Primer

Patterning the *Drosophila* embryo

Theodora Bloom

The Nobel Prize for Physiology and Medicine was awarded to Edward Lewis, Christiane Nüsslein-Volhard and Eric Weischaus for their pioneering work on the genetics of pattern formation in the fruitfly *Drosophila melanogaster*. Their work and that of numerous others over the past thirty years or so has led to the most complete picture for any organism of the genes required to set up patterns in the developing embryo that will determine the shape and features of the adult organism. Many of the principles their work uncovered — and indeed many of the genes — are conserved among species from the nematode worm to mouse and man. This primer will review one aspect of pattern formation in the fruitfly, namely the formation of individual segments with specialized functions along the anterior–posterior (head-to-tail) axis, and will consider some of the implications of studies on this subject for workers in other fields.

The genetics of fruitfly development

The development of the adult fruitfly from a fertilized egg takes about nine days, passing through three larval stages in which the basic segmented pattern of the adult body is apparent (Fig. 1). During these stages, certain adult structures, such as the eyes, legs and wings, develop in specialized bags of cells — the imaginal discs — within particular segments. Lewis performed classical genetic analyses of mutations affecting the character of individual segments. Weischaus and Nüsslein-Volhard carried out an enormous screen for mutations in genes expressed in the embryo that affect patterning during development. In their screen, male fruitflies were treated with a chemical to induce mutations in a proportion of their sperm. The offspring were then interbred and the next generation of offspring examined to identify any recessively acting mutations that had disrupted segmental pattern, apparent as changes in the pattern of bristles and segments visible on the larval cuticle.

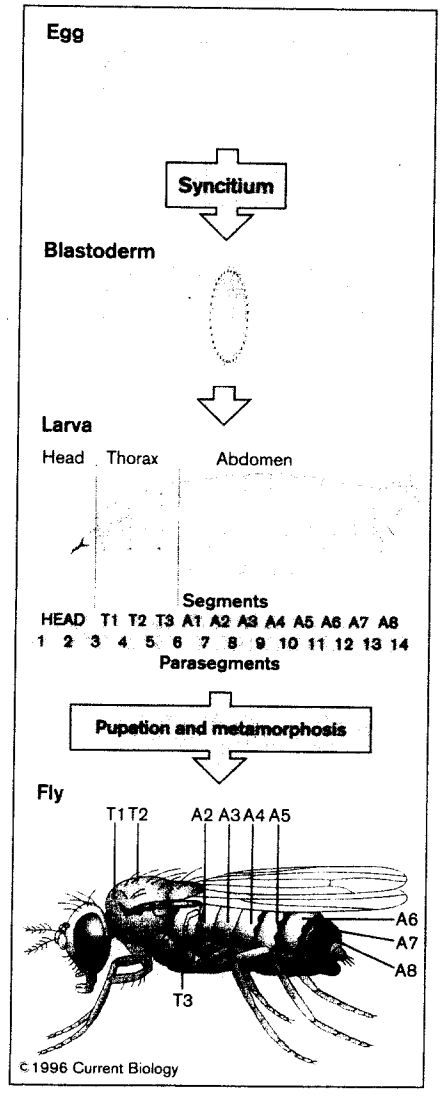
Weischaus and Nüsslein-Volhard were able to identify three groups of 'segmentation genes', mutations in which affect the pattern of segments in the larva (these genes are discussed in more detail below). It was already well-established that the unfertilized egg has within it the positional information required to differentiate the two main axes, anterior to posterior and dorsal (back) to ventral (belly). Later genetic screens identified the maternal-effect genes. Mutations in these are manifest only in the eggs produced by mutant mothers, because the encoded proteins are involved in patterning the egg while it lies within the ovary. These maternally encoded factors, or determinants, are involved in establishing initial gradients of activity along each main axis, usually from a source at one point (see Fig. 2). Once cells have 'perceived' these gradients, they are then acted on by the segmentation genes; the combination of gene activities sequentially subdivides the embryo into strips made up of small groups of cells that have had identical patterns of gene expression and that prefigure the segments of the embryo and adult.

The rules underlying development

Although from looking at an insect larva one might suppose that the segment is the basic unit of its body, the genes determining pattern in fact operate within parasegments (Fig. 1), each made up of the posterior part of one segment and the anterior part of the next one back. The problem of patterning is then two-fold: to divide the body up into repeating units, the parasegments, and to give each parasegment a different fate, according to its position. So, a parasegment develops, for example, wings appropriate to one thoracic segment or the antennae characteristic of the head.

