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Role of segment polarity genes in the definition and maintenance of cell states in the *Drosophila* embryo

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Summary

Segment polarity genes are expressed and required in restricted domains within each metameric unit of the *Drosophila* embryo. We have used the expression of two segment polarity genes *engrailed* (*en*) and *wingless* (*wg*) to monitor the effects of segment polarity mutants on the basic metameric pattern. Absence of *patched* (*ptc*) or *naked* (*nkd*) functions triggers a novel sequence of *en* and *wg* patterns. In addition, although *wg* and *en* are not expressed on the same cells absence of either one has effects on the expression of the other. These observations, together with an analysis of mutant phenotypes during development, lead us to suggest that positional information is encoded in cell states defined and maintained by the activity of segment polarity gene products.

Key words: *Drosophila* embryo, pattern formation, gene expression, segment polarity, *engrailed*, *wingless*.

Introduction

The establishment of 'fields' is considered an important strategy used by developing systems to generate and elaborate body plans (Huxley & de Beer, 1934; Weiss, 1939). In terms of positional information (Wolpert, 1969), a field can be defined as a bounded group of cells whose fates are specified with respect to the same coordinate system (Wolpert, 1969; French *et al.* 1976). More operationally, a field is 'the domain within which changes in the presumptive fates of cells (regulation) can occur in response to surgical manipulations' (reviewed in French *et al.* 1976).

In *Drosophila*, a variety of experimental evidence indicates that imaginal discs, and therefore imaginal segments, regenerate and intercalate, in a manner similar to the segments of other insects (reviewed in French *et al.* 1976; Bryant, 1978; Lawrence, 1973, 1981) and can thus be considered as fields. In contrast to the imaginal discs, *Drosophila* embryos display little or no regeneration after local damage (Lohs Schardin *et al.* 1979; Underwood *et al.* 1980). Nonetheless, the existence of fields in these embryos has been suggested by analogy with the imago (Garcia Bellido *et al.* 1979). Furthermore, the phenotypes of mutants of the segment polarity class, in which there are pattern deletions and associated duplications within every metameric unit (Nusslein Volhard & Wieschaus, 1980), provide indirect evidence for the behaviour of embryonic metameres as fields.

In insects, the existence of global properties that determine the differentiation of any given cell on the basis of its position in the field has been extrapolated from a large body of experimental evidence (reviewed in Lawrence, 1973). There are two contrasting hypotheses as to the nature of these global properties: gradients of diffusible morphogens (Locke, 1960; Stumpf, 1966; Lawrence, 1966) and interactions between neighbouring cells (French *et al.* 1976; Nubler Jung, 1977; Lewis, 1987; Mittenthal, 1981). In both cases, a cell at a given position within the field has a precise identity which is expressed in its pattern of differentiation. According to the gradient hypothesis, this identity is provided by the concentration of a diffusible substance distributed from a source in a graded manner across the field. In the case of nearest neighbour interactions, the differentiation pathway of a cell is determined, in part, by the differentiated pathways of its neighbours. In both cases, cells respond homeostatically to perturbations

either by restoring the initial gradient, or by reassessing their neighbours and intercalating the missing values.

The phenotypes of *Drosophila* segment polarity mutants suggest that the products of these genes mediate some of these properties. Although it is believed that some of their products are required and expressed in restricted regions of the metameres, it is generally considered that many aspects of the mutant phenotypes are secondary effects of these primary localized requirements (Nusslein Volhard *et al.* 1982; Martinez Arias, 1985; Martinez Arias & Ingham, 1985; Wieschaus & Riggleman, 1987; Perrimon & Mahowald, 1987). The physiological bases for these indirect effects have never been discussed.

Some genes of the segment polarity class have been cloned and shown to be expressed in restricted, homologous but different, domains of every metamere (*engrailed*, *en*: Kornberg *et al.* 1985; Fjose *et al.* 1985; *wingless*, *wg*: Baker, 1987; and *gooseberry*, *gsb*: Bopp *et al.* 1986; Cote *et al.* 1987). Transcripts from *en* and *wg* accumulate in adjacent narrow rows of cells on either side of the parasegment boundary (Baker, 1987; Ingham *et al.* 1988). Despite this localized expression, absence of either gene product causes pattern defects throughout the whole metamere (Kornberg, 1981; Nusslein Volhard & Wieschaus, 1980; Baker, 1987). To investigate the relationship between this expression pattern and the mutant phenotypes, we have examined the expression of the *en* and *wg* genes in embryos that lack the function of other segment polarity genes. Here we show that absence of segment polarity genes leads to a chain of regulative events probably involving local cell interactions. We surmise that in the wild type these interactions maintain and elaborate the patterns of gene expression established in cells of the blastoderm, whereas in mutants they serve to generate alternative stable cell states.

Materials and methods

Stocks

All mutants used in this study have been described before. Mutant embryos were generated by crossing flies carrying the corresponding alleles to a Canton S stock and using the F_1 heterozygotes to generate the mutant embryos. The different mutations and alleles are as follows: *engrailed*: $Df2(R)en^B$ (Eberlein & Russell, 1983; Gubb, 1985); *naked*, nkd^{7E89} and nkd^{7H16} (Jurgens *et al.* 1984); *patched*, ptc^{IN108} (Nusslein Volhard *et al.* 1984); *wingless*, wg^{CX4} (Baker, 1987). In the cases of *en* and *wg*, mutant embryos were identified by hybridizing alternate sections with the probe of interest and with the probe absent in the deficiency. In the case of *nkd* and *ptc*, mutant embryos were identified by the abnormal patterns of *wg* and *en* expression. In either case, the number of mutant embryos amounted to one fourth of the total. The alleles used in this study are null; either they are deficiencies, in the case of *en* and *wg*, or their phenotype is indistinguishable from that of deficiencies for the particular region, *ptc* and *nkd*. The $Df(2R)en^B$ eliminates *en* and two other loci: shavenoid, *sha* and schnurri, *shn*. The mutants *sha* and *shn* have no effect on the development of the ventral side of embryos and in the case of *sha* the homozygous animals are viable as adults (Nusslein-Volhard *et al.* 1984). Consequently, we consider that the effects we observe in homozygous $Df(2R)en^B$ are due solely to the absence of *en*. The wg^{CX4} is a small deficiency in the 5′ region of the *wg* transcript (Baker, 1987), which we find to lack expression of the RNA encoded in pwgc14a, a cDNA spanning most of the *wg* transcript (see Baker, 1987 and Rijsewijk *et al.* 1987 for details).

In situ *hybridizations*

The manipulation of embryos and probes for and during these experiments were as described previously (Ingham *et al.* 1985; Akam & Martinez Arias, 1985). The probes used were cDNAs from the *engrailed* (Poole *et al.* 1985) and *wingless* (Baker, 1987) regions, subcloned into standard vectors to produce antisense RNA. The relative location of the transcripts was performed by hybridizing adjacent sections with different probes, superimposing landmarks (the cephalic furrow, parasegmental grooves or tracheal pits) and establishing the relative location of the transcripts with respect to those landmarks.

