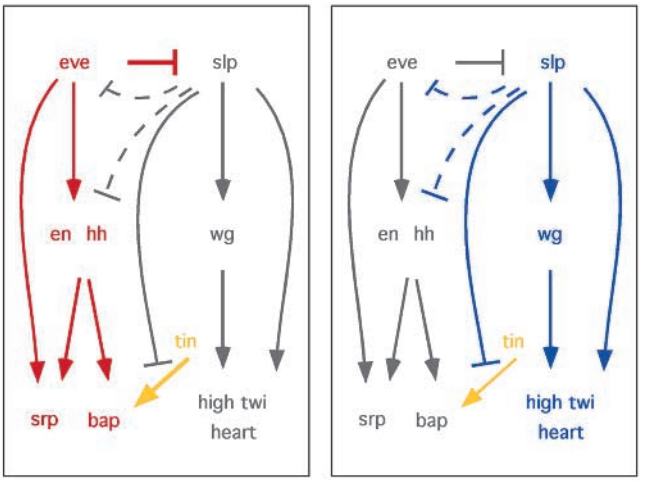

Fig. 6. Diagram of gene activities in mesoderm segmentation. The *eve* and *slp* domains are shown as boxes. In each box, the genes active in that domain as well as the interactions among them are shown. Active genes and pathways are in colour, inactive ones grey; positive interactions are indicated by arrows, negative interactions by lines ending in a bar. The broken line from *slp* to *eve* indicates repression of *eve* by *slp* in part of the domain (the Eve stripe is slightly broader in *slp* mutants than in the wild type) (Cadigan et al., 1994). See text for details.

A model for mesoderm segmentation

A model for mesoderm segmentation consistent with the data reported here and by others is shown in Fig. 6. The mesoderm can be divided into alternating domains in which cell fates are controlled by *eve* and *slp*. *eve* is required for the development of visceral mesoderm and fat body, while *slp* is required for the heart and somatic mesoderm. *slp* and *eve* act partly through their downstream targets *wg*, *en* and *hh*, but they must also have direct effects, or effects mediated by other genes (i.e. not segmentation genes), since *wg*, *en* and *hh* mutant embryos have much weaker mesodermal defects than *slp* or *eve* mutants. For example, the effect of *slp* on Twist levels is probably partly, but not completely mediated by *wg*. *wg* mutant embryos show a premature and ectopic decay of Twist (Fig. 2, see also Bate and Rushton, 1993), but not to the same degree as we see in *slp* embryos. Moreover, whereas patches of cells expressing high levels of Twist are initially established in *wg* mutant embryos, we see no Twist in the trunk region of *slp* embryos after stage 11 at all. Even more strikingly, *bap* expression is expanded to fill the whole segment in *slp* mutants, but not in any other segmentation mutant tested (Azpiazu et al., 1996).

The three genes *eve*, *slp* and *dpp* are sufficient to explain the patterning of at least the dorsal part of the mesoderm. The dorsoventral subdivision of the mesoderm is regulated by Dpp signalling from the ectoderm (Staehling-Hampton et al., 1994; Frasch, 1995; Bate and Baylies, 1996). Dpp is required for the maintenance of *tin* expression in the dorsal mesoderm, which in turn is essential for the development of the dorsal derivatives of the mesoderm. *tin* activates *bap* expression within the *eve* domain and promotes cardiac and dorsal muscle fates in the *slp* domain (Azpiazu and Frasch, 1993). Slp may control *bap* expression by binding to the *bap* promoter and acting as a repressor interfering with the action of a transcriptional activator of *bap*. The only known activator of *bap* is Tin (Azpiazu and Frasch, 1993), which is in fact expressed in all those cells that express *bap* when *slp* function is removed. Thus, a reasonable model would have Tin as a necessary and sufficient activator of *bap*, whose action is blocked in the *slp* domain by Slp acting as a *bap* repressor. This model is consistent with the findings of Staehling-Hampton et al. (1994), which show that ubiquitous ectopic expression of *dpp* in the invaginating mesoderm leads to a pair-rule rather than ubiquitous expression of *bap*. It is likely that *slp*, which is expressed in a pair-rule pattern during gastrulation, restricts Dpp activation of *bap* to areas not expressing *slp* in this situation. However, ectopic *slp* expression in a cell competent to express *bap* is by itself insufficient to repress *bap* transcription, suggesting a modulating effect of other segmentation genes on *slp* function.

Analogously, in the *eve* domain, *bap* requires *eve* function, but the effect of *eve* is only partly mediated by *en* and *hh*, since even in *en hh* double mutants *bap* expression is not completely lost (Azpiazu et al., 1996). Together these results show that *slp* and *eve* are jointly responsible for setting up domains in which *tin* can exert its activating effect on *bap*.

