Development 119, 971-976 (1993)
Printed in Great Britain © The Company of Biologists Limited 1993

Drosophila segmentation: after the first three hours

Peter A. Lawrence and Javier Sampedro

MRC Laboratory of Molecular Biology, Hills Road, Cambridge, CB2 2QH, UK

Key words: *Drosophila*, segmentation, parasegment, pair rule genes, segment polarity genes, gradient

**"The instruments of darkness tell us truths,
Win us with honest trifles, to betray's
In deepest consequence"**

Not long ago, there were streams of illuminating discoveries about early *Drosophila* development and these have built a coherent picture of the first three hours (Nüsslein-Volhard, 1991). Now these streams have slowed: attempts to find clarifying principles have been held up at a particular phase in development, the period when segment polarity genes begin to take over the task of patterning the segment. At that time, the pair rule genes (Nüsslein-Volhard and Wieschaus, 1980) have already laid down the segmented body plan. It was suggested that the prime function of pair rule genes is "to **locate** boundaries that delimit fields or gradients of positional information" (Lawrence, 1987). The purposes of this speculative essay are to assess this hypothesis with respect to new evidence about the segment polarity genes, to consider the functions of these genes and to question some modern trends. (There is a swarm of reviews in this field; please see them for primary references: e.g. Martinez-Arias, 1989; DiNardo and Heemskerk, 1990; Ingham, 1991; Hooper and Scott, 1992; Ingham and Martinez-Arias, 1992; Peifer and Bejsovec, 1992; Woods and Bryant, 1992).

There is now good evidence that the parasegmental borders are the first and most fundamental signs of segmentation. They are delineated by the anterior boundaries of expression of the pair rule genes *fushi tarazu* and *even-skipped*. They coincide with the parasegmental grooves. There was evidence that the parasegmental border coincided with a lineage restriction, a compartment border (Garcia-Bellido et al., 1973) and now this has been demonstrated by observing the division pattern and gene expression of cells directly (Vincent and O'Farrell, 1992). This means that, by the end of the blastoderm stage, cells are unequivocally allocated to specific parasegments. The parasegmental boundaries become the limits of expression and requirement for homeobox genes such as *Ultrabithorax*, while the mechanisms that position and fix these limits are becoming clearer (see Müller and Bienz, 1991).

Although the pair rule genes locate the parasegment boundaries, they cannot maintain them beyond the first three hours, for many of the pair rule genes switch off and their products disappear around that time. Yet, in our view, these boundaries have to be maintained; for example to impede adjoining populations of dividing cells from mixing, to delimit selector gene expression and to contain gradients of positional information. We propose that the segment polarity genes are largely responsible for these boundary properties. We mean that their products would be instrumental in cell adhesion and cell affinities (lineage restriction?) and act as components of intercellular junctions (limits of fields?). The gradients of positional information set up in relation to these boundaries would subsequently determine cell fate and cell polarity.

There are two main types of evidence consistent with our view. First, the pattern of expression of segment polarity genes, if limited to parts of the parasegment, is either to abut the boundary (*cubitus interruptus, engrailed, hedgehog, patched, wingless*) or form a narrow stripe overlapping the border (*gooseberry*). Second, **all** the mutant phenotypes can be interpreted as due to border failures or to extra borders. To understand this, we need to look back to earlier work.

GRADIENT MODELS

Although most of the experiments on insect segmental gradients were done 20-30 years ago, they have not been superseded, nor have the phenomena found any other explanation. They are concerned with exactly the same type of patterns seen in the *Drosophila* embryo after the first three hours; these are the array of cell types within the segment, their arrangement along the anteroposterior axis and their polarity as indicated in the orientation of anisotropic structures in the cuticle. The models generated from these studies can be applied directly to the *Drosophila* patterns even though they were not designed for *Drosophila*. The system that we envisage is a gradient, probably though not necessarily, a concentration gradient of a morphogen. In the model, the level of the gradient gives the cells information of their position in the segment, determines the patterns of gene expression and thus the types of differentiation. The direction of slope of the gradient gives the cells their polarity. The gradient is reiterated in each segmental unit. Thus, if one segment has a broader band of denticles than another, this is not because the gradient differs in each segment. Rather, common fields of positional information are interpreted uniquely in each segment, the interpretation depending on the subset of homeotic genes that are active

there. Any changes in the gradient, whether brought about by mutations or experiments, would therefore apply equally to all the segments and produce consistent changes in the pattern of each one (reviewed in Lawrence, 1992).