Cuticle preparation and antibody stains

Larvae were removed from the vitelline membrane and mounted in Hoyers: lactic acid (see Wieschaus & Nusslein Volhard, 1986). The antibody used in this study was the *Ubx* monoclonal FP.3.38 (White & Wilcox, 1984) and labelling was performed according to published protocols (White & Lehmann, 1986; Ingham & Martinez Arias, 1986).

Results

(A) *The phenotypes of segment polarity mutants*

In the wild-type first instar larva, the epidermis is characterized by a denticle belt motif repeated every metameric unit. Each abdominal belt, except the first one, is composed of six rows of denticles each with a characteristic size and polarity (Lohs Schardin *et al.* 1979; Fig. 1A). In this study, we have looked at four segment polarity mutants: *engrailed* (*en*), *naked* (*nkd*), *patched* (*ptc*) and *wingless* (*wg*). Each of these mutants causes a characteristic alteration of the larval cuticular pattern in a defined region of every metameric unit (Fig. 1); together the mutant domains for these genes span the whole metameric repeat. These phenotypes are in some cases associated with cell death and might therefore be a regulative consequence of this process. Nevertheless, prior to cell death, alterations in pattern can be visualized by

monitoring a modulation in the expression of *Ubx* products across each abdominal parasegment (White & Lehman 1986; Ingham & Martinez Arias, 1986; Martinez Arias & White, 1988; Fig. 1B).

First instar larvae lacking the *wg* gene are smaller than wild type and each segment is covered with denticles of different polarity (Fig. 1C). Their reduced size is due partly to epidermal cell death during germ band shortening. Earlier, these embryos lack parasegmental grooves (Perrimon & Mahowald, 1987) and the epidermal cells have lost the distinct and different levels of *Ubx* expression that characterize the wild-type pattern (Fig. 1D).

The complete absence of *en* causes a local increase in the levels of *Ubx* within the region where *en* product would have been present (Martinez Arias & White, 1988; Fig. 1F). This pattern is grossly altered during germ band shortening because of cell death and rearrangements thus leading to the terminal aberrant cuticular phenotype (Fig. 1E).

Absence of *nkd* activity results in embryos of small size whose cuticle lacks most denticles (Fig. 1G). The terminal phenotype of *nkd* embryos is also due partly to cell death after germ band shortening (A.M.A. unpublished data). In the extended germ band stage, these embryos display alterations in the pattern of *Ubx* expression: most obviously the regions of lowest and highest levels of *Ubx* antigen are enlarged at the expense of the middle region with intermediate levels (Fig. 1H).

Embryos mutant for *ptc* show no cell death other than wild type at any time during embryogenesis (A.M.A. unpublished data) and the terminal phenotype is characterized by a mirror-image transformation of the middle third of the segment into the most anterior one (Fig. 1I). In the extended germ band of these embryos, the *Ubx* distribution shows clear local defects: in contrast to the graded wild-type distribution, every *Ubx* metamere displays a sequence of low, intermediate, low and high levels of *Ubx* antigen (Fig. 1J). An obvious morphological feature of these embryos is the presence of two parasegmental grooves: one approximately in the wild-type position relative to the *Ubx* metameres, the other one in a similar position to the tracheal pit with which it often fuses to produce a very deep groove (Fig. 1J).

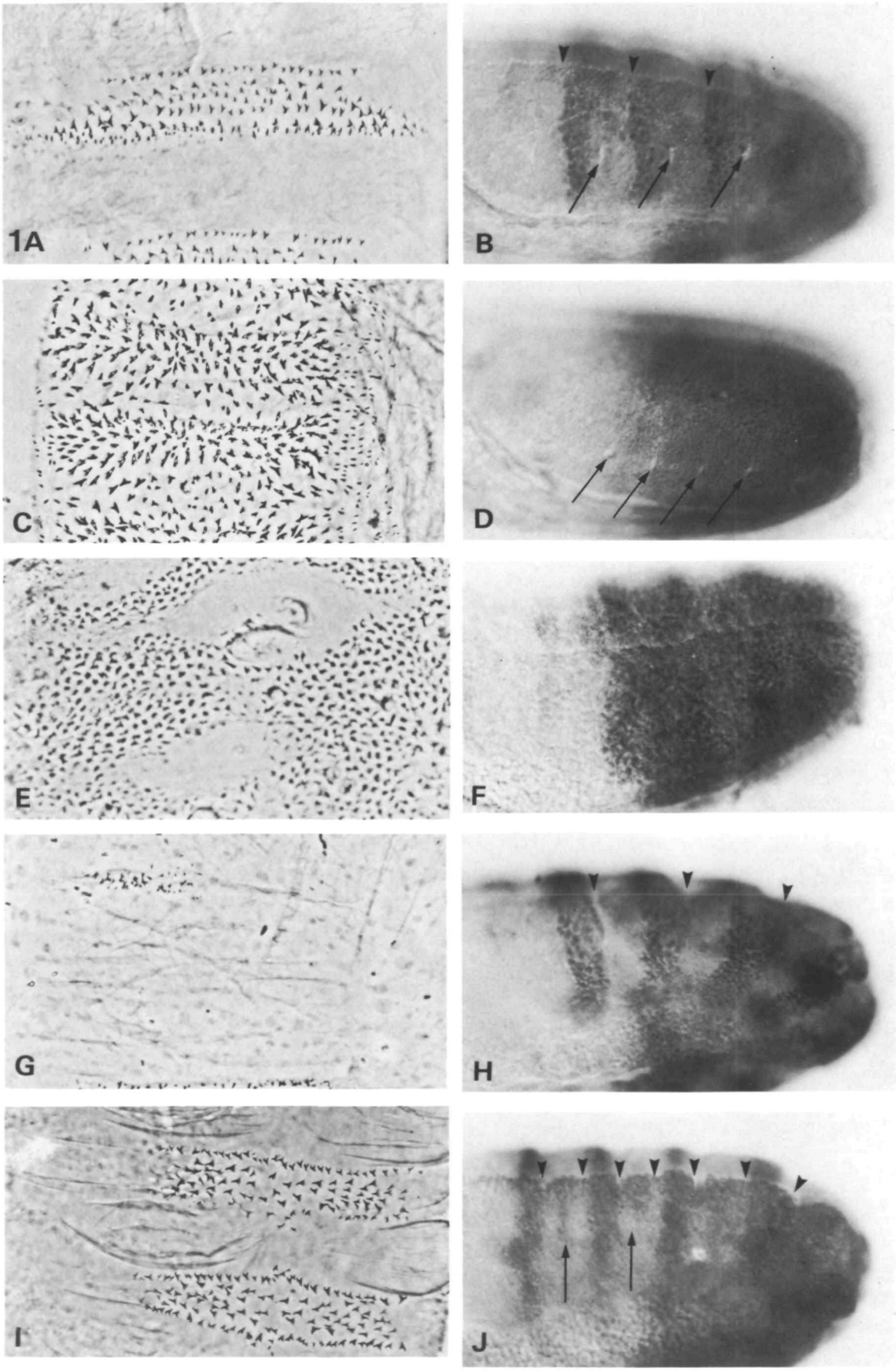
(B) *The pattern of* wg *expression in* ftz *and* eve *mutant embryos changes during development*

