Regulation of segment polarity genes in the *Drosophila* blastoderm by *fushi tarazu* and *even skipped*

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During the late cellular blastoderm stage of *Drosophila* embryogenesis the segmentation genes *engrailed*, *en*, and *wingless*, *wg*, become expressed in two series of 14 stripes[1–4] which will subsequently coincide with the anterior[5] and posterior[4] limits of each parasegment[6]. Previous studies[7–10] have shown that the establishment of the pattern of *en* stripes depends upon the activity of the homoeobox-containing pair-rule genes[11] *fushi tarazu*, *ftz*[12] and *even skipped*, *eve*[8,13]. Here we show that these two genes also control the spatial expression of *wg*. Whereas *ftz* and *eve* behave as activators of *en* we find that both genes are required to repress *wg* expression, so that *wg* becomes expressed only in the narrow stripes of cells which come to separate the *ftz* and *eve* bands at the end of the blastoderm stage. In contrast, we propose that the precise positioning of the *en* stripes depends upon signals generated in a combinatorial manner[14] by the overlaps between the *ftz* or *eve* domains and those of other pair rule genes, specifically *odd paired*, *opa*[15] and *paired*, *prd*[11,16,17].

In the early *Drosophila* embryo, the transcripts of several pair-rule genes accumulate in a series of rapidly evolving and partially overlapping stripes along the antero-posterior body axis[8,9,17–19]. Although some of these genes encode functions primarily involved in the refinement of these patterns[7,20,21] others appear to play a more direct role in establishing the final subdivision of the embryo into parasegments. Good candidates for the latter class are the *ftz* and *eve* genes both of which encode homoeo-domain proteins[8,12,13] and act as regulators of *en* expression[7–10]. By the end of the blastoderm stage, transcripts of both genes are present in a series of regularly spaced stripes, three nuclei wide, which are precisely out of phase with one another[8,9]. The evolution of both patterns requires the function of the two 'primary' pair-rule genes *hairy* and *runt*[7,21]. As the stripes of these and other pair-rule genes undergo a continuous narrowing[8,9,18,19] it is not clear when and where their expression is critically required. It has recently been suggested that only the anterior margins of the *eve* and *ftz* stripes have an instructive role[22], serving to define the boundaries of parasegments by the activation[7–9] of the *en* gene. Such a model raises the question as to how the precise domains of other segment polarity genes, such as *wg*[4], are defined in the blastoderm. To investigate this question we have analysed the effects of *ftz* and *eve* mutations on the activation of *wg* expression.

Expression of *wg* in mutant *ftz* and *eve* blastoderms was monitored by hybridizing tritiated antisense *wg* RNA to sections of embryos derived from parental flies heterozygous for transcript-minus mutant alleles of *ftz* (*ftz*^{W20} and *Df (3R) 4Scb*) or *eve* (*Df* (2*R*) *eve*$^{1.27}$). Mutant embryos were identified by hybridizing adjacent sections with labelled *ftz* or *eve* probes, respectively. Typical patterns of *wg* expression in both mutants are shown in Fig. 1. In both cases there is a reduction in the number of *wg* stripes established; most of those remaining are significantly wider than in wild type, in most cases spanning five cells rather than one. The simplest way of explaining how such patterns might arise is to suppose that each novel broad stripe is generated by filling in between consecutive pairs of normal *wg* stripes. In the case of *ftz*, this would occur between stripes 1 and 2, 3 and 4, 5 and 6 and so on, and in the case of *eve*, between stripes 0 and 1, 2 and 3, 4 and 5 and so on. (See Fig. 1*d* and *e*). This 'filling in' would be due to the failure to repress *wg* expression within the normal *ftz* or *eve* domains in the absence of either gene. The exceptionally broad anteriormost stripe seen in *eve* embryos would presumably reflect not only the requirement for *eve* repression between alternate pairs of *wg* stripes but also the premature decay of the first *ftz* band seen in *eve* embryos[9,20].

