Pattern formation in the *Drosophila* embryo: allocation of cells to parasegments by *even-skipped* and *fushi tarazu*

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Summary

The first sign of metamerization in the *Drosophila* embryo is the striped expression of pair-rule genes such as *fushi tarazu* (*ftz*) and *even-skipped* (*eve*). Here we describe, at cellular resolution, the development of *ftz* and *eve* protein stripes in staged *Drosophila* embryos. They appear gradually, during the syncytial blastoderm stage and soon become asymmetric, the anterior margins of the stripes being sharply demarcated while the posterior borders are undefined. By the beginning of germ band elongation, the *eve* and *ftz* stripes have narrowed and become very intense at their anterior margins. The development of these stripes in *hairy*, *runt*, *eve*, *ftz*, and *engrailed* embryos is illustrated. In *eve* embryos, the *ftz* stripes remain symmetric and lack sharp borders. Our results support the hypothesis (Lawrence *et al.* Nature 328, 440-442, 1987) that individual cells are allocated to parasegments with respect to the anterior margins of the *eve* and *ftz* stripes.

Key words: *even-skipped*, *fushi tarazu*, parasegments.

Introduction

When the *Drosophila* egg is laid it is already polarized in the anteroposterior axis, a polarity that becomes translated into a gradient of positional information (Sander, 1960; Driever & Nüsslein-Volhard, 1988). Eventually a series of genetic and epigenetic steps (for example, see reviews by Nüsslein-Volhard *et al.* 1987; Ingham, 1988) divide up the main part of the body, both ectoderm and mesoderm, into fourteen parasegments, each of which will construct a precisely defined part of the larva and adult (Martinez-Arias & Lawrence, 1985). As parasegments are defined, so is each individual cell irrevocably allocated to a compartment of cells that all share one genetically determined fate (García-Bellido *et al.* 1979).

Of the number of segmentation genes classified by Nüsslein-Volhard & Wieschaus (1980), *fushi tarazu* (*ftz*) and *even-skipped* (*eve*) may be complementary because there is evidence that these genes act together to delimit parasegments — *ftz*+ being directly responsible for the anterior borders of the even-numbered parasegments and *eve*+ for the anterior borders of odd-numbered parasegments. Drawing these 'lines' through the blastoderm may allocate each and every cell in the trunk of the embryo to a specific parasegment (Lawrence *et al.* 1987).

Our purpose is to describe the pattern of *eve* and *ftz* expression in greater detail than hitherto. We use staged embryos and study the patterns of expression of both gene products at cellular resolution. We show that the *eve* and *ftz* stripes are asymmetric from midblastoderm, that is the anterior edges of the stripes are more definite than the posterior edges. Later, both *eve* and *ftz* stripes decay from the posterior edge, the anterior boundaries stabilize and sharpen as expression of the genes becomes locally more intense. By the time the germ band is extending, expression of both genes is confined to ragged rows of cells at the anterior borders of each parasegment. In embryos lacking the *eve* gene, the *ftz* stripes are strong and broad but lack asymmetry and do not sharpen. Such *ftz* stripes fail to make metameres suggesting that, in the wildtype, it is the sharp and persistent anterior edges that are the elements of the stripes responsible for this function.

Materials and methods

Antibody labelling

We have used affinity-purified rabbit anti-*eve* and anti-*ftz* sera generously given by Manfred Frasch and Henry Krause (see Frasch *et al.* 1987; Krause *et al.* 1988), as well as anti-β-galactosidase and anti- *engrailed* monoclonal antibodies kindly sent by Chris Doe and Kevin Coleman, respectively. Eggs were dechorionated, fixed in 4% paraformaldehyde/heptane, the vitelline membrane was removed in heptane/methanol and the embryos stored in methanol. The anti-*ftz* serum gave better results if it was preabsorbed — it was diluted x100, mixed with about 20% volume of eggs aged approx. 6-16 h, agitated overnight at 4°C and used at 1:5. The anti-*eve* serum was used at 1:20.