The fat body primordium appears to be defined in a different way than the visceral mesoderm. Although, like *bap* expression, *srp* expression is also lost in *eve* mutants, *eve* is not an essential positive regulator of *srp* expression, since *eve slp* double mutant embryos are able to express *srp*. Both in *slp* and in *slp eve* double mutants, we observed a striking correlation of the changes in *en* and *srp* expression, with *srp* in each case expressed exactly in the same pattern as *en* in the ectoderm. There is no indication of a direct, negative effect of *slp* on *srp* expression. Instead, *srp* appears to depend only on positive input from *eve*, *en* and *hh* (Azpiazu et al., 1996; V. R., K. P. Rehorn, R. Reuter and M. L., unpublished data).

The model in Fig. 6 shows that *slp* and *eve* each have activating and repressing effects that may interfere with the other's function. These include the early repression of *slp* by *eve* as well as the effect of each gene on downstream segment polarity genes (not shown in this figure). Apart from repressing *slp*, *eve* must also act at another level to block *slp* function, since ectopic expression of *slp* in the whole segment does not lead to a change of cell fates in the *eve* domain. Thus, even when *eve* repression of *slp* is bypassed, *slp* is still unable to activate the developmental programme of the *slp* domain in the *eve* domain. A similar interplay between permissive and prohibitive activities on mesodermal gene expression occurs at the level of the segment polarity genes *hh* and *wg* (Azpiazu et al., 1996). Thus each step of the pathways leading to the segmental determination of cell fates in the mesoderm appears to have multiple back-ups and relies on more than one mechanism for ensuring correct gene expression in each specific group of cells.

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APPENDIX

In the version of this paper first submitted for publication, we used the term 'anterior' and 'posterior' domain to refer to the repeated units in the mesoderm, as did Azpiazu et al. (1996). Based on the objections of one reviewer, Michael Bate, and the correspondence resulting from the editor sending the review to Manfred Frasch for comments, as well as further discussion between M. Bate, M. Frasch and us, we have renamed the domains after the genes that control their development. We have also changed Fig. 1 to include all boundaries and names that have been used to describe the subdivision of the mesoderm. Since M. Bate and M. Frasch give a very clear account of the reasons for adopting their respective nomenclatures, we felt it would not be sensible to rewrite their discussion for the paper in our own words. Instead, they and the editor kindly agreed to include their correspondence as an appendix to this paper. It appears in a slightly edited form below.

Review by Mike Bate

I urge the authors to think again about the nomenclature that they use for the different mesodermal domains that they describe. As it stands, the way the authors use 'anterior' and 'posterior' to describe domains will be bound to lead to confusion and misunderstanding.

The authors refer to segments and to anterior (muscles, heart) and posterior (visceral mesoderm, fat body) domains within these segments, for example: 'The anterior domain of each segment gives rise to the dorsally located heart precursors and to the majority of

somatic muscles...'; 'In the posterior domain of each segment, the most dorsally located group of cells gives rise to the visceral mesoderm'

Is this confusing? Yes, to the average punter. Two examples will suffice. We think of cells in the embryo in relation to other cells. In this instance, a convenient way of thinking about where the *bap*-expressing cells lie is that they are immediately beneath the invaginating tracheal pits. Where are the tracheal pits for the average embryologist? 'In the *anterior* third of segments T2 through A8' (Manning and Krasnow, 1993, in *The Development of Drosophila melanogaster*, edited M. Bate and A. Martinez-Arias, 1993, Cold Spring Harbor Laboratory: Plainview, NY pp. 609-685). However, according to the proposed nomenclature, the mesoderm immediately beneath them belongs to the '*posterior* domain'.

A second example refers to the other, supposedly 'anterior' domain which include the precursors of most, perhaps all, of the somatic muscles. As the authors point out this corresponds with the high Twist domain. According to Dunin Borkowski et al. (1995), the ventral prolongation of this high Twist domain lies between neuroblast rows 5 and 6. According to all maps of the neuroblasts (and fitting with the results of lineage analysis in terms of what happens to the progeny of the neuroblasts), these two rows lie posteriorly in the segment. Yet, if we follow the proposed scheme, the mesoderm overlying them will form part of the 'anterior' domain in the segment.