According to this interpretation, *wg* would normally only be expressed in the regions between the *eve* and *ftz* bands, which, at the end of the cellular blastoderm stage, are three cells wide and separated by one cell[8,9,13]. To test this interpretation we have mapped the *wg* domains with respect to the *eve* domains in *ftz*$^-$ embryos. As *eve* expression is independent of *ftz* function[9] we would expect that within the metameric region of *ftz*$^-$ embryos all cells will express either *eve* or *wg*. An example of a section of a *ftz*$^-$ embryo hybridized with *wg* and *eve* probes is shown in Fig. 2*c*. As expected, all the cells in the metameric region are labelled, the signal due to hybridization to *wg* transcript being weaker than that due to hybridization to *eve* transcript.

If each *wg* domain coincides with the posterior limit of each parasegment at the cellular blastoderm stage, as it does in extended germ-band embryos[4], then it follows from these results that the anterior margin of each *ftz* and *eve* transcriptional domain should be precisely in register with the anterior border of each parasegment. Such an inference is consistent with the finding that, in the extended germ-band embryo, the stripes of cells which express *en* protein coincide with the anteriormost cells expressing the β-galactosidase gene under the control of the *ftz* or *eve* promoters[22]. To confirm this, we hybridized wild-type blastoderm embryos with a mixture of *ftz* and *en* probes. We find that the *en* stripes, which define the anterior margin of each presumptive parasegment, do indeed coincide with the anterior margins of the *ftz* stripes (Fig. 2*d*).

Previous studies have shown that the activation of the even-numbered *en* stripes depends on *ftz*$^+$ function[7]. By contrast, *eve*$^+$ function is required for the establishment of both the odd-numbered and even-numbered *en* stripes[8,9] (see Fig. 3*a*). This latter function of *eve* can be ascribed to its expression in seven additional minor stripes which lie within the *ftz* domains and first become visible at the end of the blastoderm stage[8,13]. There are, however, *eve* alleles which retain some activity and remove only the odd-numbered *en* stripes[10], suggesting that the principal function of the gene in its major domains of expression is analogous to that of *ftz*. Such a congruity is supported by our finding that both genes regulate the spatial expression of *wg* in an analogous fashion.

It has been suggested that the main function of the *ftz* and *eve* stripes is the demarcation of the parasegment boundary and that expression of either gene away from this boundary may be immaterial[22]. Our finding that removal of *ftz* or *eve* activity leads to the de-repression of *wg* in all nuclei which normally express these genes argues strongly against this view. Also it is clear that the dynamic properties of the *ftz* and *eve* expression patterns are crucial to the function of these genes, the narrowing of each pair-rule stripe from four to three nuclei being the necessary condition for the establishment of the *wg* domains. By contrast, *en* is activated within the *ftz* or *eve* stripes, but in only one of the three nuclei which express either pair-rule gene (see Fig. 3*b*). This suggests either that *en* responds to a threshold value of *eve* or *ftz* activity within their respective domains or alternatively, that the *en* stripes are specified by the combined expression of *ftz* or *eve* and some other pair-rule gene activities (see ref. 7). A good candidate for generating such a combinatorial signal is the *paired*, *prd*, gene[11,16,17], because absence of *prd* function results in the elimination of the odd-numbered

