Control of cell fates and segmentation in the Drosophila mesoderm

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(with an appendix by Michael Bate and Manfred Frasch)

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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 (Gorzyczka 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; Rehborn 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 CyO34B P[enA]A208.1M2 both slp loci are deleted, on CyO346 P[km2]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 eve211, which, like fushi-tarazu7B and wgJHD2, was from the Tübingen stock collection, the eve, slp double mutant (Df(2L)edSZ1, eve211) 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-Poxmeso (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 CoCl2 and NiSO4 (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 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 Ndel and converting the Ndel site to an Xbal site using the large (Klenow) fragment of DNA polymerase I and (TGCTCTA-GAGCA) linkers. Following digestion of the resulting clone with Xbal and EcoRI, a 1.5Kb fragment containing the entire open reading frame was inserted into pUAST. To create the construct pUAS:Slp1, the Ndel site within the Slp1 cDNA was converted to an Xbal site as described above. The EcoRV site of the clone containing the new Xbal site was converted to a BglII site by addition of (GGAAGATCTTCC) linkers and subsequently a 1.3 kb BgIII/Xbal 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 ppx 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; Gisselfrech 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
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 (Δ34B) 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) pos meso, stage 10. pos 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 pos meso (brown) and en (blue) to demonstrate the location of the pos 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.
reduced numbers of slp-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.
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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REFERENCES


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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...
The authors have followed Azpiazu et al. (1996) who make an argument unless this leads to confusion or is simply wrong. In this case, the 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 ectodermal 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.