The Polar Coordinate Model Goes Molecular

Peter J. Bryant

One of the fascinations of animal development is that cells appear to know their physical position in the embryo and behave (differentiate, grow, divide, migrate, die) accordingly. But what is the frame of reference for such a positional information system, and how is it generated? Conventional descriptions of embryos and limbs in terms of anterior-posterior and dorsal-ventral axes imply that a Cartesian system of orthogonal coordinates specifies position in developing systems. However, an alternative idea, which came from studies of limb regeneration in insects and vertebrates, was the polar coordinate model, proposed in 1976 (*1*). In this model the position of limb cells is specified by one coordinate corresponding to the proximal-distal axis of the limb and another corresponding to position on one of a concentric series of circles at different proximal-distal levels. The molecular basis of the polar coordinate system has always been elusive, but important clues are now coming from several sources. One of these sources is the genes that control the primary segmentation pattern of the *Drosophila* embryo. Several of these genes also participate in limb development, and their expression patterns and mutant phenotypes provide striking evidence in favor of a polar coordinate system of positional information. Here I concentrate on the developing leg (Fig. 1); the slightly more complex situation in the wing is discussed elsewhere (*2*).

Genes that potentially specify the circumferential coordinate have been found in the segment polarity class. In the embryo, these genes are expressed as patterns of stripes of less than a segment in width; their mutations cause alterations of segment pattern and polarity (*3*). In the *wingless* subclass of segment polarity genes, mutations cause deletion of the posterior region of each embryonic segment and its replacement by a mirror-image duplication of the anterior denticle belt. It is difficult to test these genes for a subsequent effect on imaginal disc patterning because the mutants die as embryos, before imaginal disc growth. However, analysis of the phenotypes of weak alleles, and of the phenotypes of homozygous cell clones in mutant heterozygotes, has shown that at least four of the *wingless*-subclass genes function again in setting up the limb pattern (*4*, *5*).

The author is at the Developmental Biology Center, University of California at Irvine, Irvine, CA 92717.

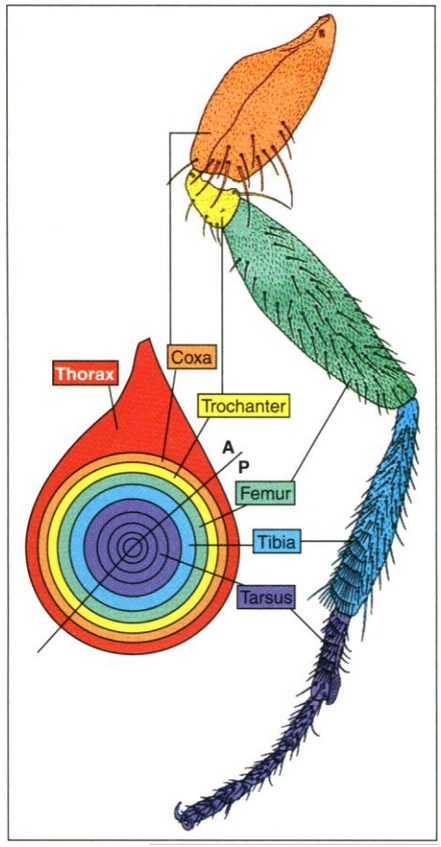

Fig. 1. The leg imaginal disc of *Drosophila* develops into the adult leg. During metamorphosis, the imaginal disc dramatically elongates, the center of the disc becomes the distal tip of the leg, and the periphery becomes the proximal boundary.