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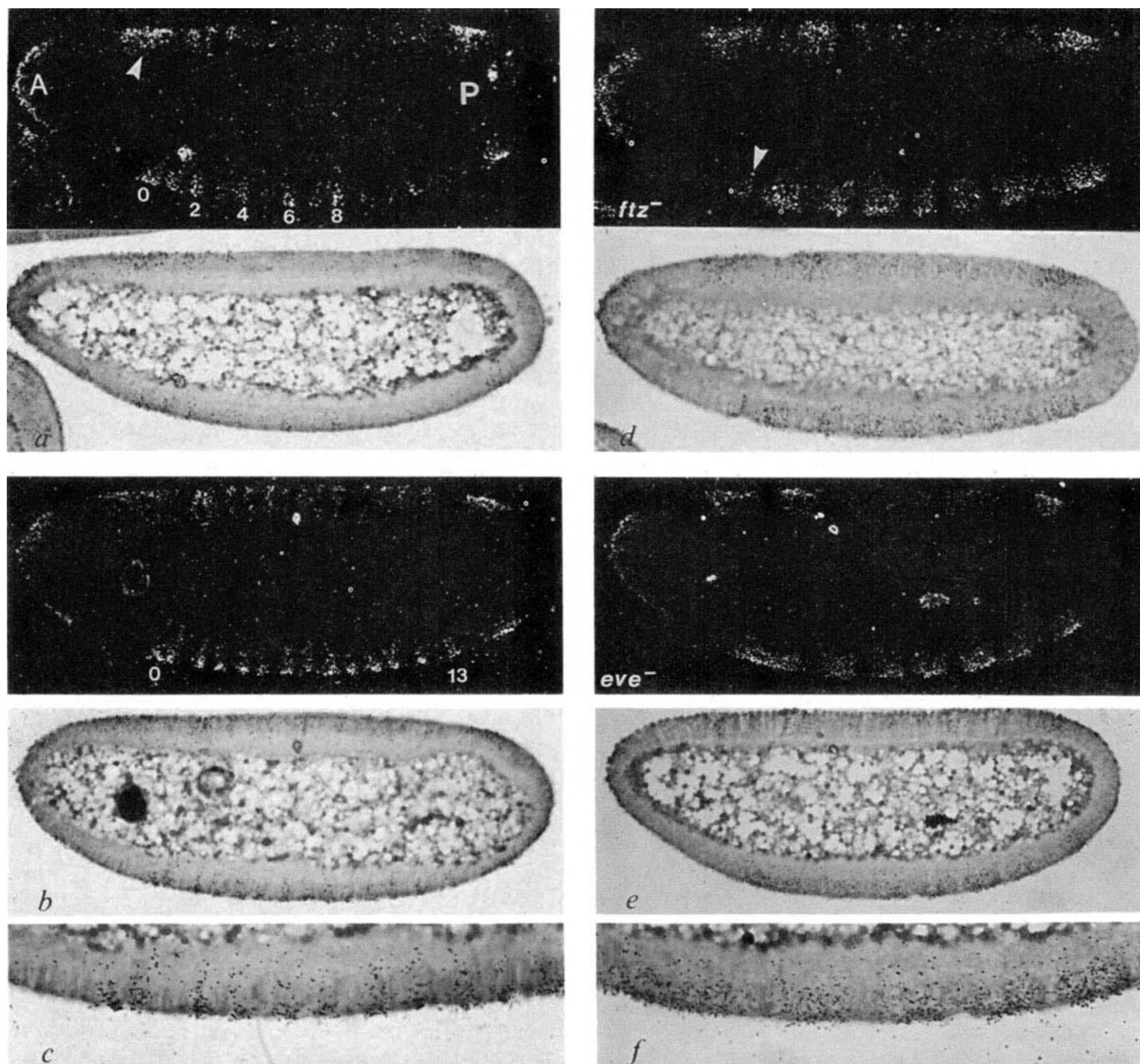

Fig. 1 Dark-field and bright-field images of wild-type (*a–c*) and mutant (*d–f*) embryos. The onset of *wg* expression in the wild-type blastoderm (*a*) is characterized first by the appearance of two sites of transcript accumulation: around the anterior pole (A), and in a broad stripe near the posterior pole (P). Subsequently a third region of accumulation is established antero-dorsally (arrowed). This expression precedes the generation of 14 stripes throughout the presumptive metameric region. The stripes, numbered 0–13 to indicate their parasegmental location, appear in an antero-posterior progression, the odd-numbered ones appearing slightly in advance of their even-numbered neighbours (see *a*). By the onset of gastrulation (*b*) 14 stripes are present between 15% and 75% EL (EL—egg length 0%—posterior pole). Each stripe spans about one nucleus (see detail in Panel *c*). In $ftz^{W20}/Df(3R)$ *4Scb* embryos (*d*) only the most anterior stripe (arrowed) is of normal width. Posterior to the cephalic fold, seven broad stripes of *wg* expression form. Each of these spans about five nuclei. *e*, Expression of *wg* in a *Df* (2*R*) $eve^{1.27}$ blastoderm. As in the case of the *ftz* mutants, fewer but broader stripes of *wg* expression are present. In this case the most anterior stripe is very broad, spanning 12–13 nuclei. This region corresponds to that demarcated by stripes 0 and 3 in the wild-type. Posterior to this are a further five stripes, each around five nuclei wide (see detail in Panel *f*). An identical pattern of expression is seen in embryos hemizygous for the allele eve^{R13} (data not shown).

