The role of the *hairy* gene during *Drosophila* morphogenesis: stripes in imaginal discs

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The *Drosophila* pair-rule embryonic segmentation gene *hairy* is also required later in development to suppress the formation of certain adult sensory structures. Striking patterns of nuclear *hairy* protein distribution emerge during larval and pupal imaginal disc morphogenesis. *hairy* is expressed transiently in the cells just anterior to the morphogenetic furrow as it passes across the eye disc and in stripes along the proximo–distal axis of the leg discs and the developing veins of the wing disc. *hairy* expression is correlated with disc cell-cycle changes and is excluded from the sensory neurons that differentiate near or among the rows of *hairy*-expressing cells. It is likely that *hairy* regulates genes affecting neuronal determination, such as *achaete*, and is regulated by genes that specify anteroposterior polarity within imaginal discs.

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Through systematic screens for mutations in loci that visibly perturb the organization of the *Drosophila* embryo, geneticists have been able to identify and categorize several sets of genes that control fundamental aspects of embryonic pattern formation. Much emphasis has been placed on the characterization of genes controlling segment formation and determination (for reviews, see Akam 1987; Nüsslein-Volhard et al. 1987; Scott and Carroll 1987), dorsoventral positional information (Anderson and Nüsslein-Volhard 1986), and neurogenesis (Lehmann et al. 1983). However, *Drosophila* is a holometabolous insect; the adult form does not emerge from the growth of the embryo but from the complete metamorphosis of the larval imago. Relatively little is known about the genes that control adult pattern formation in the larval imaginal structures.

Some genes, particularly those of the Bithorax and Antennapedia homeotic class (Lewis 1978; Peifer et al. 1987), are known to affect both embryonic and adult segmental determination. Recessive loss-of-function homeotic gene alleles cause transformations of embryonic segment identity, whereas partial loss-of-function or dominant gain-of-function alleles may transform adult segmental fates. Molecular analyses of homeotic gene expression have shown that many homeotic genes are expressed throughout development, although it remains unknown as to how the expression of homeotic genes influences the external morphology of either embryonic or adult segments.

The *hairy* gene of *Drosophila* is another locus that affects both embryonic and adult pattern formation (Ingham et al. 1985). Early in embryogenesis, *hairy* acts

as a pair-rule gene and controls the formation of pattern elements at double-segment intervals. It has been shown that a major part of *hairy* function is to control negatively the expression of the *fushi tarazu* (*ftz*) pair-rule gene. Mutations that disrupt early embryonic *hairy* activity cause a derepression of *ftz* expression in regions where *ftz* is not normally expressed (Carroll and Scott 1986; Howard and Ingham 1986), whereas ectopic induction of *hairy* can extinguish normal *ftz* expression (Ish-Horowicz and Pinchin 1987). Later in development, *hairy* is required to suppress ectopic bristle formation on many parts of the adult body. Ingham et al. (1985) have characterized four classes of *hairy* mutations, based on their complementation of the adult viable *h*¹ allele and an embryonic lethal allele. These four classes consist of alleles that (1) affect only the bristle pattern of adults; (2) affect bristle pattern and have weak effects on segmentation; (3) have some effect on bristle pattern and strongly affect segmentation; and (4) strongly affect both processes. Based on their genetic analysis, Ingham et al. (1985) proposed that the first three types of mutants probably identified different regulatory regions of the *hairy* gene controlling early and late expression of a common coding region. Mutations in the protein-coding region were presumed to cause the fourth most severe class of phenotypes.

hairy is the only pair-rule or gap gene besides *giant* (Simpson and Morata 1980) known to be required or expressed during later development. It is most likely that *hairy* is involved in a different regulatory pathway in imaginal structures than in embryos. Bristle development involves the differentiation of mechanosensory

neurons of the peripheral nervous system (PNS). The ectopic bristle phenotype of adult viable *hairy* mutant and *hairy*⁻ mutant clones induced by mitotic recombination suggests that the late *hairy* function is to suppress bristle formation. It is likely that part of this suppression is mediated through genes of the *achaete-scute* gene complex (AS-C), principally the *achaete* locus. Loss of *achaete* function mutations result in vast reductions in the number of small bristles (microchaete) on the adult body (for review, see Ghysen and Dambly-Chaudière 1988). In the presence of extra doses of *achaete*, *hairy* becomes dominant, i.e., extra bristles appear, indicating that the balance between the amount of the *hairy* and *achaete* gene products regulates the decision to form bristles in certain regions of the body (Botas et al. 1982).