The wild-type patterns of transcription of *wg* and *en* indicate that from the onset of their expression (Fig. 2A,B) the two genes are transcribed in different and adjacent groups of cells; the *wg* expressing cells being those at the most-posterior part of every parasegment and the *en* expressing cells those at the most-anterior part (Fig. 2; Baker, 1987; Ingham *et al.* 1988). This can be clearly seen in the extended germ band when the transcriptional domains can be mapped with respect to the parasegmental grooves (Baker, 1987 and Fig. 2G,H) and earlier in the early gastrula where the cephalic furrow can serve as a morphological landmark (Fig. 2C,D). After germ band extension, the areas expressing *en* widen in a manner consistent with the cellular rearrangements and proliferation which follow gastrulation; by contrast, the regions expressing *wg* remain constant in width (Fig. 2E,F). This suggests that while it is likely that the descendants of all cells expressing *en* at blastoderm express *en* after germ band extension, the transcription of *wg* may be subject to influences by neighbours.

Further support for this observation is found in the expression of *wg* in *ftz* and *eve* mutant embryos. We have shown previously that, during the late cellular blastoderm, the products of the *ftz* and *eve* genes repress the transcription of *wg* (Ingham *et al.* 1988). In the absence of *ftz*, *wg* is activated in seven broad stripes during blastoderm and a similar pattern of *wg* expression is observed, but in a complementary frame, in the absence of *eve*. These patterns persist during gastrulation but are not maintained during subsequent embryonic stages. In the epidermis of *ftz* mutant embryos, the broad stripes of *wg* transcription become narrow as embryogenesis proceeds and, by the time parasegmental grooves are visible, *wg* expression is restricted to a small region at the posterior end of every double-width metamere (Fig. 3). This region has approximately the same width as the normal *wg* stripe at the same stage and appears to be maintained during subsequent development. In *eve* mutant embryos, the expression of *wg* fades in the epidermis (data not shown). These observations stress that the normally stable pattern of *wg* transcription is not simply a static consequence of the blastoderm pair-rule prepattern, but the outcome of continuing regulatory interactions. We suspected that these interactions might be mediated by the products of other segment polarity genes and, to test this more directly, we have studied the expression of the *wg* and *en* genes in segment polarity mutant backgrounds.

(C) *Reciprocal requirements of the* wg *and* en *products for the maintenance of their expression*

The localized transcription of *wg* and *en* in the early stages of embryogenesis contrasts with the extensive disruption of pattern observed in the terminal phenotypes of embryos mutant for these genes. This suggests that absence of either product has effects on the physiology of neighbouring and transcriptionally distinct cells. To investigate this relationship, we have monitored the expression of *wg* and *en* in embryos lacking the *en* or the *wg* gene respectively (see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Materials and methods). Absence of the *wg* gene has no effect on the initiation of *en* expression. However, after germ band extension, the absence of either gene results in the loss of expression of the other in the epidermis (Fig. 4). In *wg* mutant embryos, often there is residual expression of *en* in the gnathal region (Fig. 4). Thus, although the two genes are transcribed in different cells, each appears to be necessary to stabilize the expression of the other. This observation indicates that in normal development, signals are exchanged between the *wg* and *en* transcribing cells.

(D) *The expression of* wg *and* en *requires the activity of other segment polarity genes*

The cuticular phenotypes of other segment polarity mutants suggest that these mutations might stimulate interactions between cells analogous to those observed in *en* and *wg* mutant embryos. To test this, we have analysed the expression of *en* and *wg* in two other segment polarity mutants, *naked* (*nkd*) and *patched* (*ptc*). Each of these affects a region of the metamere where neither *en* nor *wg* are expressed (Figs 1, 7).

In *nkd* mutant embryos, the pattern of *en* expression is first changed shortly after gastrulation. The *en* stripes become broader and each covers approximately one half of a metameric unit; alternate sections hybridized with *wg* do not show any deviation from the wild-type pattern at this stage (Fig. 5). This novel pattern of *en* expression is conserved at least until germ band shortening. After the germ band has extended, at a stage when epidermal cells have probably divided once, an extra stripe of *wg* expression appears in most segments. This stripe lies next to the cells expressing *en* ectopically. In some metameres, this pattern is not stable and later evolves to a sequence of adjacent *en* and *wg* stripes (data not shown). The relative position of the *en* and *wg* transcriptional domains with respect to the parasegmental grooves is inverted in these embryos, and suggests that the parasegmental groove does not form at the normal *wg*:*en* interface but at the ectopic one.

In *ptc* mutant embryos, during germ band extension *wg* is expressed in a broad domain which occupies half of the metameric unit. At this stage, adjacent sections probed with *en* show a normal