The answer to the first part of the patterning problem is that the segmentation genes lay down the basic pattern of parasegments with distinct identities. The determinants of anterior–posterior pattern in the fruitfly can be roughly divided into

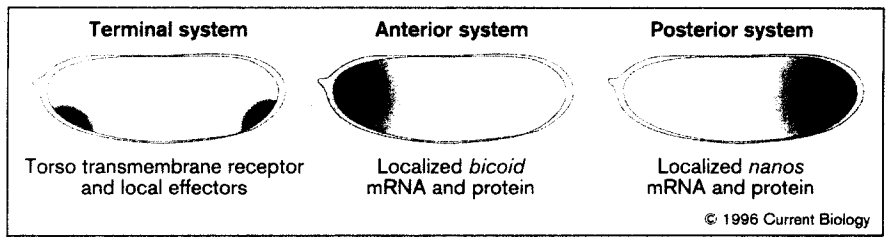
Figure 1



Development from an egg to an adult fruitfly. The nucleus of the fertilized egg initially divides many times to produce a multinucleate syncitium; this then becomes divided up into cells.

Figure 2

Localized signals laid down within the egg itself are central to determining the later pattern of the embryo, but the three region-specific systems responsible for patterning different parts of the body plan do not all use the same mechanism.



those that affect the anterior, the posterior or the terminal tips of the body pattern (Fig. 3a). Within each of these areas, a localized signal in the oocyte determines the localization of proteins and the transcription of genes that are active within that area of the embryo (Fig. 2).

It is during the earliest syncitial stages of development of the fertilized egg through to the blastoderm stage that the three classes of segmentation gene come into play. First, half a dozen or so gap genes coarsely divide the embryo up into blocks (Fig. 3b). Overlaid onto this is a repeating striped pattern of expression of six or seven pair-rule genes in overlapping and offset patterns that, between them, mark out each parasegment from the next. Finally, the segment polarity genes act and lead to the differentiation of anterior from posterior identities within each parasegment. So, for example, the segment polarity gene products Wingless and Hedgehog signal back and forth across parasegment boundaries and so maintain sharp parasegment boundaries. These molecules are sources of information for patterning the remainder of the parasegment, and together with further cell–cell signalling molecules allow the specification of the future fate of strips of cells only a single cell wide.

Ultimately, within each group of cells that share a pattern of segmentation gene expression, 'homeotic selector genes' act to specify the body parts that should be made there. In effect, a series of 'genetic addresses' is laid down for each strip of cells along the anterior–posterior axis, determining which homeotic selector genes should be expressed in each small group of cells. For example, the *Antennapedia* gene is normally expressed in the thoracic and abdominal parts of the fruitfly, and expression is at its highest in the thorax, where legs are made on each segment. Inappropriate expression of *Antennapedia* in the head causes legs to be made there instead of antennae. Thus, the region of the head that normally makes an antenna is set aside to make an appendage (from an imaginal disc), and altering the expression of a single 'master' gene can change that appendage from antenna to leg.

The process by which a mutation can cause one part of the body to be replaced by one appropriate for another position was originally described as 'homeosis' early this century. Lewis's model for the activity of different combinations and increasing numbers of homeotic genes within successively more posterior segments of the fruitfly, giving a detailed genetic address to each segment, has been central to understanding in the field for the past twenty years. We now know that not only are individual selector genes expressed in just the sorts of bands across the embryo from head to tail that were predicted by Lewis's model, but the organization of homeotic genes in clusters along the fruitfly chromosome mirrors the order of their expression along the body. DNA sequence analysis also revealed that all selector genes have a similar domain, the homeobox, which is responsible for binding to DNA.