To shed more light on the function of this key regulatory gene, previously we have studied its expression in embryos using specific antibodies (Carroll et al. 1988). *hairy* is expressed in a striped pattern of cell nuclei encircling the early embryo where it appears to regulate *ftz* expression. Although *hairy* is expressed around the tracheal pits and a few other regions of the embryo at distinct times during embryogenesis, it is not expressed in the embryonic nervous system. Here, we show that *hairy* expression in imaginal discs is associated with morphogenetic events in the eye, leg, and wing and that it is lost or absent from cells that will differentiate into sensory structures. The striking spatial patterns of late *hairy* expression are associated with cell-cycle changes along several regions that will become morphological boundaries in the discs. The distinct patterns of *hairy* expression during morphogenesis suggest that *hairy* is under the control of other genes that specify positional information in the developing discs.

Results

We have investigated the pattern of *hairy* protein expression in many larval and pupal tissues by staining fixed whole-mount preparations with a *hairy*-specific antibody (Carroll et al. 1988). We find that *hairy* is expressed in all imaginal discs examined and in their tracheal branches. Here, we present detailed studies on the expression of *hairy* during the morphogenesis of the major imaginal discs.

The eye: hairy is transiently expressed in a line of cells anterior to the morphogenetic furrow as it traverses the disc

In surveying those third-instar larval imaginal discs that expressed *hairy*, we were surprised to observe that the highest level of *hairy* protein accumulation occurred in the eye portion of the eye–antennal disc (Fig. 1a). In middle to late third-instar discs, intense nuclear *hairy* staining is observed in a band three to five cells wide, stretching dorsoventrally across the entire apical surface of the eye disc. On the anterior side of this band, nuclei are visible that are expressing lower levels of *hairy* pro-

tein (Fig. 1b, arrow). On the posterior side of the band, there is no trace of *hairy* expression.

Morphogenesis of the eye occurs as a wave across the disc epithelium (for review, see Tomlinson 1988). A depression in the disc surface, the morphogenetic furrow, moves across the third-instar eye disc from the posterior to the anterior (from the optic stalk toward the antennal part of the disc). Posterior to the furrow, the photoreceptor cells begin to differentiate and mitosis is restricted to a narrow band of cells, whereas the cells remain largely undifferentiated and continue to divide anterior to the furrow. To determine the position of the band of *hairy* staining relative to the morphogenetic furrow, we double-labeled eye discs with the rabbit anti-*hairy* antibody and the mouse monoclonal antibody 22C10, which stains the developing axons of the photoreceptor cells (Fujita et al. 1982). The morphogenetic furrow lies between the field of 22C10-labeled photoreceptor cells and the band of *hairy* expression (Fig. 1c).

Next, we sought to understand how *hairy* expression changed with respect to movement of the morphogenetic furrow. We double-labeled late second instar; early middle, and late third instar; and pupal eye discs with the *hairy* and 22C10 antibodies. The second-instar eye discs are very small, as several more rounds of cell division and growth occur during the third instar. The second-instar discs do not express much, if any, 22C10 antigen when the morphogenetic furrow is only ~25% of the way across the eye disc, but they do express *hairy* in a band of cells across the disc and anterior to the morphogenetic furrow (Fig. 2a). By middle to late third instar, the morphogenetic furrow is ~50% of the way across the disc and each antibody strongly labels cells on opposite sides of the furrow (Fig. 2b). In early pupal eye discs [0–2 hr after puparium formation, (APF)], the furrow is ~60% of the way across the disc and the *hairy* staining is beginning to decrease anterior to the furrow (Fig. 2c). *hairy* protein is still detectable at 8 hr APF in a thin band of cells at the far anterior end of the eye, near the base of the everted antennal disc (data not shown). From these staining patterns we can infer that *hairy* is expressed in cells as the furrow approaches and is then rapidly lost as the furrow reaches these cells and they differentiate into the various cells of the eye ommatidia. The weak level of *hairy* staining in nuclei six to eight cells anterior to the furrow and the absence of staining posterior to it indicate that both a *hairy* activation signal, as the furrow approaches, and a repression or destruction mechanism, to eliminate *hairy* from cells within the furrow, probably exist. The combined effect of activation and turnover or repression is the creation of a transient wave of *hairy* protein expression. On the basis of studies of morphogenetic furrow movement and ommatidial assembly (Ready et al. 1976; Campos-Ortega and Hofbauer 1977), which determined that the furrow moves one ommatidial row every 2 hr, we deduce that cells accumulate *hairy* for a period of 4–6 hr and lose it within 30–45 min.