Figs 1 and 2 show an example of a gradient model, in which parasegment borders form barriers between adjacent gradients. It suggests a simple way to interpret all segment polarity phenotypes. These phenotypes, either produced by mutation or by universal expression of the genes, can be arranged into two classes. In the first, the borders 'leak', resulting in a flatter gradient landscape. As shown in Fig. 1, this failure can be massive (as in null alleles of *wingless* and *hedgehog*, or when very little *armadillo* protein is present) or slight (as in *gooseberry*$^{-}$, or when there is some *wingless*, *hedgehog* or *armadillo* proteins). Most segment polarity mutants belong to this class. In the second class, which includes *naked*$^{-}$ and *patched*$^{-}$, ectopic borders appear and these are associated with altered patterns and polarity (Figs 1, 2). For example, in the *naked*$^{-}$ mutant embryo, the cuticle loses the denticle bands and ectopic grooves mark the new boundaries. Using the gradient model, all these changes in the cuticle, in each segment, find a common cause in an altered landscape, which itself derives from the persistence of the original border and the arrival of a new one (see Figs 1, 2). The gradient model provides one explanation for the many patterns in the different segments including such curious features as the elimination of all patches of denticles **except** the beard in the first thoracic segment and not only why the beard persists but also why it has a reflected pattern of denticles (compare Figs 2 and 3).

This suggests that all segment polarity genes are needed for normal parasegment borders. If this is so, what are their wild-type functions? Some gene products might be involved in making a special kind of 'sealing' junction at the border (Sampedro et al., 1993). This special junction might depend on intracellular localisation or stabilisation of the *armadillo* protein (a component of adherens junctions, Peifer et al., 1993), a localisation or stabilisation that requires *wingless* (Riggleman et al., 1990). After generalised expression of *wingless*, the altered localisation of *armadillo* protein can be seen in every cell (Noordermeer et al., 1992), but borders only appear at their normal location and at an ectopic site in the middle of the parasegment. One possible explanation is that normal and ectopic borders might form only where cells with different adhesion properties or 'affinities' meet