For double labelling (Lawrence *et al.* 1987), batches of
embryos were treated overnight in anti-ftz primary antibody in PBS buffer plus 0·1 % BSA and 0·1 % triton ("BBT"). After washing in BBT and in BBT containing 2 % goat serum they were treated for 2 h in biotinylated goat anti-rabbit IgG (Vectorlabs, preabsorbed against Drosophila eggs of 0–16 h old and used at a total dilution of 1:500). After washing, the embryos were reacted with ABC reagent and then with DAB in PBS buffer plus 0·1 % BSA and 0·1 % triton ("BBT"). After washing in BBT and in BBT containing 2 % goat serum they were treated for 2 h in biotinylated goat anti-rabbit IgG as before. Finally, cobalt and nickel ions (0·03 %) were added to the DAB reaction to give a dark grey colour (Adams, 1981).

For labelling of β-galactosidase, ftz and eve antibodies were applied together as the first step and, after reaction for both with DAB, Doe's monoclonal antibody against β-galactosidase was preabsorbed with 6–16 h embryos and used at 1:10, preabsorbed biotinylated horse anti-mouse IgG (Vectorlabs) was used at 1:500 and the embryos stained with DAB plus Co²⁺ and Ni²⁺. The same procedure was followed for studying en embryos, except that a monoclonal antibody against engrailed was used in the second step — the ascites fluid was preabsorbed against 6–16 h embryos and used at 1:130.

Mounting embryos
The stained embryos were washed, dehydrated and cleared in methyl salicylate. Individual embryos were placed on a slide between two no. 1 (thickness approx. 140 µm) coverslips in Araldite or Canada balsam and then roofed over with a no. 1½ (approx. 170 µm) coverslip. Sideways movement of the grooving coverslip rolls the eggs to the required orientation. For detailed examination of the cells, the eggs were broken open with a fine needle along the midventral axis, the insides removed as far as possible with the needle and the pieces mounted flat. The roofing coverslip was supported either by no. 0 (approx. 100 µm) coverslips, or by glass beads, diameter 40–75 µm.

Photography
Photographs were taken under bright-field or DIC optics using Zeiss WL and Zeiss Axioptoph macroscopes. Ektachrome 64 and Fuji 50 were used, the latter gave somewhat greater colour contrast. For the wildtype series of pictures (Figs 3–6, 15–18) all embryos were taken from one batch of double-stained wildtype embryos and all photographed and printed under standard conditions. Increases in colour intensity with age seen in the stained cells should therefore reflect a genuine increase in the amount of gene products.

Staging
We have restricted this study to cell cycle 14 and onward. Although ftz and eve are transcribed from about cell cycle 12 (Hafen et al., 1984; Macdonald et al., 1986) there is no clear striping of proteins until beyond cell cycle 14.

After cell cycle 14 there is a long interphase; living embryos can be staged by observing the plasma membranes (under DIC optics) which grow down between the peripheral nuclei, eventually cutting up the cortical cytoplasm into individual cells (Foe & Alberts, 1983; Edgar et al., 1987). This whole period (which lasts from some 2 h 30 min – 3 h 15 min after egg laying at 22°C) is called stage 5 (Bownes, 1975; Campos-Ortega & Hartenstein, 1985; Wieschaus & Nüsslein-Volhard, 1986) and we have subdivided it into 3 substages depending on the amount the membranes have grown in, each of about 15 min duration (see Fig. 1). Thus stage 5(1) indicates the first third of stage 5 and 5(3) the last. Gastrulation then follows and this is divided up into stages 6 and 7, each some 15 min long (op. cit. and own observations on living embryos). The germ band begins to extend (stage 8) and our present interest in the process ends with the loss of ftz and eve expression — this occurs as germ band extension is completed. A useful landmark is when the germ band is only partially extended, and when the expression of ftz and eve is still strong, we call this 8(1) and it is some 15 min after the end of stage 7 (i.e. at about 4 h after egg laying at 22°C). Thus, the whole process of stripe maturation is accomplished within about 90 min of development, which we divide into six stages, each of about 15 min (Fig. 1).

Quantification
Although we hope the pictures speak for themselves, we did try and quantify the degree of asymmetry in the stripes of ftz and eve expression. Each of a different batch of embryos was split down the dorsoventral midline, mounted and a specific region was studied under bright-field optics (×63, 1·4NA lens). This included ftz stripes ps 4, 6, 8 and eve stripes ps 5, 7 and 9 (note stripes are numbered according to the parasegments to be defined by the anterior margin of the stripe). An area including these 6 stripes of about 15 cells across was studied for both left and right halves.