Fig. 1. Pattern formation in segment polarity mutants represented by *Ubx* expression and denticle belt formation. (A) Wild-type denticle belt of an abdominal segment. The first row lies in the most posterior part of every segment, by the criterion that the segment boundary lies where most of the muscles attach; denticles in the first and fourth rows point, mostly, anteriorly, while denticles in the other rows point mostly posteriorly (Lohs Schardin *et al.* 1979). (B) *Ubx* expression in the abdominal region of a wild-type extended germ band. Numbers indicate parasegments. In this region, because of the morphogenetic movements associated with germ band extension, the most anterior part of every metameric unit is to the right and the most posterior to the left of the picture. This holds for all the panels showing embryos in this and subsequent figures. Notice that the expression of *Ubx* increases posteriorwards between the parasegmental grooves (arrowheads) with a relatively large increase in expression in the last third of the metameric unit; arrows indicate tracheal pits. (C) Denticle belt pattern of a homozygous wg^{CX4} larva. The animal is smaller than wild type and the posterior part of every metamere is deleted. Metamerism is still obvious and the denticles tend to orient towards the midline (see Baker, 1987, 1988 for further details). (D) Expression of *Ubx* in a wg^{CX4} embryo during the extended germ band stage. The embryo lacks parasegmental grooves but has tracheal pits (arrows, and see also Perrimon & Mahowald, 1987). The intrametameric modulation of *Ubx* expression is absent. (E) Denticle belt pattern of a $Df(2R)en^{B}$ larva. The denticles are very small probably due to the *shn* mutant and, although there is a tendency to orient towards the midline, their pattern varies from animal to animal. (F) Expression of *Ubx* in the extended germ band of an embryo as E. Some metamerism and modulation of *Ubx* expression are visible; the regions with high levels of *Ubx* correspond to the more posterior regions of the wild-type parasegments (see Martinez Arias & White, 1987 for details); notice the increase of *Ubx* products in the most anterior part of every metamere (compare with B). (G) Denticle belt pattern characteristic of a nkd^{7E89}/nkd^{7H16} heterozygote. The larva is similar in size to *en* or *wg* mutant larvae. For the most part, the cuticle lacks denticles; occasionally animals have some denticles, but their position and 'identity' are rather variable. (H) Expression of *Ubx* in the extended germ band of a *nkd* mutant embryo. In every metamere, the regions of highest and lowest *Ubx* expression are expanded at the expense of regions with intermediate levels. Notice that the relative position of *Ubx* expression and the parasegmental grooves (arrowheads) has been changed; in these embryos, the grooves lie on the same position as the tracheal pits with which they sometimes fuse. The parasegments are, therefore, out of frame with the *Ubx* metameres. (I) Denticle belt pattern of a ptc^{IN108} homozygous larva. The animal is of almost wild-type size. The patterns of denticles suggest a substitution of the posterior half of the denticle belt by a mirror image of the most anterior half (Nusslein-Volhard & Wieschaus, 1980; compare with A). (J) Expression of *Ubx* in the extended germ band of a ptc^{IN108} embryo. There are slight alterations in the pattern of *Ubx* expression, notably there is a small region of high levels in the middle of the low levels region (arrow). Notice the presence of two parasegmental grooves (arrowheads); using the region with high levels of *Ubx* expression as a reference, one of the grooves is at the normal position and the other is at the position of the tracheal pit with which it often fuses.

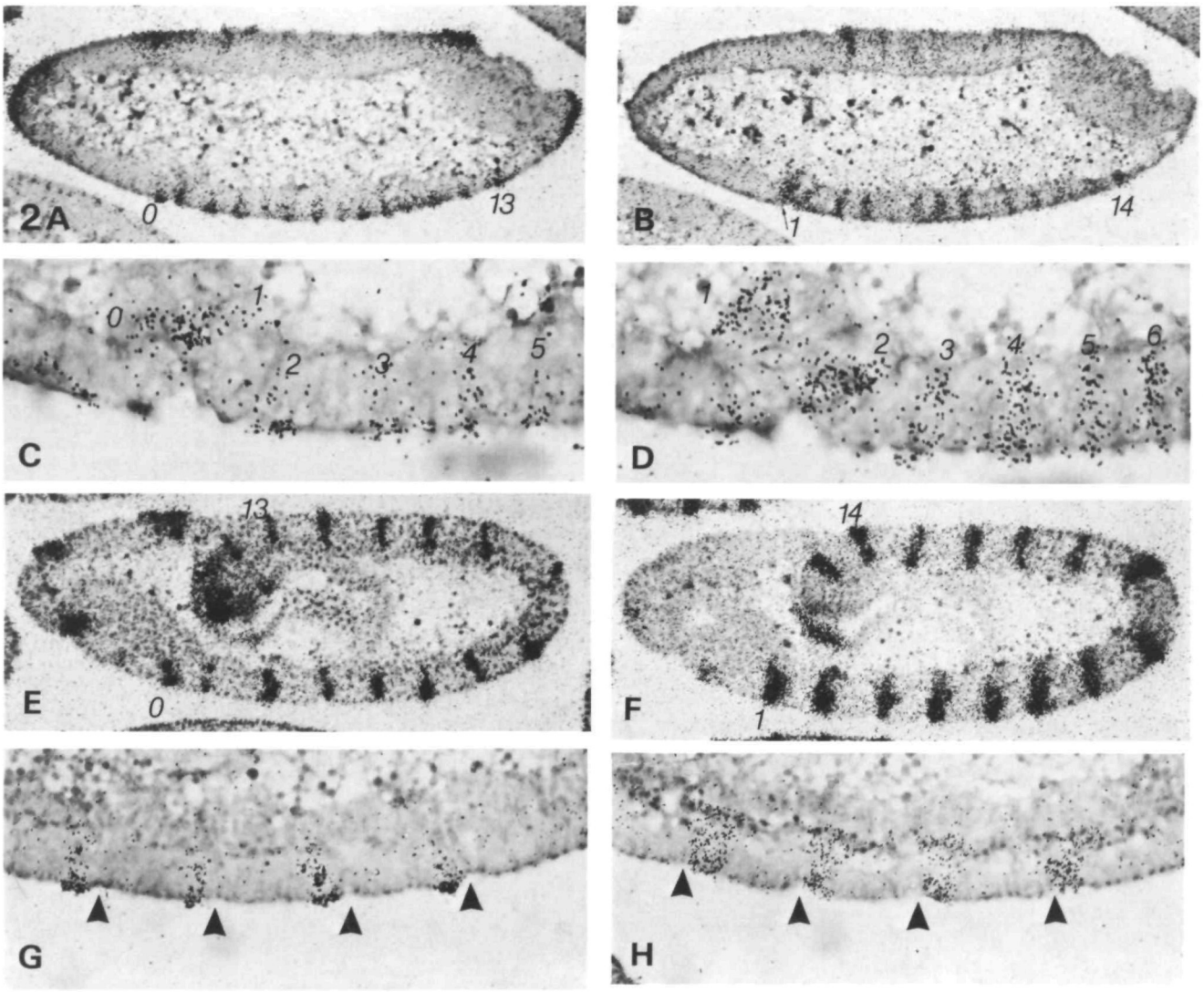

Fig. 2. Transcription of the *wg* and *en* genes in the wild type. (A,B) Alternate sections of a late gastrula hybridized with ^{35}S-probes for *wg* (A) and *en* (B) (see Materials and methods for details of the clones used to generate probes for the data presented in this and subsequent figures). In the region between 10 % and 80 % egg length, there are 14 domains of transcription. Numbers indicate reference to parasegments. Superimposition of these sections will produce a partial overlap of the *en* and *wg* stripes. This is largely due to the scatter of the ^{35}S-probes; when using ^{3}H-probes and morphological landmarks as a reference (see C,D), it is clear that the overlap is negligible. (C,D) Details of an embryo similar to the one presented in A and B, hybridized with ^{3}H-probes. Notice that using the cephalic furrow as a landmark it is clear that the domains of transcription are different. At this stage the domains are similar, approximately one nucleus wide. (E,F) Adjacent sections of an embryo approximately 2 h after gastrulation hybridized with ^{35}S-probes for *wg* (E) and *en* (F). The relative position of the stripes is similar to earlier stages, but the width of the domains has changed; while the *en* domains are broader, the *wg* domains are relatively unaltered. (G,H) Details of adjacent sections of an extended germ band hybridized with ^{3}H-probes for *wg* (G) and *en* (H). Notice the relative position of the transcriptional domains with respect to the parasegmental grooves (arrows), in adjacent but different groups of cells.

pattern of expression. Changes in this early pattern are observed shortly after the cells divide when an extra stripe of *en* expression appears adjacent to the ectopic domain of *wg* (Fig. 6). This pattern is maintained at least until germ band shortening. In these embryos, there are twice the number of parasegmental grooves in the cellular width equivalent of a normal parasegment; one of them is at the normal *wg*:*eg* interface, and one at the position of the tracheal pit (see Fig. 1J).