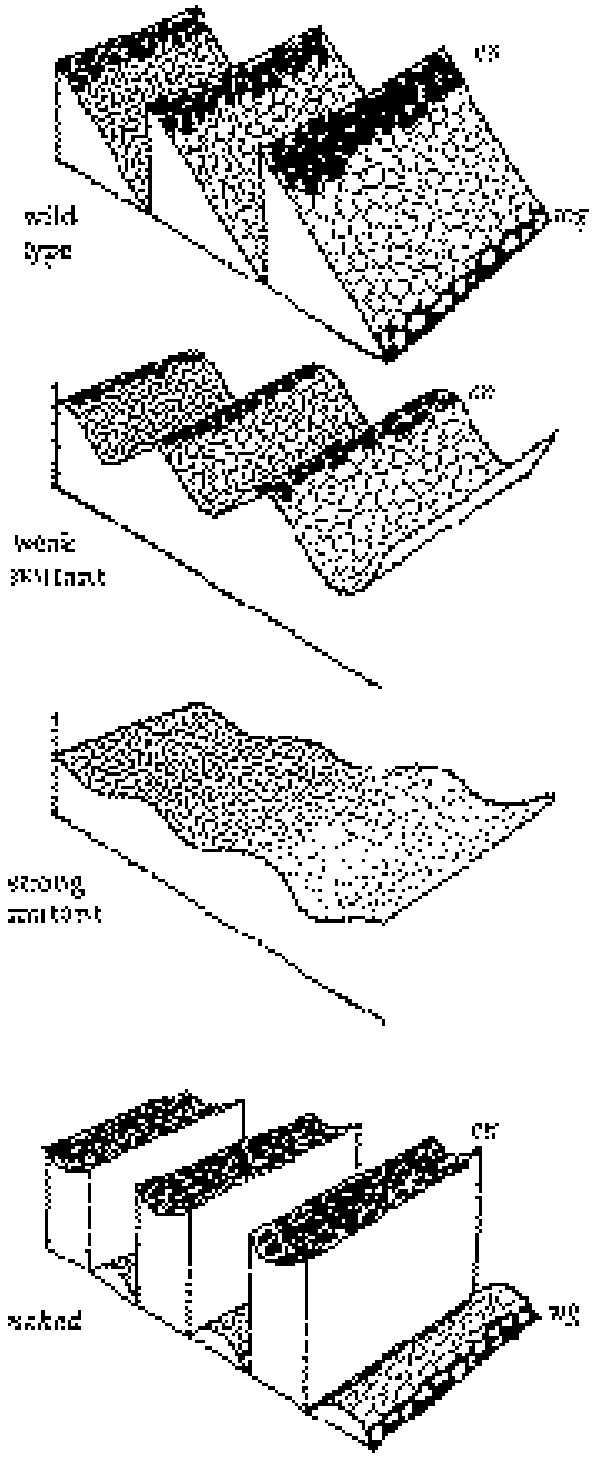

Fig. 1. A possible gradient model (note that there are other ways of drawing the model that would be formally equivalent, for example the slope could be drawn with opposite sign). Gradient profiles of three consecutive parasegments in the wild type and several mutants. Cells are represented as if physically raised by the height of the gradient (which is not the case in real life!). Black dots represent cells expressing *engrailed* (*en*); black background surrounding white dots represents cells expressing *wingless* (*wg*).

The wild type. Parasegment borders coincide with precipices at which cells with the lowest and highest gradient values are juxtaposed. Cells with high values express *engrailed*; cells with low values express *wingless*.

Weak mutants. In a number of segment polarity mutants, the parasegment borders fail to isolate adjacent gradients so that the high values 'leak' into the low and a wavy profile results, with no precipices. Peaks are still high enough for some cells to express *engrailed*, but valleys are not deep enough for *wingless* expression.

Strong mutants. More severe failure of the parasegment borders lead to a flattish landscape without good peaks and valleys, and hence without *engrailed* or *wingless* expression.

naked mutant. In a different kind of mutant phenotype, such as *naked*$^{-}$, inverted extra borders form in the middle of every parasegment, leading to altered landscapes as shown. As a consequence of the new landscape, *engrailed* stripes are broader than normal and ectopic stripes of *wingless* form.