We first counted all the cells stained darkly for each antigen and then those that were palely stained, separately noting those that were at the anterior or the posterior edge of the

Fig. 1. A summary of the criteria used to stage the embryos (see text). For subdividing stage 5 the ingrowing membranes are observed, for stages 6 and 7 the ventral and cephalic furrows provide a guide, and for stage 8(1) the extent of germ band elongation is used (see methods). Timings are approximate (22°C).
strips. Unstained cells were also counted (these could be compared with cells anterior to the first eve stripe which express neither eve nor ftz). The staining for ftz was clearer than for eve so the ftz figures are more reliable. This could not be done before stage 5(2) as all the cells stain so weakly. Some raw data are shown in Table 1.

**βgalactosidase expression**

We used ftz-βgal (Hiromi et al. 1985) and eve-βgal constructs (Lawrence et al. 1987) to see if the native ftz and eve patterns of expression are identical, cell-by-cell, to that of the βgalactosidase. Unfortunately, in these stocks, the βgalactosidase does not stain sufficiently intensely until the beginning of germ band elongation. By that time the two gene products are coextensive at the anterior margins of the stripes, but βgalactosidase (presumably because it accumulates over a longer period as it is much more persistent (compare Hiromi et al. 1985; Edgar et al. 1986)) is graded and is detected further back (Fig. 24 for ftz and ftz-βgal, eve-βgal not shown). The anterior margins of stripes of both βgalactosidase (Lawrence et al. 1987) and the native ftz protein (Carroll et al. 1988b) are coextensive with the anterior margin of the engrailed stripe. These results confirm that the βgalactosidase pattern reflects an integration over time of the pattern of expression of the native ftz and eve genes (Lawrence et al. 1987).

**Fly stocks**

For eve− embryos, eveR13/Balancer and Df(2R)eveR1−27/Balancer were crossed (eveR13 is a null mutation and Df(2R)eveR1−27 lacks the entire gene, Nüsslein-Volhard et al. 1984, 1985).

For hairy− (h−) embryos, hL79k/Balancer and hE32/Balancer were crossed (both these hairy alleles are considered to be nulls, Ingham et al. 1985a).

For runt− embryos runLBS/Balancer and Df(1)B102, run−/Balancer were crossed (runLBS is considered to be a null, Gegen & Wieschaus, 1986).

For ftz− embryos, ftzW20/Balancer were crossed to Df(3R)4 Scb/Balancer (ftzW20 is considered to be a null allele (Weiner et al. 1984) and Df(3R)4 Scb lacks the entire gene). Embryos from these four crosses were collected and double-stained for ftz and eve as before. The eve− embryos could be recognized as they lacked all eve antigen. The ftz− embryos had a few scattered weakly stained brown cells, which were not simply background as they were only found in the domain where ftz is expressed (up to 70% egg length). The h− and runt− embryos were identified by their markedly abnormal staining patterns.

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**Table 1. The staining of a midlateral set of cells in presumptive parasegments 4–9 inclusive**

<table>
<thead>
<tr>
<th>Stage</th>
<th>Estimated age*</th>
<th>Cells in ftz stripes</th>
<th>Weak ant. cells</th>
<th>Weak post. cells</th>
<th>Cells in eve stripes</th>
<th>Weak ant. cells</th>
<th>Weak post. cells</th>
<th>Unstained cells</th>
<th>Total cells</th>
</tr>
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<tr>
<td>5(2)</td>
<td>180</td>
<td>45</td>
<td>1-3</td>
<td>5-0</td>
<td>39</td>
<td>1-8</td>
<td>7-1</td>
<td>2-2</td>
<td>638</td>
</tr>
<tr>
<td>5(3)</td>
<td>195</td>
<td>41</td>
<td>1-0</td>
<td>5-6</td>
<td>33</td>
<td>1-5</td>
<td>9-7</td>
<td>17</td>
<td>616</td>
</tr>
<tr>
<td>6</td>
<td>210</td>
<td>36</td>
<td>0</td>
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<td>7-0</td>
<td>27</td>
<td>683</td>
</tr>
<tr>
<td>6</td>
<td>210</td>
<td>35</td>
<td>0-6</td>
<td>5-4</td>
<td>30</td>
<td>0-3</td>
<td>7-0</td>
<td>22</td>
<td>681</td>
</tr>
<tr>
<td>7</td>
<td>225</td>
<td>33</td>
<td>0-3</td>
<td>5-9</td>
<td>26</td>
<td>0-6</td>
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<td>28</td>
<td>0-3</td>
<td>7-0</td>
<td>22</td>
<td>680</td>
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<tr>
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<td>28</td>
<td>0</td>
<td>5-1</td>
<td>25</td>
<td>0</td>
<td>6-5</td>
<td>35</td>
<td>629</td>
</tr>
</tbody>
</table>

*In minutes after egg laying at 22°C.