Discussion

The phenotypes of segment polarity mutants of *Drosophila* (Nusslein Volhard & Wieschaus, 1980) suggest that the normal products of these genes are

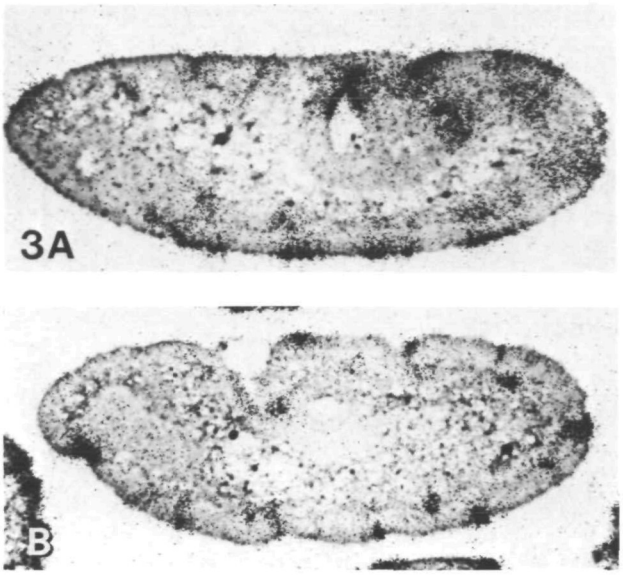

Fig. 3. Transcription of the wingless gene in *ftz*$^{-}$ embryos during development. (A) During germ band extension, the blastoderm pattern (Ingham *et al.* 1988) is maintained and the *wg* gene is transcribed in seven broad stripes. (B) After cellular proliferation, the stripes of transcription have narrowed and resemble wild-type stripes at a comparable stage.

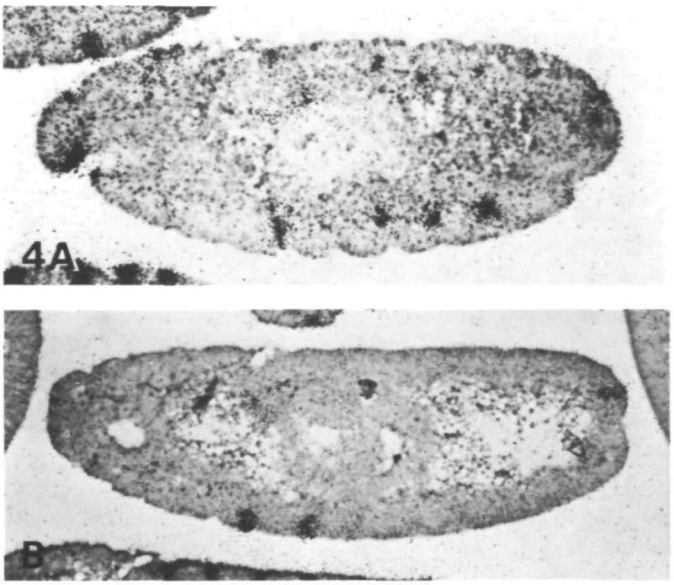

Fig. 4. Transcription of *wg* (A) and *en* (B) in *Df(2R)en*^{B} and *wg*^{CX4} mutant embryos. (A) Transcription of *wg* in an *en* mutant embryo during the extended germ band. No expression is detected in the epidermis except for the presumptive germ band. No expression is detected in the epidermis except for the presumptive gnathal region but some metameric expression is detected in the developing nervous system. (B) Transcription of *en* in a *wg* mutant embryo during the extended germ band. Expression has disappeared from most of the epidermis; though essentially normal transcription can still be observed in the gnathal region. Mutant embryos were identified by hybridizing adjacent sections with probes for the deleted genes.

involved in the definition and maintenance of positional information within each metameric unit. Several of the segment polarity genes have now been shown to be transcribed in defined regions of each segment such that each metameric unit can be considered to be composed of a series of cell states defined by gene activities.

Our results show that the terminal cuticular phenotypes of segment polarity mutant embryos are not the simple consequence of the local absence of their products, but the result of regulative processes. For instance, in the absence of *ptc*, an initial response is generated by the ectopic activation of *wg*; this generates an interface between cell states which is not present in the wild type and, probably as a consequence, cells at this interface change their fate and express the *en* gene (Figs 6, 7). This array is stable and is allowed to differentiate (Fig. 7). Similar regulatory processes lead to the generation of new interfaces between cell states in *nkd* mutant embryos (Figs 5, 7) and it is likely that analogous events are responsible for the changes in the pattern of *wg* transcription observed in *ftz* and *eve* mutants.

We consider these observations to represent the molecular correlate of the intercalation observed in classical experiments on insects segments or in imaginal discs. In what follows, we will discuss our results in the light of these experiments and propose a mechanism for the generation and maintenance of positional information in the embryonic epidermis of *Drosophila*.

A cellular basis for positional information

Experimental manipulation of the insect epidermis indicates that segments behave as fields of positional information (reviewed in Lawrence, 1973). The response of epidermal tissue to excisions, rotations and transplantations has led to the idea that gradients of morphogens are organized by sources and sinks located at the segment boundaries (Locke, 1960; Stumpf, 1966; Lawrence, 1966). In this view, the segment boundary is envisioned as a place where large discontinuities in positional information are stably maintained and as having special organizing properties. The body of an insect is thus considered as a series of fields joined at their boundaries (see Lawrence, 1981). In an elegant series of experiments, Wright & Lawrence (1981*a*,*b*) demonstrated that the segment boundary behaves very much as any other pattern element associated with a particular positional value and concluded that the insect body can