Adult viable *hairy* mutants do not exhibit any discernible phenotype in the eye so we do not know the func-

Figure 1. Expression of *hairy* protein in the third-instar larval eye-antennal imaginal disc. (a) Whole-mount eye-antennal disc stained with antibody to the *hairy* protein, as described in Methods. A line of *hairy* expression extends across the anterior portion of the eye disc (bottom), whereas several patches of *hairy* expression are discernible in the antennal portion of the disc complex (top, arrows). (b) Higher magnification view of *hairy* protein expression in the eye disc. There is strong expression of *hairy* in the cells on the posterior edge of the line (bottom), which has not yet become as strong in more anterior nuclei (solid arrow). Note the absence of any trace of *hairy* protein posterior (bottom) of the line. (c) Eye disc that was double-labeled with *hairy* antibody and monoclonal antibody 22C10. The anterior line of *hairy* staining (top) is separated from the field of developing photoreceptor cells stained with antibody 22C10 by the morphogenetic furrow (mf). Bars, 50 μ m.

tion of *hairy* in the eye disc. *h¹/h¹* homozygous larvae exhibit a normal spatial pattern of protein accumulation in the eye that appears to be just a small degree weaker than wild-type Oregon-R larvae (data not shown). Perhaps the high residual level of *hairy* expression in *h¹/h¹* mutant eyes is sufficient to provide full *hairy* function (see Discussion). Because strong *hairy* alleles are embryonic lethals, we must generate *hairy⁻* clones in the eye to assess what role, if any, the observed transient expression of *hairy* has in the proper development of the compound eye.

The leg: a complex spatial distribution hairy in third-instar discs emerges into a pattern of stripes during eversion of pupal discs

Many cells within each of the third-instar leg discs stain strongly with *hairy* antibody. In each compacted disc, only a subset of the total *hairy* pattern is discernible in any single focal plane; this is shown for the mesothoracic (second leg) disc in Figure 3, a–c. *hairy*-labeled cells are found within most or all of the presumptive leg segments (for a general fate map of the leg, see Bryant 1975, 1978) in various sized patches and concentric rings of

cells that follow the contours of the folds within the disc. Because of these folds and the thickness of the structure, out-of-plane staining appears as fuzzy gray to black clouds. Optical sectioning of the disc resolves many of these clouds when the appropriate focal plane is reached (Fig. 3a–c). The prothoracic (first leg) and metathoracic (third leg) discs exhibit patterns that are similar to the mesothoracic leg (Fig. 3d,e). The different sizes and contours of the legs make it difficult to establish whether there are exact correspondences between *hairy* expression in each leg at this state of development. After eversion, we can determine some relationships more clearly.

Shortly APF, the leg discs begin to unfold and evert to produce an elongated tubular structure (for details, see Fristrom and Fristrom 1975; Fristrom 1976). The center of the larval disc forms the most distal elements of the pupal leg, the tarsal segments. During eversion, the concentric rings of *hairy* expression evolve into longitudinal rows and transverse patches of stained cells (Fig. 4a–c). From the examination of staged leg discs, it appears that the level of *hairy* expression is increasing during early evagination, with some cells becoming more intense and others turning on *hairy*. As each leg disc everts, it can be

Figure 2. The fate of *hairy* protein expression during eye development. Eye discs at different stages of development were double-labeled with the *hairy* and 22C10 antibodies. Arrows indicate the position of the morphogenetic furrow in each disc as it moves from the posterior (left) to the anterior (right). (a) Second-instar larval eye disc with furrow ~25% of the distance across the disc. There is little or no 22C10 staining posterior to the furrow, but there is *hairy* expression in a line anterior to the furrow. Note the relative size of this disc compared to the older discs. (b) Third-instar larval eye disc with furrow ~50% of the distance across the disc. There is strong expression of both the 22C10 antigen within the developing photoreceptor cells posterior to the furrow and the *hairy* protein in the undifferentiated cells just anterior to the furrow. (c) Early pupal eye disc with furrow ~60% of the distance across the disc. The *hairy* line has moved and remains anterior to the morphogenetic furrow. Bars, 50 μ m.