† eve staining too difficult to score at this stage.

Figures are percentages of total cells scored (right hand column), there are two different embryos for each of stages 6 and 7, to show consistency of results.

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**Fig. 2.** Measures of stripe maturation in the wildtype. ordinate: % of total cells scored.

As development proceeds the proportion of cells strongly expressing ftz and eve diminishes. Some of the cells at the edges of the stripes express ftz and eve only weakly, the majority of these are at the posterior edge, with a diminishing number at the anterior. The slope marked A indicates the proportion of all weakly stained cells that are located at the anterior edge of the stripe; it falls from 20 to 0 % over the period studied. Compare with Figs 3–5 and 15–18. See Table 1.

For en− embryos, eggs from a Df(2R)en−8/Balancer stock (see Gubb, 1985) were collected and labelled as described under antibody labelling.

For studying ftz-βgal (Hiromi et al. 1985) and eve-βgal (Lawrence et al. 1987) distribution, stocks carrying either one or both these constructs were used as described under antibody labelling above.

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**Results**

The main results are the Figs 3–6 which show the refinement of the ftz and eve stripes from the beginning of the 14th interphase in the blastoderm to the middle of germ band extension. Low-power pictures of embryos are shown in Figs 15–18, these give an overview of the process. Initially, the ftz (brown stain) and eve (grey stain) gene products are widely distributed but by stage 5(2) (Figs 3,15) weak stripes are visible. By 5(3)
(Figs 4,16) some unlabelled cells show between the stripes and, as the stripes intensify, these gaps widen. The stripes are asymmetric (sharper anterior boundaries, softer posterior ones) from stage 5(3) but this becomes more obvious as they mature. By the beginning of gastrulation the distribution of both ftz and eve appears graded, stain is more concentrated in cells anteriorly, less so posteriorly.

Sharpening of the different stripes does not occur at the same rate – for example, the eve stripe corresponding to parasegment (ps) 1 is relatively sharp, and the ftz stripe corresponding to ps4 relatively fuzzy – details of this sort can be seen in the figures and they are reproducible. Unstained cells begin to appear first in the posterior regions of the presumptive even-numbered parasegments about stage 5(3) (Figs 4,16) and our impression is that this is due to loss of eve label (Figs 15,16). By stage 8(1) extension of the germ band is associated with increasing cell cross-sectional area, and cell rearrangement (Figs 6,18). At this stage the parasegments may vary from 2 to 6 cells across.

We have monitored the proportion of cells expressing ftz or eve (Fig. 2). Table 1 (see Methods) also shows how the stripes sharpen, with the number of weakly staining cells at the posterior edge of the stripes always being far more than at the anterior edge; weakly stained anterior cells drop to none by stage 8(1) – see Fig. 2.

Mutant phenotypes

There are many aspects of the altered ftz and eve expression that could be discussed but as we suspect that the crucial aspect of these stripes in the wildtype is the sharp anterior edges (because they delimit the parasegment borders, Lawrence et al. 1987) we will look particularly at the presence or absence of such edges in the various mutant phenotypes.

Pattern in hairy~

The hairy gene is required for proper expression of ftz and eve (Carroll & Scott, 1986; Howard & Ingham, 1986; Frasch & Levine, 1987; Carroll et al. 1988a). Figs 7,8,19 and 20 show expression of ftz and eve in hairy~ embryos at stages 5(2) and 6. Initially, the ftz and eve expression are strongly overlapping, contrasting with the wildtype where such overlap is weak and transient. As the pattern resolves, many more cells than normal express ftz and only a few come to express eve strongly.

From stage 6, it is clear there is almost no vestige of the eve stripes except for ps1, while the ftz stripes (presumably those equivalent to ps2, 4, 6, 8, 10, 14 in the wildtype) have fairly well developed anterior edges – although, with the possible exception of ps8, they are not as sharp or as straight as the wildtype (Fig. 20). The ftz stripes do narrow and sharpen with time, although not as much as the wildtype.

Thus, apart from ps1, which should develop almost normally, the hairy~ embryos should have only the anterior margins of even-numbered parasegments – and these should, with the exception of ps8, be less sharp and straight than in the wildtype.