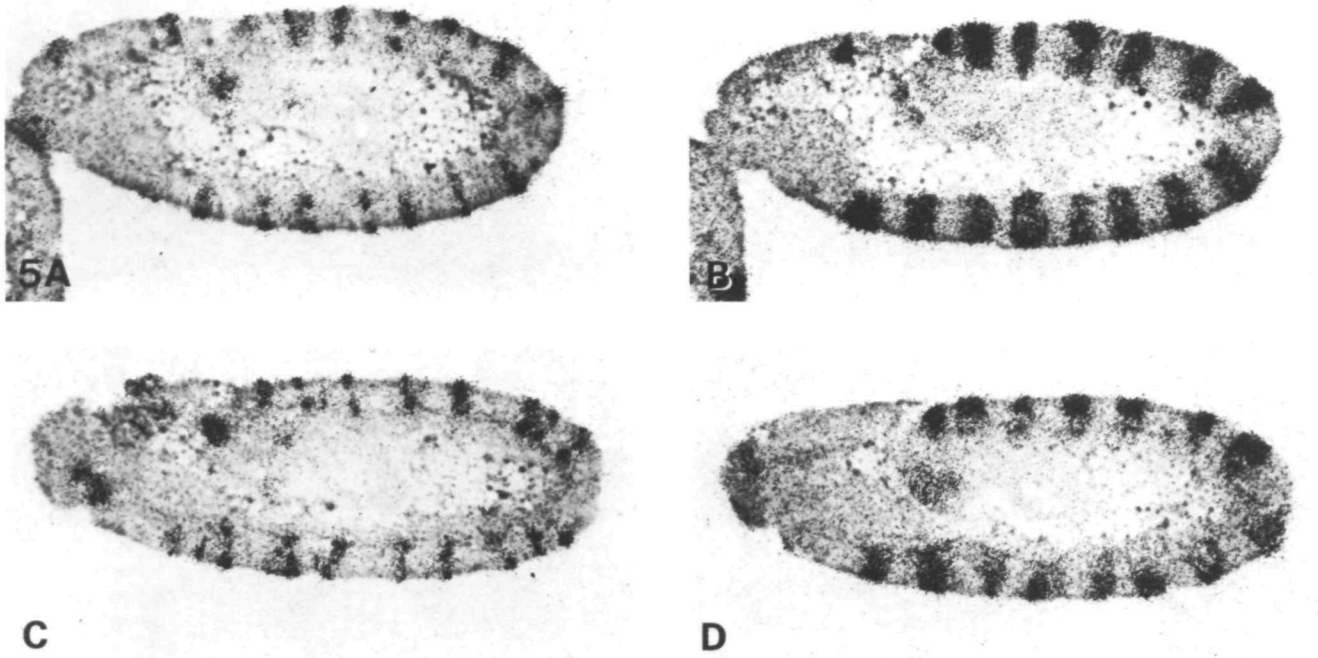

Fig. 5. Transcription of *wg* and *en* in nkd^{7H16}/nkd^{7E89} mutant embryos. (A,B) Adjacent sections through a young - extended germ band probed for *wg* (A) and *en* (B) expression with ^{35}S-probes. While the pattern of *wg* expression is as in wild type, the pattern of *en* expression is altered; the bands are much broader than wild type at a similar stage. The relative positions of *en* and *wg* are maintained. (C,D) Older extended germ bands hybridized with probes for *wg* (C) and *en* (D). While the pattern of *en* has remained unaltered, there are extra stripes of *wg* activity; these stripes flank the broader *en* stripes.

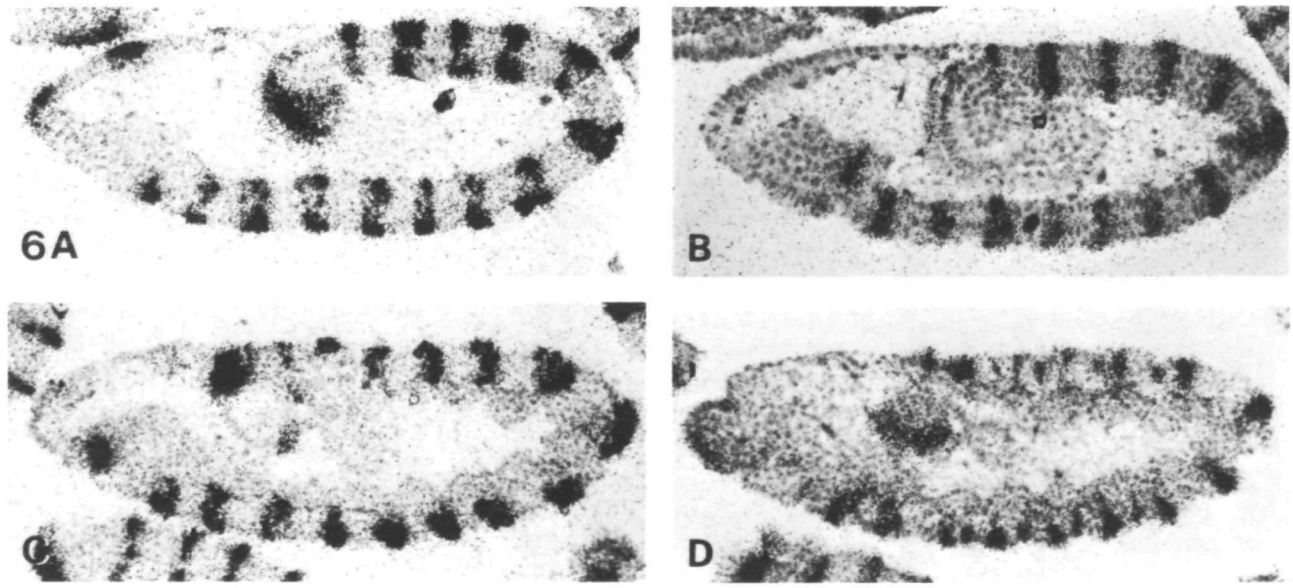

Fig. 6. Transcription of *wg* and *en* in ptc^{IN108} mutant embryos. (A,B) Adjacent sections of extending germ bands hybridized with ^{35}S-probes for *wg* (A) and *en* (B). While the pattern of *wg* expression is similar to the wild-type one (compare with Fig. 1), *wg* is transcribed in a much broader region. Addition of the two domains still leaves a region in every metamere. (C,D) Adjacent sections through an extended germ band hybridized with *wg* (C) and *en* (D). Cell proliferation has taken place; while the pattern of *wg* is similar to the early one (A), a second band of *en* has appeared in every metameric unit, just anterior to the enlarged *wg* domain. Mutant embryos were identified by their altered patterns of *wg* and *en* expression and represented, approximately, one quarter of the total population.