seen that the overall patterns of *hairy* expression are similar. Each leg exhibits four rows of cells along the longitudinal axis of the femur and tibia that continue both proximally toward the coxa and distally into the basitarsus and the four other tarsal segments (Fig. 4a–c, see corresponding lines between tibia and tarsal segments). In the tarsal segments, there are both transverse rings and patches of cells that are oriented perpendicularly to the longitudinal rows. One ring is most evident in the fourth tarsal segment (Fig. 4a,b). The overall *hairy* expression patterns are similar between the different pupal legs in that the main longitudinal lines are present consistently. The slightly different shapes of each leg and the lack of an independent constant landmark make it difficult to determine whether there are strictly conserved elements to each leg pattern. However, the types of cells that express *hairy* are very constant from leg to leg (see below).

hairy is excluded from leg neurons

A major question concerning *hairy* function deals with the types of cells that express it. The ectopic bristle phenotype of adult viable homozygous mutants and the *hairy* genetic interaction with the AS-C suggests that *hairy* works to oppose neuronal determination that is positively controlled by the AS-C (Botas et al. 1982; Ghysen and Dambly-Chaudière 1988). During late larval and early pupal development, much of the adult PNS is formed. We have examined the spatial relationship of *hairy* expression to these events in the leg and wing.

By 4 hr APF, many neurons and their axons are clearly evident in the developing legs. The monoclonal antibody 22C10 labels the axons extended by all peripheral neu-

rons. We double-labeled pupal legs with anti-*hairy* and the 22C10 antibody to determine the relationship between the peripheral neurons and *hairy* expression. *hairy* is not expressed in 22C10-labeled cells in all legs at all times. This relationship is clearest when one inspects the pattern of *hairy* staining in the tarsal segments of a well-everted leg disc. In many places, there are patches of cells exhibiting high levels of *hairy* staining but one or two cells in the patch are unlabeled (Fig. 5a, arrows). Some cells that do not express *hairy* are labeled by 22C10 (Fig. 5b, arrows). We have not been able to determine whether these neurons differentiate from what were previously *hairy*-expressing cells or whether all neuronal precursors are set aside before the *hairy* pattern is established. On the basis of the spatial relationship of the *hairy*-expressing cells to the leg neurons (for a high magnification view, see Fig. 5c,d) and the fact that *hairy* staining in the larval leg precedes neuronal differentiation in the pupal leg, it is possible that the neurons are born from cells that may have once expressed *hairy*.

The wing: hairy is expressed along the presumptive wing veins and excluded from peripheral neurons

The third-instar wing and haltere discs consistently exhibit weaker levels of *hairy* expression. This is not due to problems with the penetration of antibodies, because other nuclear antigens are well stained in our protocols. In the wing, there are several areas that are lightly labeled by the *hairy* antibody (Fig. 6a). These include a large portion of the thoracic region of the disc that will give rise to parts of the notum (Fig. 6a, th). The staining pattern is less distinct but above background on the ven-

Figure 3. The patterns of *hairy* expression in larval leg imaginal discs. In all frames, proximal is to the *left* and distal is to the *right*. (a–c) Different focal planes through a mesothoracic leg disc stained with *hairy* antibody. The more proximal focal plane exhibiting the outer concentric rings of *hairy* expression is shown in a; the more distal plane is shown in c. Note the change in the clarity of the patch of *hairy*-labeled cells as the focal plane moves distally (small arrow). As the distal focal plane is reached, the tarsal segments of the leg come into focus; sharp nuclear staining can be observed in regions that appear fuzzy gray when out of focus (large arrow in c). (d) Prothoracic leg disc, proximal focal plane. Overall, there is a resemblance to the spatial pattern of *hairy* expression in a; however, it is impossible to determine whether there is precise correspondence between the two leg discs. (e) Metathoracic leg disc, distal focal plane. There is a resemblance to the pattern shown in e, but a precise comparison is not possible. The dark nuclei toward the center of the disc belong to the tarsal portion of the disc. Bars, 50 μ m.

tral portion of the disc, near the region that will serve as the wing hinge between the dorsal and ventral surfaces of the wing and near the midline of the wing blade. Both the lower level of staining and the contours of the folds within the disc hinder a more precise determination of the *hairy* pattern at this stage of development.