Pattern in runt~

The runt gene is also required for the proper expression of ftz and eve (Carroll & Scott, 1986; Frasch & Levine, 1987) and from the beginning the patterns are altered in a complex way (Figs 9,10,21,22). Initially the ftz stripes corresponding to ps4, 8, and 14 are well developed, but 2, 6, 10, 12 are fading away. Later (Figs 10,22) ftz expression becomes limited to a few irregular rows of cells and a variable pattern of eve stripes survives, with irregular boundaries, some of which (such as that of ps13) are quite sharp. Some of these stripes appear to be sharpening at both anterior and posterior edges, suggesting there is some local reversal of polarity. The cuticle pattern of runt~ embryos does show zones of opposing polarity (Nüsslein-Volhard & Wieschaus, 1980).

Pattern of ftz expression in eve~

As described by Carroll & Scott (1986), it is surprising that the pattern of expression of ftz is not much altered in eve~ embryos, even though such embryos produce larvae which show little residual metamerization (Nüsslein-Volhard et al. 1984), and do not express most of the engrailed stripes (Harding et al. 1986). However, the ftz stripes are abnormal in that they remain broad and do not sharpen at their anterior edges; weakly stained cells persist at both margins and the stripes remain symmetric (Figs 11,12,23).

Pattern of eve expression in ftz~ embryos

Staged ftz~ embryos (distinguished from siblings because they failed to express ftz) appeared to express eve~ quite normally, with the eve stripes maturing as in wildtype (Figs 25,26).

Pattern of ftz expression in en~ embryos

As striped engrailed expression begins soon after that of ftz and eve (Weir & Kornberg, 1985; DiNardo et al. 1985) it seemed possible that the coexpression of engrailed with ftz and eve in the anterior parts of the parasegments (Lawrence et al. 1987; Carroll et al. 1988a) might be essential for the narrowing and shar-
Figs 11,12. *ftz* expression in *eve*~−~ embryos (stages 5(3) and 7). Note that the stripes are broad and symmetric, they do not narrow or sharpen. The spacing of stripes varies from embryo to embryo (compare Fig. 23). ×1250.

Figs 13,14. Young extending germ band 8(1) to show *ftz* stripes marking anterior margin of ps4, 6 and 8. These embryos have been stained for *ftz* (brown) and for *engrailed* (grey). Fig. 13 is *en*~+~ and 14 is *en*~−~, the nascent *engrailed* stripe which is easy to see elsewhere in the embryo, is just detectable in Fig. 13 (arrows). We can detect no difference in the *ftz* stripes.

Figs 7,8. Midlateral view of *hairy*~−~ embryos, stained for *ftz* and *eve* as in Figs 3–6. Fig. 7 stage 5(2). Note that *eve* and *ftz* overlap considerably giving a grey–brown colour. Fig. 8, stage 6. Only few cells now express *eve*, while *ftz* expression has intensified but is patchy. Staining is best assessed by comparing nuclei stained for *eve* (e), *ftz* (f) or both (ef) with the background colour seen in unstained nuclei (b). The grey–brown colour which is due to coexpression of *eve* and *ftz* is also clearly seen in Figs 19 and 20.

Figs 9,10. Similar view of *runt*~−~ embryos at stages 5(2) and 6. The *ftz* stripes narrow while *eve* expression becomes patchy (compare Figs 19 and 20). All ×1250.
Figs 15–18. Wildtype half embryos stained for ftz and eve as before. Stages 5(2), 5(3), 6 and 8(1). Fig. 17 shows the ftz stripes numbered according to the parasegments their anterior margins delimit.

Figs 19, 20. hairy~ embryos stained for ftz and eve as before; stages 5(2) and 6. Arrows mark the presumed anterior margins of indicated parasegment borders.

Figs 21, 22. rnt~ embryos stained for ftz and eve as before; stages 5(2) and 6.

Fig. 23. ftz expression in an eve~ embryo, stage 7.

Fig. 24. Lateral view of embryo (stage 8(1)) stained for both ftz and eve (both brown) and then with βgalactosidase (dark grey). The stock carried a ftz-βgal construct, and the picture purports to show cell-by-cell correspondence of the βgalactosidase and ftz staining at the anterior margins of the even-numbered parasegments. This correspondence of staining, which is more easily seen down the microscope by focusing up and down, can be pictured by the sympathetic reader if he or she carefully compares the even-numbered stripes (ftz, brown nuclei plus grey cytoplasm) with the alternating ones (eve, brown nuclei only).