The expression pattern of, and requirement for, the *wingless* gene in the developing imaginal discs are described by Couso and co-workers in this issue of *Science* (*6*). When it is required for limb patterning, *wingless* is expressed in a narrow ventral-anterior sector in the leg disc (Fig. 2). In flies carrying an allele of *wingless* that is lethal at the pupal stage (*4*, *7*), or a heat-shocked temperature-sensitive heteroallelic combination (*6*), ventral structures are replaced by a mirror-image duplication of dorsal structures. The deleted region includes not only the ventral-anterior sector but also flanking areas. This nonautonomous effect of the mutation outside the region where the gene is expressed is consistent with the idea that the gene product diffuses away from the cell that makes it and is required for normal development of other cells. Small *wingless* mutant clones in a region requiring *wingless* can be rescued by surrounding wild-type cells, confirming that the gene product can affect cells that do not express *wingless*. Indeed, the *wingless* product is a secreted protein that is transferred between cells in vesicles at the apical end of the cell (*8*, *9*).

Other genes in the *wingless* subclass may encode additional elements of the *wingless*-mediated signalling pathway. The *armadillo* gene is expressed over the entire leg disc, but appears to be required only in a wide ventral sector that includes the narrower region where *wingless* is expressed (*4*). This larger sector may correspond to the region over which the *wingless* signal is effective, and *armadillo* may function in the response of cells to the *wingless* signal (*4*). Another element in the response to the *wingless* signal is the *engrailed* gene, a member of a different subclass of segment polarity genes. In embryonic segment patterning, *wingless* and *engrailed* are expressed in adjacent domains, and each is required to maintain the expression of the other (*10*). Both *wingless* and *engrailed* products seem to be essential for the initial establishment of the leg imaginal discs in the embryo, because mutations in these genes completely (*wingless*) or almost completely (*engrailed*) prevent the formation of leg imaginal discs in cultured embryos (*11*).

In the leg disc, *engrailed* is expressed in a 180° sector adjacent to the *wingless*-expressing sector (*12*) (corresponding to the posterior compartment), and *hedgehog* shows a similar expression pattern. Loss of *engrailed* function causes transformation of posterior into anterior structures (*13*). The *engrailed* gene encodes a homeodomain-containing transcription factor (*14*) and appears to be responsible for repressing the expression of *cubitus interruptus* in the posterior compartment (*12*), leaving it expressed only in the anterior compartment.

The circumferential coordinate of the leg disc pattern could be specified by localized expression of these and other segment polarity genes, just as anterior-posterior position within the embryonic segment is specified by local expression of the same genes. The expression patterns of *wingless* and *engrailed* in embryonic segments evolve quite directly into the imaginal disc patterns, as shown by Couso and co-workers (*6*). The phenotypes of the adult-viable segment polarity gene mutants (*15*) are consistent with their being involved in controlling the circumferential coordinate. Mutations, not only in *wingless*, *engrailed*, and *cubitus interruptus*, but also in *patched* and *fused*, each affect one or a few longitudinal bristle rows, corresponding to a specific part of the circumferential dimension. This result suggests that these genes will also have sectorial patterns of expression.

Proximal-distal levels of the developing

leg pattern are also associated with the local expression of specific genes (Fig. 2). In some cases expression is in a single ring, for example, the *teashirt* gene at a very proximal level (coxa-trochanter) and the *apterous* gene at a distal level (fourth tarsal segment). One gene, *rotund*, is expressed in a larger region of the proximal-distal axis (tarsal segments 1 to 4). In other cases—*odd-skipped*, *disconnected*, and *Distal-less*—expression is seen in a number of concentric rings, each one usually corresponding to part of the proximal-distal extent of a given leg segment (*16*).