As the wing disc everts, part of the spatial pattern of *hairy* expression becomes much clearer and stronger. By 6 hr APF, *hairy* is clearly visible in two longitudinal lines across the dorsal surface of the presumptive wing blade (Fig. 6b), as well as in patches in the thorax (data not shown). On the basis of the position of these lines with respect to the rows of neurons that form along the anterior wing margin on longitudinal vein L1 and the three neurons that arise early on L3 (Murray et al. 1984), we can place these lines of *hairy* expression along the 'preveins' of L2 and L3. By 8 hr APF, the L3 staining is the most prominent (Fig. 6C). We double-labeled pupal wing discs with both *hairy* antibody and the neural-specific monoclonal antibody 22C10 to examine the spatial relationship of neuronal differentiation to *hairy* expression. As was the case in the legs, some cells in the longitudinal rows along L3 that do not express *hairy*, but are surrounded by *hairy*-containing cells (Fig. 6d, arrows), are labeled with the 22C10 antibody (Fig. 6e, arrows).

These observations are the most relevant with respect to previous descriptions of the *hairy* mutant phenotype.

In homozygotes for adult viable alleles, such as *h*¹, ectopic bristles are observed on the wing blade, predominantly along veins L2 and L3 (Ingham et al. 1985). The expression of *hairy* in the cells that will form these veins certainly correlates with the genetic requirement for *hairy* to suppress ectopic bristle formation in L2 and L3. The comparatively low level of *hairy* expression in the preveins suggests that these structures may be very sensitive to a reduction in *hairy*⁺ function and could largely explain why the *hairy* mutant phenotype is most evident in the wings and not on the legs or eyes, where much higher levels of *hairy* protein accumulate. We examined *hairy* protein accumulation in homozygous *h*¹/*h*¹ larvae and pupae and observed essentially identical patterns to those in the wild-type eye, leg, and wing (Fig. 4d). However, it appeared that there was a slight reduction in the amount of *hairy* antigen in some mutant discs. This reduction could partially explain the *h*¹/*h*¹ phenotype and the sensitivity of the wing bristle pattern to *hairy* and AS-C dosage (see Discussion).

The extent of *hairy* protein expression APF was unexpected also. Previous studies on the requirement for *hairy* in suppressing bristle development in clones of cells induced during larval and pupal life concluded that *hairy* was not required beyond 8 hr before puparium formation (BPF) (Garcia-Bellido and Merriam 1971). There may be several explanations why *hairy* could appear to

Figure 4. Emergence of an ordered *hairy* pattern during eversion of pupal imaginal leg discs. In all frames, proximal is to the left and distal is to the right. (a) Prothoracic leg disc 4 hr APF. There are four longitudinal lines of *hairy* expression. Three lines (1–3) in the tibia (tib) are in focus and extend in a discontinuous pattern into lateral (1,3) and medial (2) regions of the tarsal segments (small arrows). A fourth line (4) is located medially but is out of focus on the opposite side of the disc. There are five tarsal segments (1,5); the fourth exhibits a strong transverse band of *hairy* staining. (b) Mesothoracic leg disc 4 hr APF. Three of four longitudinal lines of *hairy* expression are in focus in the tibia (tib, small arrows); these are similar to the lines in a. In the fourth tarsal segment, there is also a large transverse band of *hairy* expression (arrow) as in the prothoracic leg above. (c) Metathoracic leg disc 4 hr APF. Three of the four longitudinal lines of *hairy* expression are visible in the tibia (tib, small arrows). The tarsal pattern resembles those of the prothoracic and mesothoracic leg discs above. (d) Leg disc from homozygous *h¹/h¹* pupa 7 hr APF. The level of *hairy* expression is comparable to that found in wild-type animals. Note the long line of *hairy* expression extending from the proximal tibia to the tip of the tarsus. Bars, 50 μ m.