Methods. Embryos were fixed, embedded and sectioned as previously described[19]. Single-stranded RNA probe, labelled with ^{3}H, was prepared from the T3 promoter of plasmid pwg-c14a (ref. 4), and hybridized to the sections as described[19]. Mutant embryos, were identified by hybridizing adjacent sections with probes for either the *ftz* or *eve* transcript which have been described elsewhere[7,8].

en stripes[10,23] (see Fig. 3*a*). The expression pattern of *prd* in the late cellular blastoderm differs from that of *ftz* or *eve*, being expressed in 14 bands each two cells wide (with the exception of the most posterior band)[17]. Double labelling experiments have suggested that alternate *prd* stripes lie adjacent and posterior to each *ftz* stripe[17]. As each *prd* stripe is two cells wide, it follows that alternate stripes will overlap the anterior part of each *eve* band by one cell (see Fig. 3*b*). Thus the combination of *eve* and *prd* expression could serve to define the position of the odd-numbered *en* stripes that require the activity of both genes for their establishment. By symmetry, we suggest that a similar signal is provided by the combined expression of *ftz* and the pair-rule gene *opa*, to establish the even-numbered *en* stripes. Indeed, absence of opa^+ function results in the elimination of these stripes[23,24] (see Fig. 3*a*) and the *opa* phenotype is approximately the reciprocal of that of *prd*[25]. We therefore surmise that *opa* will exhibit a reciprocal pattern of expression (see Fig. 3*b*). These patterns of expression give rise to at least two other combinations of gene activities, namely *ftz* + *prd* and *eve* + *opa*, which may be used to specify the domains of expression of other segment polarity genes. Absence of prd^+ or opa^+ function also results in the elimination of alternate stripes of *wg* expression (data not shown; see Fig. 3*a*). This function of *prd* and *opa* appears redundant with respect to the specification of

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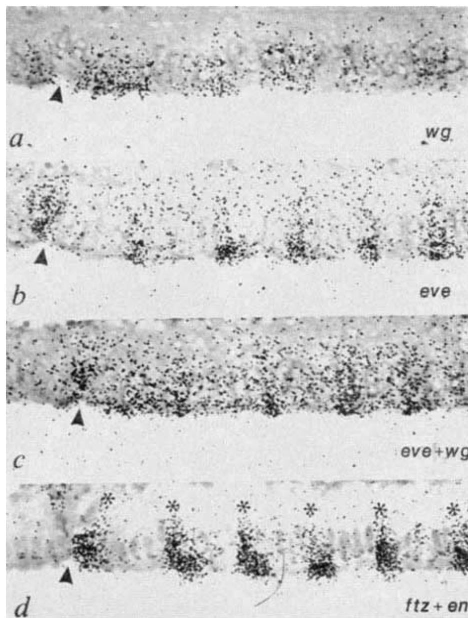

Fig. 2 *a*, Part of a saggital section of a $ftz^{W20}/Df\ (3R)\ 4Scb$ embryo hybridized with *wg* probe showing the broad bands of *wg* transcript accumulation. The adjacent section of this embryo was hybridized with *eve* probe (*b*), revealing the seven-striped pattern of *eve* expression, which is independent of ftz^+ function. The next section (*c*) was hybridized with a mixture of *wg* and *eve* probes. All of the cells are labelled, indicating that the broad stripes of *wg* expression lie in between the *eve* stripes and therefore correspond to the regions where *ftz* would normally be expressed. Arrowheads, position of the cephalic fold. *d*, The relationship between the *ftz* and *en* transcriptional domains. A saggital section through the ventral region of an embryo which has just begun gastrulation. Arrow head, cephalic fold. The section was hybridized with a mixture of tritiated *en* and *ftz* probes. The grains due to hybridization of the *en* probe extend deep into the cytoplasm of the cells whereas the *ftz* signal is restricted to the periphery. At this stage only the even-numbered *en* bands can be clearly visualized. The anterior margin (to the left) of each *ftz* domain can be seen to coincide with the even-numbered *en* stripes (*).