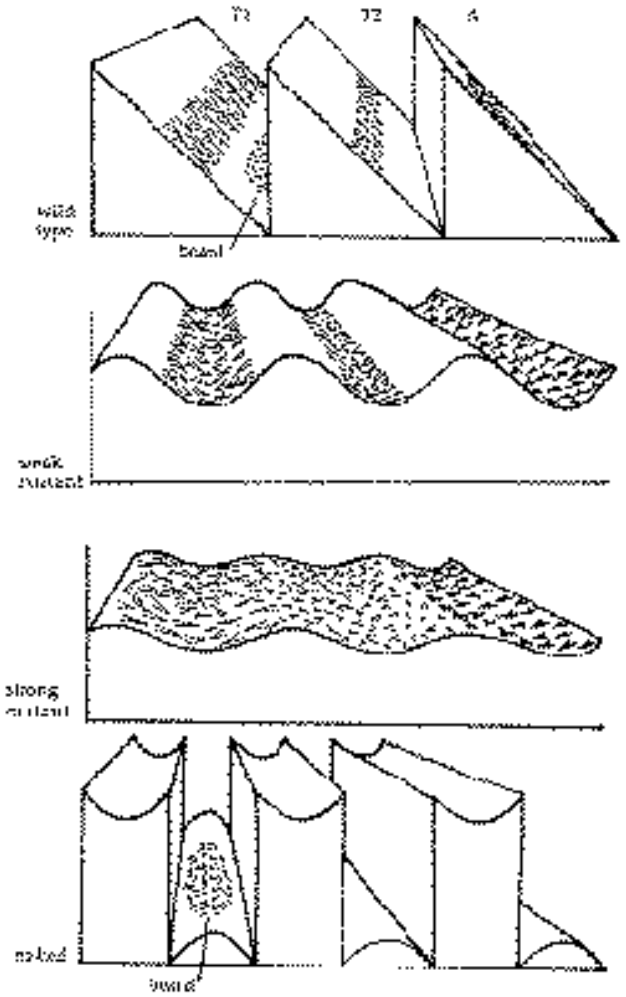

Fig. 2. The same gradient model applied to the cuticle patterns. Gradient profiles of two thoracic parasegments (T1, T2) and a typical abdominal one (A) in the wild type and the same mutants as in Fig. 1. In the model, denticles and naked cuticle are determined between characteristic levels of the gradient, and here they are drawn consistently at those levels. Note also that the slope of the gradient defines the polarity of the denticles.
The wild type. Although the gradient landscape is the same for all parasegments, it is interpreted in specific ways in each of them. This is evident in the varying structure and amount of denticles in the different units. Also note the 'beard', a patch of small denticles specific to part of T1.
Weak mutant. As in Fig. 1, 'leakage' through the parasegment borders results in a wavy profile, with consequent changes in the denticle pattern as shown. Although this phenotype is quite complex (there are stripes of naked cuticle in the thorax but not in the abdomen, planes of symmetry run through the middle of each denticle belt irrespective of the belt size), a simple curve models all these features.
Strong mutant. A more severe failure of the borders produces a flatter landscape. Values for naked cuticle are reached nowhere. Denticle type and polarity reversals still define the different parasegments. (The real mutant embryos are much smaller than shown here, due to cell death. Cell death might be a consequence of the gentle slopes, see Lawrence, 1992).
naked mutant. Inverted extra borders and associated gradient reshaping result in the landscape shown. Values appropriate for denticles are not present, with the exception of the 'beard' in T1 as shown in Fig. 3.

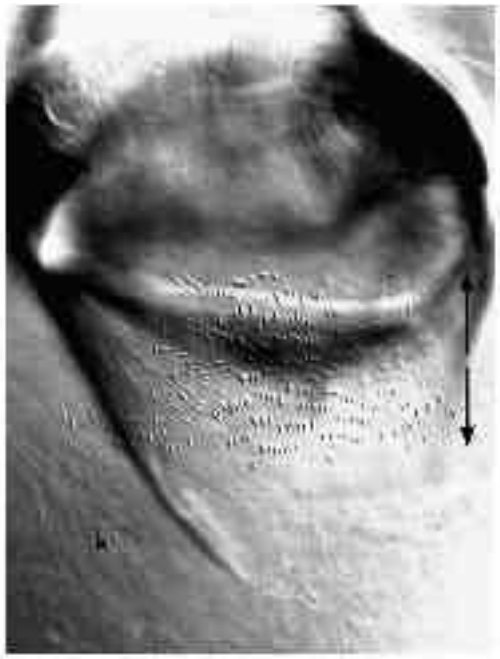

Fig. 3. Interference contrast picture of part of the ventral thorax of an embryo to show the *naked*$^{-}$ phenotype. Note the beard in T1 which shows oriented denticles as predicted by the gradient model (arrows) and the Keilin's organ (*k*). Anterior to top.

(Morata and Lawrence, 1975). Other segment polarity gene products might themselves be instrumental in cell affinities.