Figs 25, 26. Lateral view of wildtype and ftz~ embryos stained as before for ftz and eve (stage 7). In the ftz~ embryo (Fig. 26) scattered cells show ftz staining but they are not striped. The eve stripes are similar in both wildtype and ftz~. All the 14 parasegments that are basic to the body plan (Martinez-Arias & Lawrence, 1985) are indicated in Fig. 25.

pening of the ftz and eve stripes. This was not the case for ftz however; en~ embryos, easily detected at stage 8(1) because they lacked any engrailed protein, had ftz stripes that were indistinguishable from their en~ siblings (Figs 13–14). We did not test for effect of en~ on eve stripes.

Discussion

The development of the ftz and eve stripes has been extensively studied by in situ hybridization (Hafen et al., 1984; Ingham et al., 1985b; Martinez-Arias & Lawrence, 1985; Weir & Kornberg, 1985; Harding et al., 1986; Macdonald et al., 1986), by means of antibodies (Carroll & Scott, 1985; Frasch et al., 1987; Frasch & Levine, 1987; Carroll et al., 1988a; Frasch et al., 1988; Krause et al., 1988) and the use of reporter gene constructs (Hiromi et al., 1985; Lawrence et al., 1987). From these studies, only some of which approach cellular resolution, cellular interpretations have been made (Duncan, 1986; Gergen et al., 1986; O'Farrell & Scott, 1986; Akam, 1987; DiNardo & O'Farrell, 1987; Scott & Carroll, 1987; Frasch et al., 1988; Ingham, 1988; Ingham et al., 1988; Martinez-Arias et al., 1988; Weir et al., 1988). We have provided photographs of staged embryos that describe the process at cellular resolution, so that theoretical descriptions and models can be given a firmer basis. We have also described some mutant phenotypes.

It is clear from the photographs and from cell counts (Table 1, Fig. 2) that the eve and ftz protein stripes can be first resolved midway through the blastoderm stage (stage 5(2) – for stages see Methods). By stage 5(3) they are already somewhat asymmetric. The asymmetry increases as their anterior edges stabilize and sharpen by the beginning of gastrulation about 30 min later. The definitive anterior boundaries must become established around the time of cellularization because there are so few weakly stained anterior cells at that time (stage 5(3)–6). During gastrulation and thereafter, the stripes intensify at the anterior edge, and narrow from the posterior edge. This behaviour of the stripes was predicted from the anteroposterior gradient of intensity, and sharp and stable anterior edges of the stripes, of ftz-βgalactosidase and eve-βgalactosidase (Lawrence et al., 1987). We also suggested that one main wildtype function of the eve gene is to delimit the anterior boundaries of the odd-numbered parasegments – that is, in effect, to allocate each and every cell near to the anterior edges of the eve stripes to a parasegment. We proposed that ftz does the same with respect to the anterior boundaries of the even-numbered parasegments, so that each parasegment becomes defined as those cells that lie between one delimiting ftz boundary and one delimiting eve boundary (Fig. 6; Lawrence et al., 1987; Lawrence, 1987, 1988).

This hypothesis can be contrasted with a class of hypotheses that consider ftz and eve to label those cells in the stripes and distinguish them from those not in the stripes. Such hypotheses are that ftz, eve and other pair-rule genes (such as paired, see Kilcherr et al., 1986) are expressed in stripes that overlap and that the combination of active and inactive genes allocates each cell to a particular state which defines its subsequent development (Gergen et al., 1986; Scott & O'Farrell, 1986; Akam, 1987; DiNardo & O'Farrell, 1987; Scott & Carroll, 1987; Carroll et al., 1988a; Frasch et al., 1988; Ingham, 1988; Ingham et al., 1988; Martinez-Arias et al., 1988; Weir et al., 1988). To take just one example from many, Ingham et al. (1988) have suggested that the wingless gene (which becomes expressed in cells near the posterior edge of each parasegment, Baker, 1987) may be activated in certain cells as, and because, they become free of eve and ftz gene products. As the eve and ftz stripes narrow further, Ingham et al. suggest that activation of other genes (such as odd-paired and paired) may stop wingless becoming expressed more and more anteriorly. Models of this type treat gene expression as under digital control (Scott & O'Farrell, 1986) and complex combinatorial and hierarchical models can be built (see Martinez-Arias et al., 1988; Ingham, 1988). However, these models describe events at the cellular level, but mostly depend on descriptions of gene expression at less than cellular resolution. Thus, they treat the stripes of gene expression as if they had sharp limits and as if all cells fall clearly into or out of the stripes – as we have shown, this is not the case. Further, by elaborating beyond the careful description of Carroll & Scott (1985) the myth has grown up that the ftz stripes and interstripes are initially four cells wide and then change to being three cells on, five cells off. This digital picture has been used to build models (op. cit.) that clash with our observations that the ftz and eve stripes are asymmetric, graded, and vary
markedly in cellular width (see Figs 3–6).