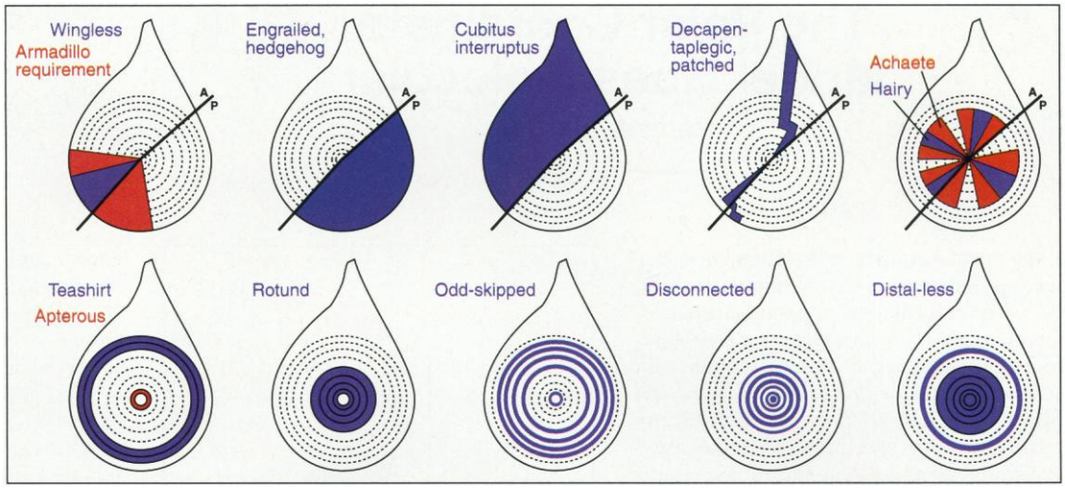

Fig. 2. Patterns of gene expression in the leg disc (late third instar). The patterns are determined from RNA in situ hybridization, protein immunolocalization, or staining for beta-galactosidase produced by a reporter gene (*21*). References: *wingless* (*6*); *armadillo* (*4*); *engrailed* and *cubitus interruptus* (*12*); *hedgehog* (*24*); *decapentaplegic* (*25*); *patched* (*26*); *achaete* and *hairy* (*27, 28*); *rotund* (*17*); *teashirt, odd-skipped, disconnected, Distal-less,* and *apterous* (*16*). The patterns for *achaete* and *hairy* are from the pupal leg. The *wingless* diagram also shows the region over which *armadillo* function is required (*4*). A/P, the boundary separating anterior and posterior lineage compartments (*16*).

These expression patterns show that cells at a given proximal-distal level share some molecular identity. But is that identity important in pattern formation? The phenotypes produced by mutations in some of these genes show that they are, in fact, essential for leg development. Thus, mutations of *rotund*, which is expressed in tarsal segments 1 to 4, cause cell death and loss of the corresponding segments (*17*). Mutations of *Distal-less*, which is expressed at high levels in the tarsal segments and distal tibia (*16*), cause loss of distal parts of the leg; the amount of loss depends upon the allele in a manner that suggests a graded requirement for the gene product along the proximal-distal axis (*18*). The *disconnected* and *apterous* genes, in spite of showing expression at specific proximal-distal levels, are not required for leg development.

Analysis of some of these genes is providing new information about how the circumferential and proximal-distal dimensions of the leg disc may be related. In the polar coordinate model, it was proposed that generation of distal structures requires a majority of circumferential positional values, and the phenotypes of several mutants support this idea. For example, the dorsal duplications produced in temperature-sensitive *wingless* animals are distally incomplete or distally branched, depending on the time of the heat pulse (*6*). Mutations of the *decapentaplegic* gene, which is expressed as a narrow proximal-distal stripe along the anterior-posterior compartment boundary, cause loss of distal structures, the extent of loss depending on the allele (*19*) Clones of homozygous mutant *hedgehog* cells in the posterior compartment of the disc, where the gene is expressed, give rise to distal defects in the legs that include anterior as well as posterior structures (*20*).

Additional genes showing local expression in imaginal discs have been identified in "enhancer trap" lines—strains of flies in which a reporter gene has been transposed into random locations in the genome and used to detect genes that have interesting spatial patterns of expression (*16, 21*). Some of the most common spatial patterns in the leg disc are single rings and concentric rings (*22, 23*), suggesting that additional genes will be found that function at distinct levels of the proximal-distal axis. Radial sectors and arcs of expression have also been found in some of these lines, again consistent with the idea of a polar coordinate system of positional information.