If the segment polarity genes are mainly responsible for borders, which genes are responsible for the segmental gradient itself? We mean gene products such as those that make the morphogen, are receptors for it or allow it to be transferred or transduced from cell to cell. We conjecture that the activities of these genes need not be localised (since the placing of boundaries alone might be sufficient to determine the positions, extent and shape of the gradients). Unlocalised gene products are usually deposited in the egg by the mother; possibly, therefore, maternal effect genes may include the genes responsible for making positional information – a search for maternal effect mutations that give segmentation phenotypes has already proved fruitful (Perrimon et al., 1989).

We imagine that, even though the patterns of expression of the segment polarity genes are initially determined by the pair rule genes, they later become dependent on the gradient that they themselves have helped to generate. Once the gradient is established, it is the height, the scalar, of the gradient that locates subsequent gene expression. In this way, the gradient landscapes model not only the abnormal cuticle patterns but also the changed patterns of gene expression found in the mutants (Figs 1, 2). In the gradient model, in strong mutants such as *wingless*$^{-}$, the borders fail, the gradient cannot form properly and a flattish landscape results at a level normally found near the middle of the segment (Fig. 1). In the absence of *engrailed*, the landscape is also flattened. These flattened landscapes mean that neither *engrailed* nor *wingless* can be expressed any longer – because expression of each of these genes is maintained only between certain characteristic levels of the gradient and

those levels are no longer there. In other words, *wingless*- and *engrailed*-expressing cells are postulated to be mutually interdependent because they each perform essential tasks in the maintenance of the borders of the segment gradient. We suggest this feedback between borders and gradient values is also the basis of the autoregulation that has been observed for both *wingless* and *engrailed* (Bejsovec and Martinez-Arias, 1991; Heemskerk et al., 1991). In the *naked*$^{-}$ mutant, the alterations in the *engrailed* and *wingless* stripes are also modelled by the new landscape (Fig. 1).

THE MODELS COMPARED

These ideas differ radically from the various assumptions and models favoured in nearly all of the many recent publications on segment polarity genes (see reviews listed above); models that generally assume that these genes directly determine cell states within the segment: For example, in a row of cells, the products of two segment polarity genes might be present, a combination that would specify cell fate in that row (e.g. Martinez-Arias et al., 1988; Dougan and DiNardo, 1992). Most of the current models (reviews listed above) have been built up step by step, and modified bit by bit, to accommodate new experimental results (e.g. compare the model in Martinez-Arias et al., 1988 with those of Ingham, 1991 and Dougan and DiNardo, 1992). This type of model building ('carpentry', Crick, 1988) tends to produce intricate explanations that, naturally, fit the data, but can contain circular arguments and hide simple clarifying principles (if they exist!). In the following list, we compare the two ways of interpreting these genes with respect to mutant phenotypes, wild-type functions and future experiments.

(1) The segment polarity genes have been seen as subdividing the segments into qualitatively different parts, seen as defining 'domains' (as the gap genes do) or 'cell states' (as the homeotic genes do). For example, the individual denticle rows could be considered as discontinuous outcomes of discrete cell states, row 1 being qualitatively different from row 2, row 3 from 4 and so on.

In the gradient model, pattern and polarity depend on a variable that changes continuously across the segment.

(2) The segment polarity mutant phenotypes have been interpreted as if specific elements of the pattern are dependent on single genes and cut out, piece by piece, in the mutants. Thus in the *naked* mutant, the denticle belt is 'deleted'; in some *wingless* mutations, cells secreting naked cuticle are 'transformed' into cells making denticles.

In the gradient model, the phenotypes are interpreted as reorganisations of the whole pattern, which derive from effects on the boundaries. Polarised denticles and naked cuticle are produced as a direct readout of positional information.