be required early and still be expressed at such high levels later in development. First, induction of somatic clones does not reveal the time of gene action directly. In this case, 8 hr BPF was the point beyond which induction of *h¹/h¹* homozygous clones had no effect on bristle phenotype. Because the *h¹* allele is only a weak allele and the *hairy* protein probably has a significant half-life, it is possible that sufficient *hairy* protein exists for many hours in 8-hr BPF clones to suppress bristle formation. We cannot determine the moment of bristle determination and the *hairy* function in that process until more is known about where *hairy* is expressed in relation to the bristle progenitor cells. Second, it is quite conceivable that some function(s) of *hairy* is unrelated to bristle development per se. Clearly, the eye function may involve a different type of neuronal regulation. Some of the leg or wing patterns may be involved in other pathways. Either analysis of cell clones for null *hairy* alleles or identification and study of *hairy* mutations that abolish *hairy* function in the imaginal discs will be required to evaluate these possibilities.

Relationship between hairy expression and compartmental boundaries

The stripes of *hairy*-expressing cells in the leg and wing prompted us to consider whether these patterns reflected determinative boundaries in the discs. In the wing, it has been shown previously that the L3 veins and the anterior–posterior (A-P) boundary are not coincident (Schubiger and Palka 1987). We can conclude from these studies that the L2 and L3 *hairy* patterns lie within the anterior compartment of the wing.

In the leg, however, little information exists regarding lineage boundaries. To study the relationship of *hairy* expression to compartmental boundaries, we double-labeled pupal leg discs with *hairy* antibody and a monoclonal antibody to the *engrailed* (*en*) gene product, which serves as a definitive marker of the posterior compartment (DiNardo et al. 1985; Kornberg et al. 1985). We examined several 6-hr APF leg discs in detail and observed a very consistent relationship between the *hairy* and *en* protein patterns. In all legs, it could be de-

Figure 5. Exclusion of *hairy* expression from pupal leg disc neurons. Prothoracic pupal leg discs 5 hr APF were double-labeled with *hairy* and 22C10 antibodies and were visualized by bright-field (*hairy*, *a,c*) and fluorescence (22C10, *b,d*) microscopy. (*a*) Leg disc exhibiting typical *hairy* staining pattern. Note the holes in the patches of staining on several tarsal segments (arrows). (*b*) Same leg as in *a* stained with 22C10. The holes observed in *a* include neurons labeled by this antibody (arrows). (*c*) High magnification view of *hairy* expression in distal tarsus. Two holes in the *hairy*-labeled patch in T4 are indicated (arrows). (*d*) Same view as in *c*; the 22C10 pattern indicates that the holes in *c* include neurons labeled by 22C10 (arrows). Bars, 50 μ m.

terminated that of the four longitudinal lines of *hairy* expression, some were located entirely within and some completely outside of the *en* domain (Fig. 7*a,b*). There is no strict relationship between the proteins; *hairy* is not simply following the A-P compartmental lineage restrictions. Of the four *hairy* lines, one runs within the posterior compartment (line 1 in Fig. 7*a–d*), one runs along the A-P boundary (line 2 in Fig. 7*a–d*), and two lie well within the anterior compartment (arrows in Fig. 7*a,b*; line 3 in Fig. 7*c,d*). The orientation of the *hairy*-labeled rows and the *en* pattern suggests that the rows are established by a regulatory mechanism that operates parallel to the orientation of the A-P boundary. Some of the segment polarity genes that affect disc development (e.g., *wingless*, *hedgehog*) could be involved in the regulation of the spatial pattern of *hairy* expression in these longitudinal stripes. The regulation of *hairy* in discs must operate differently from the embryo; most gap and pair-rule segmentation genes are inactive during late development.

Discussion

We have shown that the nuclear product of the *hairy* embryonic segmentation gene is expressed in a striking array of patterns during the morphogenesis of the eye, leg, and wing imaginal discs. Several of these patterns were completely unexpected, as previous analyses of

hairy mutant flies had given little indication of the extent and nature of *hairy* gene expression during disc development. Our observations have several implications regarding the general role of the *hairy* protein in controlling the spatial pattern of PNS development, the nature of the genetic regulatory program that specifies positional information during metamorphosis, and the general relationship between *hairy* gene expression, disc cell-cycle changes, and the commitment to cell differentiation during disc morphogenesis.