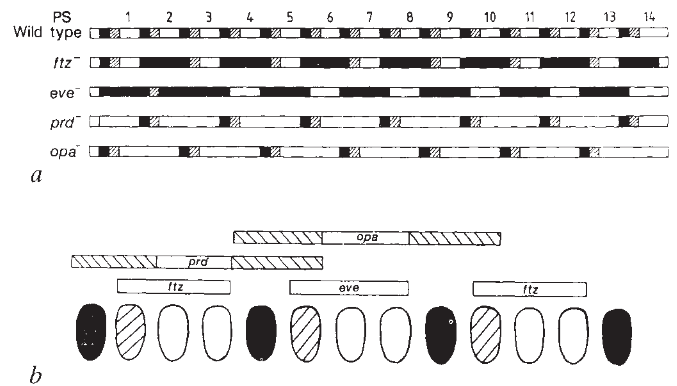

Fig. 3 *a*, Schematic representation of the patterns of *en* and *wg* expression in the wild-type cellular blastoderm and in four pair-rule mutants. Hatched bars represent *en* domains, solid bars *wg* domains. Absence of *eve* or *ftz* expression results in the expression of *wg* throughout the normal *eve* or *ftz* domains. In the case of *eve* there is an additional de-repression of *wg* between the first and second *eve* domains, presumably due to the repression of the first *ftz* stripe by the *eve* mutation[20]. In contrast, absence of *prd* or *opa* function results in the elimination of alternate *wg* bands and of the adjacent *en* bands, but the remaining bands are of normal size and position (data not shown). *b*, Schematic representation of part of the periphery of a blastoderm embryo showing the relation of the known *ftz*, *eve* and *prd* transcriptional domains, and a postulated domain of *opa* expression. At the end of the blastoderm stage the *ftz* and *eve* bands have narrowed to an average width of three cells and are separated by a single cell[8,9]. At the same time each *prd* domain becomes split into two bands each two cells wide, by the elimination of transcript from the middle (open bar) region of each domain[17]. The repression of *wg* by *ftz* and *eve* would allow *wg* expression only in the cells indicated (solid nuclei). The overlap of the *prd* and *eve* domains identifies the cell which will express *en* (hatched nuclei). That this combination specifies the odd-numbered *en* stripes is supported by the finding that their establishment depends both upon *eve* and *prd* activity. By analogy, we suggest that *opa* might be expressed in a similar, though complementary, pattern to *prd*. Thus the posterior of each *opa* domain would overlap the anterior of each *ftz* domain, thereby specifying the activation of the even-numbered *en* stripes. According to this scheme *prd* and *opa* will ultimately be expressed in identical stripes corresponding to each *en* and *wg* domain. Thus the requirement for *opa* and *prd* to activate alternate *wg* stripes is dependent upon context. The nature of this context is obscure.

the position of *wg* expressing cells in the blastoderm. Such a requirement may, however, be crucial to delimiting the domains of *wg* expression. If *wg* were exclusively under negative control, the *wg* domain might become progressively broader with time because the *eve* and *ftz* domains both continue to narrow as gastrulation proceeds. The requirement for *opa* and *prd* would thus serve to ensure that the release of *wg* from repression be restricted to the first cells to stop expressing *ftz* and *eve* (see Fig. 3*b*). Alternatively it is possible that the maintenance of the *wg* domains after the blastoderm stage depends upon regulatory interactions between other segment-polarity genes.

In terms of the foregoing formal genetic analysis, *ftz* and *eve*, both of which encode homoeo-domain proteins, act as positive regulators of *en* but negative regulators of *wg*. Also, *ftz* is required for the initial modulation of the homoeotic genes *Scr*, *Antp* and *Ubx*[24]. The specificity of each of these functions of the *eve* and *ftz* products will depend on their interaction with other gene products at particular given promoters. For example, in the case of homoeotic genes, gap-gene products might play an important role[26] whereas in the case of segment polarity genes, we suggest the specificity is set by other pair-rule genes. A situation in which the same DNA-binding protein behaves as an activator or repressor of different promoters has recently been described in detail for RAP-1 protein in yeast[27].

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