The genetic control of patterning in the developing imaginal discs is starting to resemble the much-better understood situation in the overall embryo pattern (*3*). The expression pattern of *rotund* in the leg disc may be analogous to those of gap genes in the embryo, in that the gene is expressed and required in a multisegment domain (*17*), while in other cases the expression pattern seems to be more analogous to that of the segment polarity genes.

The segment polarity genes potentially participate in specific signalling pathways in the embryo (*3*), and the positional information system in developing imaginal discs appears to utilize many of the same gene products and possibly the same pathways. Imaginal disc development is more complex than that of embryonic segments, with the addition of a proximal-distal axis and the added requirement of controlling cell proliferation in a spatially organized way. Nevertheless, with many cloned genes in hand and their expression patterns providing intriguing clues, it should now be possible to make rapid progress in understanding the genetic basis for the cell interactions underlying the polar coordinate system of positional information.

References

1. V. French, P. J. Bryant, S. V. Bryant, *Science* **93**, 969 (1976).
2. J. A. Williams, S. W. Paddock, S. B. Carroll, *Development*, in press.
3. J. E. Hooper and M. P. Scott, in *Early Embryonic Development of Animals*, W. Hennig, Ed. (Springer-Verlag, Berlin, 1993).
4. M. Peifer, C. Rauskolb, M. Williams, B. Riggleman, E. Wieschaus, *Development* **111**, 1029 (1991).
5. Y. Hatano, *Mol. Gen. Genet.* **226**, 17 (1991).
6. J. P. Couso, M. Bate, A. Martínez-Arias, *Science* **259**, 484 (1993).
7. N. E. Baker, *Development* **102**, 489 (1988).
8. M. van den Heuvel, R. Nusse, P. Johnston, P. A. Lawrence, *Cell* **59**, 739 (1989).
9. F. Gonzalez, L. Swales, A. Bejsovec, H. Skaer, A. Martínez-Arias, *Mech. Dev.* **35**, 43 (1991).
10. S. Dougan and S. DiNardo, *Nature* **360**, 347 (1992).
11. A. A. Simcox *et al.*, *Development* **107**, 715 (1989).
12. S. Eaton and T. B. Kornberg, *Genes Dev.* **4**, 1068 (1990).
13. T. Kornberg, *Proc. Natl. Acad. Sci. U.S.A.* **78**, 1095 (1981).
14. S. J. Poole, L. M. Kauvar, B. Drees, T. Kornberg, *Cell* **40**, 37 (1985).
15. L. I. Held, Jr., *Dev. Biol.*, in press.
16. S. M. Cohen, in *Development of* Drosophila, A. Martínez-Arias and M. Bate, Eds. (Cold Spring Harbor Press, Cold Spring Harbor, New York, in press).
17. M. Agnel, L. Röder, R. Griffin-Shea, C. Vola, *Roux's Arch. Dev. Biol.* **201**, 284 (1992).
18. S. M. Cohen, G. Brönner, F. Küttner, G. Jürgens, H. Jäckle, *Nature* **338**, 432 (1989).
19. P. J. Bryant, *Dev. Biol.* **128**, 386 (1988).
20. J. Mohler, *Genetics* **120**, 1061 (1988).
21. C. Wilson *et al.*, *Genes Dev.* **3**, 1301 (1989).
22. S. Goto, personal communication.
23. J. Manak and M. P. Scott, personal communication.
24. J. J. Lee, D. P. Von Kessler, S. Parks, P. A. Beachy, *Cell* **71**, 33 (1992).
25. J. D. Masucci, R. J. Miltenberger, F. M. Hoffmann, *Genes Dev.* **4**, 2011 (1990).
26. J. Hooper, personal communication.
27. S. B. Carroll and J. S. Whyte, *Genes Dev.* **3**, 905 (1989).
28. T.V. Orenic and S. B. Carroll, personal communication.