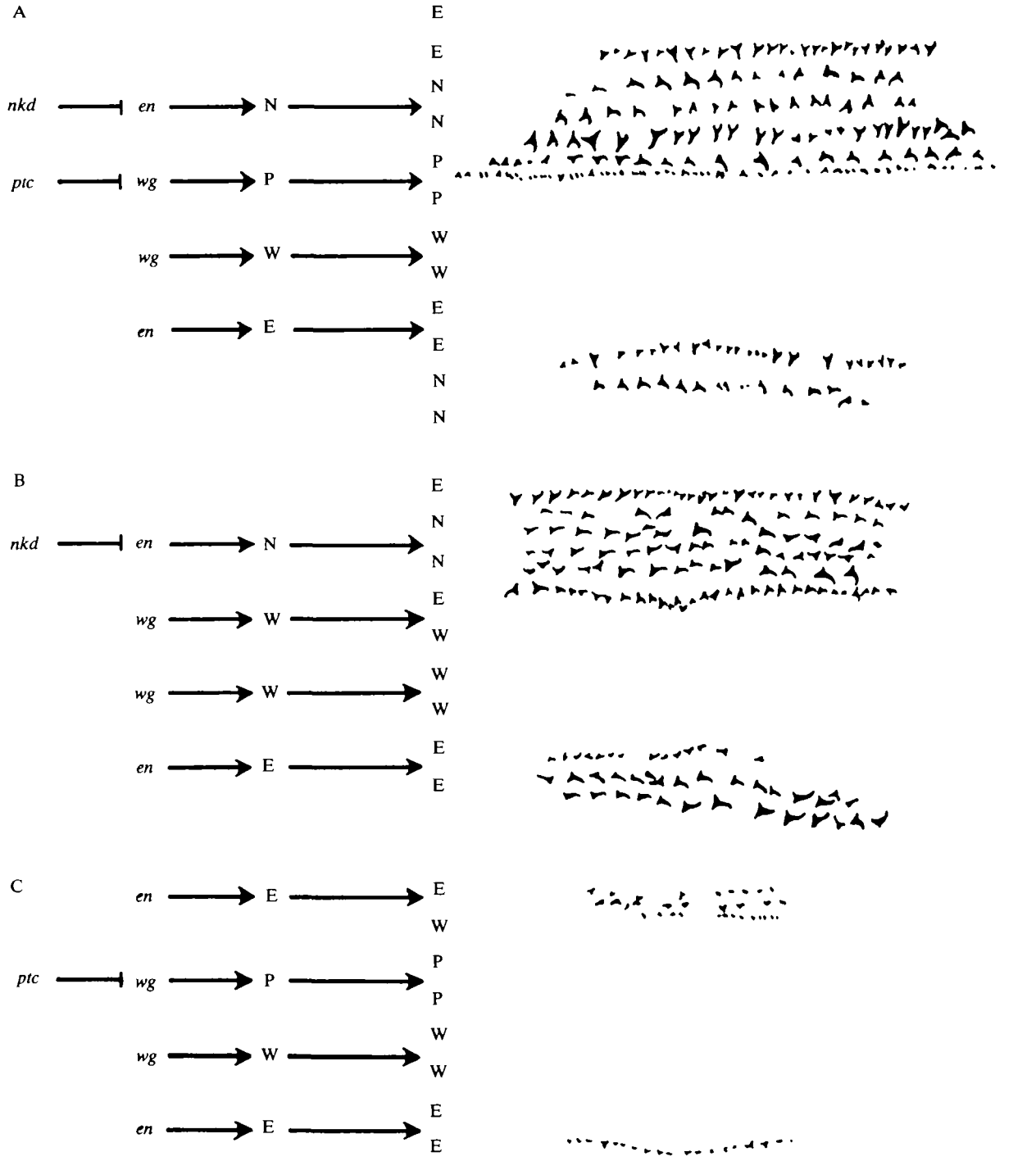

Fig. 7. Cell states and pattern formation. (A) Cell states in wild-type embryos; during the early gastrula we can define four cell states on the bases of gene expression or of the requirement of gene products for gene expression (see text). In particular, as indicated, the N state is defined by the spatially restricted requirement for *nkd* to repress *en* transcription. Similarly, P is defined by the requirement for *ptc* in the repression of *wg*. After cell divisions, these states are the bases for the differentiation of the cuticular patterns. (B) Cell states in a *ptc* mutant. The absence of *ptc* results in the ectopic expression of *wg*; as a consequence an N:W interface is generated which results in the ectopic generation of an E state. (C) Cell states in a *nkd* mutant embryo. The absence of *nkd* leads to the ectopic activation of *en* which results in an E:P interface. As a result, a new W state is intercalated, presumably to stabilize the pattern. As discussed in the text, in the generation of the pattern the state of a cell depends on its autonomous pattern of gene expression as much as on the state of its nearest neighbours.

be represented as a continuum of positional information with a basic repeat. For reasons that are associated with the definition of polarity within a field of cells and the homeostasis associated with growth, Wright and Lawrence adhered to the gradient convention and suggested discontinuities of positional information at the segment boundaries as well as a gradient as the source of positional information within the field.

Our results suggest that the germ band of the *Drosophila* embryo contains a repeating series of discrete and qualitatively different cell states. According to this interpretation, the juxtaposition of cell states that defines the segment boundary is but one of several analogous boundaries defining position within a metameric unit. This view, while in agreement with the observations of Wright & Lawrence (1981*a*,*b*), questions the existence of any positional information other than that generated by local cell interactions. Indeed, if these states are the only source of information for pattern formation, our interpretation challenges the notion of positional information as a continuum which the cells interpret to generate patterns (Wolpert, 1969). As a consequence, the metameric germ band of the early *Drosophila* embryo is not composed of continua of positional information fields but rather of a repetitious, discrete and ordered array of cell states, akin to positional values. This array can be represented as a sequence ...WENPWENPWENPWE... (Fig. 7). The cell states W and E are defined by the domains of transcription of *wg* and *en*; the states N and P are defined by the local requirements for the *nkd* and *ptc* products in the restricted expression of *en* and *wg*. Although we define cell states with reference to these four genes, we do not intend to suggest functional homologies between their four gene products. While it seems possible that the *en* products act as an autonomous 'label' for cell type (Morata & Lawrence, 1977), it seems much more likely that the *wingless* product functions extracellularly (Rijsewijk *et al.* 1987 and see below). The four postulated cell states are the minimum required to account for our observations. There may, of course, be more; for example one of the transcripts from the *gooseberry* (*gsb*) locus is transcribed in a large domain which partially overlaps with *en* (Baumgartner *et al.* 1987) and this can generate more states. A detailed analysis of the middle region of each parasegment will require the cloning of *ptc*, *nkd* and other segment polarity genes.

The WENP array of states is set up during early development and, in the case of *wg* and *en*, the initiation of the pattern depends on the prepattern governed by pair-rule gene products (Howard & Ingham, 1986; DiNardo & O'Farrell, 1987; Ingham *et al.* 1988; reviewed in Akam, 1987). We believe that, in the wild type, this basic set of states is expanded by the activation of new segment polarity genes during cellular proliferation and that this expansion depends on intercellular signals mediated by some of the segment polarity genes themselves.

At different interfaces of this array (corresponding to different values) specific pattern elements arise. For example, in the wild type the parasegmental groove arises at the W:E interface, and the segmental groove at the E:N interface. The N domain makes denticles, but the kind of denticles might be different for N cells with different neighbours, the E:N, N:N or N:P. In this view, pattern arises from the states of the cells and from the interactions between different states, without the need for an overall morphogen whose concentration is interpreted by the cells (Wolpert, 1969).