(3) Most current papers tend to avoid trying to explain pattern in the cuticle, and concentrate rather on the genetic interactions between segment polarity genes. They apply the **digital** logic of epistasis and arrange the genes in hierarchies and pathways. The logic of epistasis can be safely applied to linear pathways where a series of genes act one after the other; however, it may be precarious to apply the same logic to segment polarity genes. There are three main difficulties: First, some segment polarity genes may encode structural proteins; if the gene products join together to form structures, it is not easy, and may not be useful, to arrange them in a hierarchy. Second, the segment polarity genes act in a sheet of cells in which the neighbours change as the cells move and divide; pathways of cell interactions may easily become inscrutable. Third, these genes are expressed over a long period while both their patterns of expression and their functions, evolve (e.g. Heemskerk et al., 1991.) One unwarranted outcome of the current papers is the general perception that the main function of the segment polarity genes is to regulate each other, each one becoming an element in a network of 'cross-regulatory interactions'.

In the gradient model, the genes are involved in the function of the borders that delimit the gradients; the emphasis is not so much on how they 'interact' with each other, but on what their protein products might do, to set up the borders, to give the cell junctions particular properties, or otherwise to help establish the gradients. The 'interactions' are seen as indirect consequences of how the genes affect the borders and the gradients.

(4) One key observation is the interdependence of *wingless* and *engrailed*; remove either gene and expression of the other is no longer maintained (DiNardo et al., 1988; Martinez-Arias et al., 1988). This interdependence has contributed to the hypothesis that the *wingless* (and other *Wnt* genes) encode signalling molecules that carry messages from secreting to responding cells. The *wingless* protein is supposed to cross the parasegment border to keep the *engrailed* gene turned on in any cells that are near enough to receive the '*wingless* signal' (DiNardo et al., 1988). This signal hypothesis has good evidence in its favour; for example, the *wingless* protein is secreted and antigen can be seen in nearby cells (van den Heuvel et al., 1989; González et al., 1991); in the gut, *wingless*, when expressed in the visceral mesoderm, influences gene expression in the endoderm (Immerglück et al., 1990). Also, when *engrailed*-expressing cells are isolated from embryos, they lose *engrailed* expression, but this does not happen if they are mixed with *Drosophila* tissue culture cells that produce *wingless* protein (Cumberledge and Krasnow, 1993). There are other views of the function of *wingless*: in the leg disc, small groups of cells that express *wingless* protein ectopically can reorganise the pattern of wild-type cells nearby, suggesting that the *wingless* protein itself could be a morphogen (Struhl and Basler, 1993). Although all these results are suggestive, they do not prove that the *wingless* protein itself embodies a message. In the epidermis of the embryo, it has been shown that uniformly distributed *wingless* protein can emulate the wild-type function of the gene, undermining theories that demand that the protein be unevenly distributed – as it must be if it is to act as a morphogen gradient or as a locally instructive signal (Sampedro et al., 1993).

Because continued expression of *wingless* depends on *engrailed* function, there is postulated to be another signal passing from the *engrailed*-expressing cells to their *wingless*-expressing neighbours. It is thought that this latter signal might be the *hedgehog* protein and that this might interact with *patched* (Ingham et al., 1991).

The gradient model provides an alternative explanation for the mutual dependence of *engrailed* and *wingless* (Figs 1, 2).

(5) Digital models have led to many experiments in which segment polarity genes are eliminated in twos or even threes, in which gene products are removed or added at different stages of development and the effects on the other genes studied. Again these experiments emphasise the epistatic relations between the segment polarity genes.

The gradient model helps in the interpretation of some of these double mutants. For example, we have classified the mutants into those in which ectopic borders affect the pattern (e.g. *patched*) and those that impair border function (e.g. *hedgehog*). This perspective might suggest that a mutant of the latter class could correct the phenotype of the former class; indeed the *patched; hedgehog* double mutant has a less severe phenotype than either mutant alone (Ingham et al., 1991).