It is our hypothesis that ftz and eve are the first genes, in development of the anteroposterior axis, to allocate cells to a parasegment, that is to a limited cell fate or 'genetic address' (Garcia-Bellido et al. 1979) that is stable through cell lineage. Once parasegment borders have been defined they must be maintained and they could limit gradients of positional information (for references see Wolpert, 1969; Lawrence, 1973). Under this hypothesis subsequent expression of genes such as engrailed and wingless would result from the interpretation of the gradients, in the same general way as the segments of Galleria become subdivided into several zones bearing oriented scales (Marcus, 1962; see Lawrence, 1973). Since our Fig. 6 and the pattern of ftz expression (Fig. 24 and Lawrence et al. 1987) do suggest that ftz and eve expression become graded it is even possible that the concentrations of these gene products themselves are interpreted, directly or indirectly, by the cells (like the bicoid protein, Driever & Nüsslein-Volhard, 1988) – but many other interpretations are equally plausible.

From the theoretical bias outlined above, we can look at the results of ftz and eve expression in mutants. In h' mutants, the pattern at gastrulation has resolved into broad ftz stripes with edges of variable sharpness (Figs 8,20). Nevertheless one could draw the anterior margins of ps2, 4 and 8 fairly well, while 6, 10, 12 and 14 would be more indefinite and uneven. Apart from the first eve stripe, which has a clear anterior edge, the others have disappeared – although there are some scattered cells expressing eve. This phenotype predicts the hairy cuticle phenotype well – we expect parasegments 1, 2, 4, 6, 8, 10, 12, and 14 to develop anterior borders with variable success, and, because of the lack of the anterior borders of odd-numbered parasegments, to be initially of double width. The cuticle phenotype should therefore resemble that of weak mutant alleles of eve – with the exception of parasegment 1 – and this is the case (Nüsslein-Volhard et al. 1982; Ingham et al. 1985a,b). Our results help understand how the h phenotype appears to be a consequence of the change in domains of activity of ftz and other pair rule genes . . . (Howard & Ingham, 1986). Unfortunately, the wildtype function of hairy remains mysterious. Fig. 7 shows that, by stage 5(2), many cells express both ftz and eve simultaneously in hairy embryos (such strong overlap does not happen in the wildtype), suggesting that the hairy gene product is needed from the beginning for separating the emerging ftz and eve stripes.

The expression of eve and ftz in runt embryos is very abnormal from an early stage (Figs 9,10,21,22), and is completely different from hairy embryos. We do not understand it.

Embryos that are eve provide good evidence for our hypothesis. The ftz stripes in eve embryos are well developed but are symmetric and lack sharp boundaries. Over most of the embryo the ftz stripes appear to be completely ineffective – they lead to no expression of engrailed (Harding et al. 1986; Frasch et al. 1987) and there is no other evidence of metameterization in eve embryos (Nüsslein-Volhard et al. 1984). How the eve gene product helps sharpen the anterior boundaries of ftz expression is unclear, but we conjecture that it is the lack of a sharp boundary that makes them ineffective.

The interdependence of eve and ftz is not reciprocal, for the boundaries of the eve stripes appear normal in ftz embryos (see Fig. 26); it therefore seems unlikely that the ftz and eve genes simply compete for territory by mutual repression. It is known that eve later becomes weakly expressed in the even-numbered parasegments (Macdonald et al. 1986; Frasch et al. 1987) and thus may act directly on the ftz-expressing cells there.

Attempts to model the development of eve and ftz stripes are now being made (Lacalli et al. 1988; Edgar et al. 1988), they treat the stripes as symmetric: if our hypothesis of the importance of asymmetry – the sharp and stable anterior border and the indefinite and unstable posterior border – is correct, new models will be needed.

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