In this array, we consider the cells biochemically polarized such that the sequence WENP has a global polarity which is a consequence of the polarity inherent in the serial apposition of cells with different states. Thus polarity will be defined not by a gradient but by local interactions, as another part of the pattern encoded in the cell states. The existence of different polarities at different locations of the segment, as for example in the orientations of the different denticle rows in the larval cuticle (Lohs Schardin *et al.* 1979; Fig. 1A), is the result of interfaces between different cell states and not of the differential interpretation of 'positional information'.

The similar phenotypes of en *and* wg *mutant embryos depend on different physiological events*

The absence of either the *en* or the *wg* products causes a dramatic disruption of the final cuticular wild-type pattern. It is clear from the mutant phenotypes that the defects extend beyond the area in which the gene is transcribed. Our results indicate that this is due to effects on the cell states of neighbouring cells. In addition, alterations in *Ubx* modulation before cell death indicate that, whereas absence of *en* results in short-range defects (Martinez Arias & White, 1988 and Fig. 1F), absence of *wg* results in obvious defects several cell diameters away from its normal site of transcription (Fig. 1D).

The *en* product contains a homeobox DNA-binding domain (Fjose *et al.* 1985; Poole *et al.* 1985) and the defects caused by its absence might be a consequence of the failure to activate genes controlled by the *en* products. This defects would then spread along the positional field through nearest neighbours' interactions. By contrast, the *wg* product has been shown to be homologous to the vertebrate *int-1* product (Rijsewijk *et al.* 1987). This protein is a growth-factor-related molecule with an extracellular location (Van Ooyen & Nusse, 1984; Brown *et al.* 1987; Papkoff *et*

al. 1987) and is possibly diffusible *in vitro* (A. M. C. Brown personal communication). Such a diffusible gene product would explain the early appearance of long-range effects observed in *wg* embryos. Thus, in contrast to *en*, the absence of *wg* can be expected to have a more direct effect on cells away from its transcriptional source. On the basis of the homology with *int-1* and of experiments with temperature-sensitive alleles (Baker, 1988), it has been suggested that the *wg* product could mediate some of the effects traditionally ascribed to gradients (Baker, 1987, 1988; Cabrera *et al.* 1987). It is also possible, however, that the *wg* product mediates a growth-factor-like function (Rijsewijk *et al.* 1987) with just one functional threshold, i.e. different concentrations are not associated with different functions. In this context, it is interesting that, in the absence of *wg*, cells of the embryonic epidermis undergo proliferation over the normal period but die shortly before differentiation (unpublished observations). This cell death might be related to the similar phenomenon observed with cells in culture when deprived of differentiation factors.

Cell states and compartments

The adult *Drosophila* is composed of compartments, polyclonal groups of cells that define units of gene activity (Garcia Bellido, 1975) and probably of growth and patterning (Crick & Lawrence, 1975; Simpson & Morata, 1981). On the grounds of formal genetic analysis and more recently of homology of gene expression between the embryo and the imago, the existence of compartments has been extrapolated to the embryo. In consequence, the embryo can thus be represented as a chain of A and P compartments (Garcia Bellido *et al.* 1979; Kornberg, 1981; Struhl, 1984; Martinez Arias & Lawrence, 1985) with an underlying continuum of positional information. One problem with this model is that such a chain is apolar and thus it is not clear why a singularity such as the segment boundary should form at the PA border rather than at the AP border. To circumvent this difficulty, Meinhardt (1986) has proposed that each embryonic segment has three compartments, SAP. Accordingly, the embryonic segment should be composed of three lineages distinguished by the expression of particular segment polarity genes. Other problems with the compartmental model arise from the rigidity imposed on patterning by compartments (Gergen *et al.* 1986).

Lineage analysis has provided some evidence for clonal restrictions between, but not within, segments in the larva (Szabad *et al.* 1979) and therefore the existence of compartments in the embryo remains an open issue. Here, in contrast to these compartmental models, we have proposed a basic set of cell states which are responsible for generating polarity and pattern. Their relationship to compartments is, at present, unclear but our observations indicate that, in contrast to cell identities within a compartment, cell states, as assayed by segment polarity gene expression, are not rigidly determined and inherited but can be altered by interactions between cells during embryogenesis.

Our results show that in the *Drosophila* embryo, in addition to polarity and positional values any model has to account for the ability of cells to intercalate cell states. In the two-compartment model, we assume that this is accounted for by the existence of underlying positional information which the cells interpret according to their states and positions in the field (Crick & Lawrence, 1975); this information is thought to exist in the form of a morphogen gradient. Proposals concerning gradients in multicellular fields, while conceptually useful, are difficult to refute experimentally and thus our results cannot exclude such a model. However, if we consider cell states as the only source of information for pattern formation, both the two- and the three-state models prove inadequate to explain correctly the observed intercalation. In the first one (AP), the problem is simply that a mutant sequence PPPPP or AAAAA does not provide cells with the necessary information to generate the mutant phenotypes (Meinhardt, 1986). In the second one (SAP), although sequences APAPAP or SPSPSP are capable of producing novel patterns, they do not retain information regarding the polarity of the sequence. With a minimum of four cell states (WENP), absence of any given state will result in a locally polarized repeat capable of intercalating according to unambiguous rules. More cell states will make the rules more complicated.

In the context of the limited cell proliferation in the wild-type *Drosophila* embryo after the blastoderm stage, the capacity for epidermal cells to influence one another might seem superfluous. However, our results suggest that the blastoderm pair-rule prepattern serves to establish only a framework for the elaboration of segmental prepatterns. According to our model, interfaces between cell states are sources of information and not barriers to its flow; thus, during proliferation, the interfaces are used to generate more states through the activation of other segment polarity genes. In this manner, positional information is generated during cell proliferation. The importance of 'intercalation' in embryonic pattern formation in *Drosophila* has also been pointed out by Gergen *et al.* (1986), but in their view all positional values are present at blastoderm.

The segment polarity gene products represent the last step in the regulatory hierarchy-determining

pattern in *Drosophila*. The essential feature of pattern has been called positional information (Wolpert, 1969) and the evidence available indicates that the segment polarity genes are the molecular bases for positional information in *Drosophila*. If, as we have suggested, positional information is encoded in a discrete set of cell states, we envisage three kinds of segment polarity genes: those involved in endowing cells with a state, e.g. *en*; those responsible for a short-range communication system for that state, and a last group concerned with the long-range coordination of the cell-to-cell transmission of states and thus with the regulation of pattern; the products of *wg* could be an example of the latter class.

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Recently, we became aware of results concerning *engrailed* expression in *cis* acting and segment polarity mutants: DiNardo, S., Shear, E., Heemskerk-Jongens, J., Kassis, J. & O'Farrell, P. (1988). Two-tiered regulation of spatially patterned *engrailed* gene expression during *Drosophila* embryogenesis. Submitted to *Nature, Lond.* These results agree with and support our data and interpretation.