The gradient model leads to different kinds of experiments. Here are some examples: When dyes are injected into cells, a restriction in dye-spread shows that intercellular permeability can change at compartment borders (Warner and Lawrence, 1982; Blennerhasset and Caveney, 1984). This suggests the presence of special junctions there, or an agent that blocks intercellular channels between cells in different compartments. As far as we know, experiments on dye-spread and electrical coupling in the *Drosophila* embryo have not been reported. More attempts could be made to identify the morphogen. As it may well depend on genes which are transcribed in the mother, during oogenesis, more of these could be looked for. In general terms, the advantage of the gradient explanation is that the model is internally consistent and aims to explain coherently all aspects of the pattern including cell polarity; the disadvantage is that the molecular basis of the gradient is still unknown. However, gradient molecules are no longer mythical; in the last few years several morphogens have been identified in the *Drosophila* embryo, both in the syncytial stage before cells are formed (Driever and Nüsslein-Volhard, 1988; Struhl et al., 1992) and after cellularisation (Ferguson and Anderson, 1992).

We thank Gary Struhl and our colleagues at the LMB and other scientific institutions for their advice. J. S. is supported by a Fleming Fellowship of the Ministerio de Educacion y Ciencia.

REFERENCES

Bejsovec, A. and Martinez-Arias, A. (1991). Roles of *wingless* in patterning the larval epidermis of *Drosophila*. *Development* **113**, 471-485.

Blennerhassett, M. G. and Caveney, S. (1984). Separation of developmental compartments by a cell type with reduced junctional permeability. *Nature* **309**, 361-364.

Cumberledge, S. and Krasnow, M. A. (1993). Intercellular signalling in *Drosophila* segment formation reconstructed *in vitro*. *Nature* **363**, 549-552.

Crick, F. H. C. (1988). *What Mad Pursuit.* p60. New York:Basic Books.

DiNardo, S., Sher, E., Heemskerk-Jongens, J., Kassis, J. and O'Farrell, P. (1988). Two-tiered regulation of spatially patterned *engrailed* gene expression during *Drosophila* embryogenesis. *Nature* **332**, 604-609.

DiNardo, S. and Heemskerk, J. (1990). Molecular and cellular interactions responsible for intrasegmental patterning during *Drosophila* embryogenesis. *Sem. Cell Biol.* **1**, 173-183.

Dougan, S. and DiNardo, S. (1992). *Drosophila wingless* generates cell type diversity among *engrailed* expressing cells. *Nature* **360**, 347-350.

Driever, W. and Nüsslein-Volhard, C. (1988). A gradient of *bicoid* protein in *Drosophila* embryos. *Cell* **54**, 83-93.

Ferguson, E. L. and Anderson, K. V. (1992). *decapentaplegic* acts as a morphogen to organize dorsal-ventral pattern in the Drosophila embryo. *Cell* **71**, 451-461.

García-Bellido, A., Ripoll, P. and Morata, G. (1973). Developmental compartmentalisation of the wing disk of *Drosophila*. *Nature New Biol.* **245**, 251-253.

González, F., Swales, L., Bejsovec, A., Skaer, H. and Martinez-Arias, A. (1991). Secretion and movement of *wingless* protein in the epidermis of the *Drosophila* embryo. *Mech. Dev.* **35**, 43-54.

Heemskerk, J., DiNardo, S., Kostriken, R. and O'Farrell, P. H. (1991). Multiple modes of *engrailed* regulation in the progression towards cell fate determination. *Nature* **352**, 404-410.

Hooper, J. E. and Scott, M. P. (1992). The molecular genetic basis of positional information in insect segments. In *Results and Problems in Cell Differentiation* (ed. W. Hennig) **18**, 1-48. Berlin Heidelberg: Springer-Verlag.

Immerglück, K., Lawrence, P. A. and Bienz, M. (1990). Induction across germ layers in Drosophila mediated by a genetic cascade. *Cell* **62**, 261-268.

Ingham, P. W. (1991). Segment polarity genes and cell patterning within the *Drosophila* body segment. *Curr. Op. Gen. Dev.* **1**, 261-267.