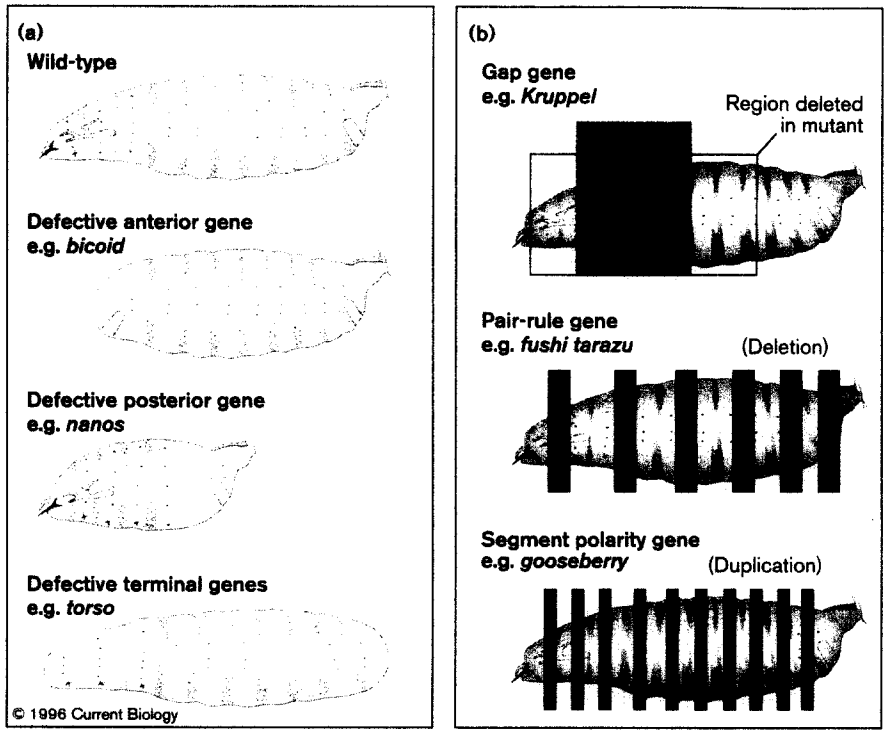
Themes and variations

Subtly different cellular and molecular mechanisms are used in the early steps of patterning the anterior, posterior and terminal parts of the body (Fig. 2). Each sets up a gradient of activity that gives cells a key piece of positional information: where they lie along the anterior–posterior axis. Once the segmental pattern of regulatory gene expression is established, it is rapidly translated into a more permanent pattern of differential selector gene activation that regulates further 'downstream' genes that will actually make legs, wings and so on. But the initial segmentation pattern itself vanishes; it can only be visualized as localized mRNA or protein for a short time window during early development. Each homeotic selector gene probably acts directly as a transcription factor: by binding to DNA at specific sequences, each can regulate the expression of downstream genes that will in turn effect changes in cell behaviour. The downstream genes ultimately regulate properties such as differential cell adhesion and the cell division cycle.

Links to other areas of biology

The modern study of pattern formation in *Drosophila*, as described here, addresses many questions common to other studies of cell and molecular biology, such as how cells interact and adhere to each other differentially, how signals are transduced from outside a cell to its interior, and how this alters cell behaviour and patterns of gene expression. But it is not only the questions being addressed that

Figure 3



(a) The cuticular phenotypes of embryos mutant for one of the anterior, posterior or terminal genes responsible for patterning. Broadly, the anterior system is responsible for patterning in the thorax (so this region is deleted in anterior gene mutants), the posterior for patterning the abdomen, and the terminal for patterning the acron and telson at the extreme tips of the larva. Adapted from Nüsslein-Volhard C and Weischaus E, *Nature* 1980, **278**:795–801. **(b)** Indication of the regions affected in larvae mutant for one of the gap, pair-rule or segment polarity genes. The regions missing from the mutant larvae are boxed in red and the region corresponding to the domain of expression of the wild-type gene (at an earlier stage) is shaded in blue. The affected regions are deleted in *Kruppel* and *fushi tarazu* mutants but replaced by duplications of the adjacent unaffected regions in *gooseberry* mutants. Adapted from St Johnston D and Nüsslein-Volhard C, *Cell* 1992, **68**:201–219.

Drosophila studies share with those of other systems. Homologues of many genes involved in determining early pattern have been found in other species, and in some cases the similarities are striking, as in the *Hox* genes of vertebrates. These homologues of the fruitfly homeotic genes mimic both the chromosomal organization and expression patterns of their *Drosophila* counterparts. In addition, the mouse homologues of two of the *Drosophila* gap genes involved in patterning the head are expressed in nested patterns on the head of the mouse. In other cases, although some of the cellular functions of homologous genes may be conserved, the spatial patterns of expression may be quite different. For example, the mouse *wnt-1* homologue of the segment-polarity gene *wingless* may well have a role in patterning, but it is not expressed in stripes along the body.

Open questions

From the results of successive mutagenesis screens, and according to models of the number of steps involved in the initial patterning of the *Drosophila* embryo, we can guess that most of the genes responsible are now known. They number about thirty: several genes are required in forming a single gradient of *bicoid*, in the anterior, for example. But clearly some are not yet known — they are apparent as gaps in schemes of the various signalling pathways and the responses to them. And even the portions of the patterning picture that seemed clear a year ago are subject to modification all the time. The biggest gap in our understanding now may be the question of how the ovary sets up the first pattern in the oocyte. More sophisticated techniques may be needed for studying the effects of mutations in genes so central to patterning the egg that all of later development fails. Finally, a major unknown is the extent to which the many homologues of *Drosophila* patterning genes found in other species have analogous functions. *Drosophila* is an excellent model organism for studying patterning molecules that seem to function in many species, but it is perhaps too much to hope that the beauty and simplicity of the segmentally repeated fruitfly body plan can be used as a precise template for understanding development in such evolutionarily distant cousins of the fruitfly as mouse and man.

Acknowledgements

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Key references

Lewis EB: **A gene complex controlling segmentation in *Drosophila*.** *Nature* 1978, **276**:565–570.

Nüsslein-Volhard C, Weischaus E: **Mutations affecting segment number and polarity in *Drosophila*.** *Nature* 1980, **278**:795–801.

St Johnston D, Nüsslein-Volhard C: **The origin of pattern and polarity in the *Drosophila* embryo.** *Cell* 1992, **68**:201–219.

Lawrence P: *The Making of a Fly.* Oxford: Blackwell; 1992.

Alberts B, Bray D, Lewis J, Raff M, Roberts K, Watson JD: *Molecular Biology of the Cell,* 3rd edn; chapter 21. New York: Garland; 1994.

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