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; Martínez Arias, 1985; Martínez 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 parasegmental 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 F1 heterozygotes to generate the mutant embryos. The different mutations and alleles are as follows: engrailed: Df(2R)en B (Eberlein & Russell, 1983; Gubb, 1985); naked, nkd T 69 and nkd M 16 (Jurgens et al. 1984); patched, ptc IN10 (Nusslein Volhard et al. 1984); wingless, wg C X 4 (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 C X 4 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 pwg14a, 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 & Martínez 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 monoclonal FP.3.38 (White & Wilcox, 1984) and labelling was performed according to published protocols (White & Lehmann, 1986; Ingham & Martínez Arias, 1986).

Results

(A) The phenotypes of segment polarity mutants

In the wild-type first instar larva, the epidermis is characterized by a denticile 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 pic 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 fzt 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 fzt and eve mutant embryos. We have shown previously that, during the late cellular blastoderm, the products of the fzt and eve genes repress the transcription of wg (Ingham et al. 1988). In the absence of fzt, 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 fzt 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 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
Gene expression in segment polarity mutants

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.

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 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 nkd¹⁵⁸⁹/nkd¹⁴⁷⁶ 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 in the same position as the tracheal pits with which they sometimes fuse. The parasegments are, therefore, out of frame with the Ubx metameres. (I) Dentine belt pattern of a ptc¹¹⁸ homzygous larva. The animal is of almost wild-type size. The patterns of denticles suggest a substitution of the posterior half of the denticate 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 ptc¹¹⁸ homozygote. 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. 1. Pattern formation in segment polarity mutants represented by Ubx expression and denticle belt formation. (A) Wild-type denticate 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 wg⁰⁴ 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 wg⁰⁴ 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) Dentine belt pattern of a Df(2R)en¹⁸ larva. The denticles are very small probably due to the enhancer 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
Fig. 2. Transcription of the wg and en genes in the wild type. (A,B) Alternate sections of a late gastrula hybridized with 35S-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 35S-probes; when using 3H-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 3H-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 2h after gastrulation hybridized with 35S-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 3H-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
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 (1981a,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*\(^{1652}/nkd^{2099}\) mutant embryos. (A,B) Adjacent sections through a young extended germ band probed for *wg* (A) and *en* (B) expression with \(35\text{S}\)-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*\(^{N106}\) mutant embryos. (A,B) Adjacent sections of extending germ bands hybridized with \(35\text{S}\)-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.
Gene expression in segment polarity mutants

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 \textit{nkd} to repress \textit{en} transcription. Similarly, \textit{P} is defined by the requirement for \textit{ptc} in the repression of \textit{wg}. After cell divisions, these states are the bases for the differentiation of the cuticular patterns. (B) Cell states in a \textit{ptc} mutant. The absence of \textit{ptc} results in the ectopic expression of \textit{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 \textit{nkd} mutant embryo. The absence of \textit{nkd} leads to the ectopic activation of \textit{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 (1981a,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 ...WENPWENP... (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 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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References


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**Note added in proof**