Ingham, P. W. and Martinez-Arias, A. (1992). Boundaries and fields in early embryos. *Cell* **68**, 221-235.

Ingham, P. W., Taylor, A. M. and Nakano, Y. (1991). Role of the *Drosophila patched* gene in positional signalling. *Nature* **353,** 184-187.

Lawrence, P. A. (1987). Pair-rule genes: Do they paint stripes or draw lines? *Cell* **51**, 879-880.

Lawrence, P. A. (1992). *The Making of a Fly*. Oxford: Blackwell Scientific Publications.

Martinez-Arias, A. (1989). A cellular basis for pattern formation in the insect epidermis. *Trends Genet.* **5**, 262-267.

Martinez-Arias, A., Baker, N. E. and Ingham, P. W. (1988). Role of segment polarity genes in the definition and maintenance of cell states in the *Drosophila* embryo. *Development* **103**, 157-170.

Morata, G. and Lawrence, P. A. (1975). Control of compartment development by the *engrailed* gene in *Drosophila*. *Nature* **255**, 614-617.

Müller, J. and Bienz, M. (1991). Long range repression conferring boundaries of *Ultrabithorax* expression in the *Drosophila* embryo. *EMBO J.* **10**, 3147-3155.

Noordermeer, J., Johnston, P., Rijsewijk, F., Nusse, R. and Lawrence, P. A. (1992). The consequences of ubiquitous expression of the *wingless* gene in the *Drosophila* embryo. *Development* **116**, 711-719.

Nüsslein-Volhard, C. (1991). Determination of the embryonic axes of *Drosophila*. *Development* (**1991 Suppl. 1**), 1-10.

Nüsslein-Volhard, C. and Wieschaus, E. (1980). Mutations affecting segment number and polarity. *Nature* **287**, 795-801.

Peifer, M. and Bejsovec, A. (1992). Knowing your neighbors: Cell interactions determine intrasegmental patterning in *Drosophila*. *Trends Genet*. **8**, 243-249.

Peifer, M., Orsulic, S., Sweeton, D. and Wieschaus, E. (1993). A role for the *Drosophila* segment polarity gene *armadillo* in cell adhesion and cytoskeletal integrity during oogenesis. *Development* **118**, 1191-1207.

Perrimon, N., Engstrom, L. and Mahowald, A. P. (1989). Zygotic lethals with specific maternal effect phenotypes in *Drosophila melanogaster*. I. Loci on the X chromosome. *Genetics* **121**, 333-352.

Riggleman, B., Schedl, P. and Wieschaus, E. (1990). Spatial expression of the Drosophila segment polarity gene *armadillo* is posttranscriptionally regulated by *wingless*. *Cell* **63**, 549-560.

Sampedro, J., Johnston, P. and Lawrence, P.A. (1993). A role for *wingless* in the segmental gradient of *Drosophila*? *Development* **117**, 677-687.

Struhl, G., Johnston, P. and Lawrence, P.A. (1992). Control of Drosophila body pattern by the *hunchback* morphogen gradient. *Cell* **69**, 237-249.

Struhl, G. and Basler, K. (1993). Organizing activity of wingless protein in Drosophila. *Cell* **72**, 527-540.

van den Heuvel, M., Nusse, R., Johnston, P. and Lawrence, P.A. (1989).

Distribution of the *wingless* gene product in *Drosophila* embryos: a protein involved in cell-cell communication. *Cell* **59**, 739-749.

Vincent, J.-P. and O'Farrell, P. H. (1992). The state of *engrailed* expression is not clonally transmitted during early Drosophila development. *Cell* **68,** 923-931.

Warner, A. E. and Lawrence, P. A. (1982). The permeability of gap junctions at the segmental border in insect epidermis. *Cell* **28**, 243-252.

Woods, D. F. and Bryant, P. J. (1992). Genetic control of cell interactions in developing Drosophila epithelia. *Ann. Rev. Genet.* **26**, 305-350.

(Accepted 20 September 1993)