Suggestion:

Normally when things are named, we retain the original nomenclature unless this leads to confusion or is simply wrong. In this case, the authors have followed Azpiazu et al. (1996) who make an argument for their A/P designations that they give based on the coincidence of the anterior margin of *bap* expression 'with the parasegmental borders of the ectoderm – thus the primordia of the midgut visceral mesoderm are largely positioned below the posterior compartments of the ectoderm'. As I have argued above this leads to real confusion when compared with the actual arrangement of things in the segments of the embryonic fly. The first naming of these domains in the mesoderm was by Dunin Borkowski et al. (1995) who used the opposite formulation (Anterior domain: low Twist, visceral mesoderm/fat body; Posterior domain: high Twist, heart progenitors, somatic muscles) which brings the mesoderm into register with the commonly used designations for ectodermal structures (tracheal pits, neuroblasts, segment borders) that lie immediately adjacent to them. Dunin Borkowski et al. (1995) were the first to draw attention to the existence of these domains and their likely significance. Is there a good reason why their common sense designation should be overturned?

Reply by Manfred Frasch

I have to disagree with Mike Bate's opinion with respect to the nomenclature of mesodermal domains. I think the confusion is mainly caused by two factors.

(1) The early mesoderm is organized into parasegments (as is the ectoderm) and the mesodermal parasegmental borders coincide with the ectodermal ones. The latter is not necessarily true for the segmental borders (if it makes any sense at all to talk about mesodermal segment borders at this stage). In other words, the sizes of what we call A and P domains differ between ectoderm and mesoderm; they are in register at their parasegmental borders but not at their 'segmental' borders.

(2) After stage 11, much of the mesoderm from the P domains moves inside,with the result that now only cells of the mesodermal A domains (perhaps with exceptions) remain in contact with the ectoderm. Thus, following stage 11, mesodermal cells of the former A domain can underly both the anterior and posterior compartments of the ectoderm.

Because of (1), some cells of the mesodermal P-domains are indeed positioned below anterior portions of ectodermal segments (since mesodermal P-domains seem to be wider than ectodermal P-compartments). Nevertheless, the majority of them are located below the posterior compartments and perhaps all mesodermal cells of the A domains are located below the anterior ectodermal compartments. Therefore, and because they share identical parasegmental borders, it makes perfect sense to give them analogous names. This nomenclature is additionally justified by the observation that A and P domains/compartments are determined by similar regulatory events in both germ layers.

I think much of the confusion can be avoided if one does not talk in terms of segmental units at these early stages, but rather in terms of parasegments. I believe that many of the seemingly contradictory examples cited by the reviewer are due to (2), since they refer to stages during or after segregation of P-cells into the interior. For example, in embryos stained for both *twist* and *bap*, the first signs of Twist modulation become apparent just when the *bap*-expressing cells start moving inside (our own unpublished observations). At this time, a sharp border of *twist*-expression develops right at the anterior borders of the *bap* patches (the parasegmental borders). This, together with the results shown in Azpiazu et al. (1996) and double stainings with mesodermal *eve*-lacZ and *bap*, disagrees with the statement in the Dunin-Borkowski paper that the sharp twist borders are located in the middle of the engrailed domains. I think that, during these cell rearrangements, the high-twist domains spread out to cover more of the ectoderm than initially. Similarly, at late stage 11 when high Twist is seen in ventral triangles, extensive tissue rearrangements have taken place, which may well bring some high-Twist cells below posterior compartments and neuroblasts of the ectoderm.

Considering all this, it seems best to me to go with the A/P and parasegmental nomenclature in the mesoderm, but reserve it for the stages prior to the cell rearrangements that create multiple layers. Segmental nomenclature can (and should only) be used for subsequent stages, particularly when the somatic musculature is considered.

Peter Lawrence to Mike Bate

I have received Manfred's letter and I agree with it. I know that people have always been confused about parasegments and segments, but this arises out of history, they were described in the wrong order, which can not be helped. But, if we want to understand things, we have to accommodate our thinking and descriptions to nature, and not vice versa. I agree with you that the authors need to make it clear that they are talking about parasegments, as they are I believe the only fundamental unit. Segments are largely a figment of our imaginations when it comes to a developmental rather than a traditional or functional description.

Mike Bate to Peter Lawrence

I agree in part with what Manfred says, but I think he misses the point of what I am trying to do, which is to make it easier for people to understand what is going on and to avoid confusing mesodermal domains with ectodermal compartments. First, there is no dispute that the obvious repeat in the mesoderm coincides exactly or approximately with the parasegment border in the ectoderm. However, there is no evidence that mesodermal groupings within either domain correspond with ectodermal compartments. This is most obvious for the *bap* and *serpent* expressing cells and by extension for the segmentally posterior cells of the high Twist domain. These high Twist cells may indeed all be located under the A compartment, but the boundaries of this domain do not correspond with the boundaries of the A compartment except at the parasegment border. Naming the domains A and P has two consequences: (1) it confuses people utterly about the position of these things in the embryo – my points about the tracheal pits and the neuroblasts and (2) it suggests (see the formulation 'compartments/domains') that there is some evidence of compartmental organisation in the mesoderm, which there is not.