Spatial and temporal regulation of hairy protein expression

The complete cycle of *hairy* protein expression [which we believe to involve only one species of polypeptide (J. Langeland and S. Carroll, unpubl.)]—first in transverse stripes encircling the embryo, then in small clusters of cells surrounding the embryonic tracheal pits, and later in the patterns described here during disc morphogenesis—illustrates an important emerging theme from the developmental and molecular analysis of *Drosophila* pattern-regulating genes. Namely, a single regulatory protein (and its potential post-translationally modified subforms) is deployed frequently in several distinct spatiotemporal patterns during the course of development and may control and be controlled by unique sets of genes at each stage and in different tissues. Several other

Figure 6. Expression of *hairy* during wing disc development. (a) Third-instar larval wing disc labeled with *hairy* antibody. Antigen levels are generally weak and can only be discerned within the thoracic (th) region of the disc and in diffuse patterns near the distal end of the disc (long arrow) and the midline of the presumptive wing blade (short arrow). (b) Wing disc 6 hr APF. Only the everted blade portion of the disc is shown. *hairy* expression is evident on the dorsal surface along the presumptive longitudinal wing veins L2 and L3. (c) Wing disc 8 hr APF. The disc blade is beginning to expand with hemolymph. L3 staining is clearly evident, and L2 staining is out of this field of view. There appear to be very weak levels of *hairy* expression in the more posterior (bottom) preveins of the disc. (d) High magnification view of L3 staining in c. Along the row of *hairy*-expressing cells are a few obvious gaps in staining (arrows); these include the three dorsal sensory neurons (see e below). (e) The disc in d was double labeled with 22C10 antibody and exhibits three individual neurons (arrows) that are located within the gaps observed in the *hairy* pattern in d. Bars, 50 μ m.

important pattern-regulating genes have been shown to be regulated independently and/or expressed in different tissues, including the ectoderm versus the central nervous system [e.g., *ftz*, *en*, *even-skipped* (*eve*) (Carroll and Scott 1985; DiNardo et al. 1985; Hiromi et al. 1985; Frasch et al. 1987)]; the ectoderm versus the PNS [e.g., *Antennapedia* (Carroll et al. 1986; Boulet and Scott 1988)]; or the ectoderm versus the mesoderm [e.g., *Ultrabithorax* (Akam and Martinez-Arias 1985; Bienz et al. 1988)]. The *hairy* pair-rule stripes are controlled by gap and pair-rule genes in the blastoderm embryo, but because these genes are inactive during disc morphogenesis, the pattern of *hairy* disc stripes must be generated by a different regulatory system. Elucidating the *cis*- and *trans*-acting regulatory machinery that generates such developmentally complex gene expression will be an important step toward understanding the functional roles of pattern-regulating genes.

Given the varying levels and duration of *hairy* expression in the different discs and the apparently unique spatial coordinates of each pattern in the three major discs, it is likely that the regulation of the *hairy* locus during disc morphogenesis is complex and involves multiple *cis*-acting elements and *trans*-acting factors. This notion is supported by several additional observations, including (1) the profile of RNA transcripts from the *hairy* locus changes during development and could involve multiple promoters and alternative RNA splicing patterns (Ish-Horowicz et al. 1985); (2) adult viable mu-

tations map outside of the coding region and range up to 20 kb away from the 5' end of the gene (Holmgren 1984; Howard et al. 1988); and (3) the embryonic regulation of *hairy* involves distinct *cis*-acting elements that are distributed over 10 kb upstream of the gene (Howard et al. 1988); these elements are most probably acted on by completely different factors [e.g., gap and pair-rule gene products) than the disc pattern elements. If disc pattern-controlling DNA elements are nested between and around the embryonic stripe-regulating elements, then a large region of DNA may be required for proper spatial control. It is also possible that some aspects of *hairy* control, e.g., its rapid turnover in the morphogenetic furrow, could be regulated post-transcriptionally at the level of mRNA or protein stability.

Function of the hairy protein during disc morphogenesis

There are two different aspects to understanding *hairy* function in imaginal disc development. First, we may consider what *hairy*'s role is in the morphogenesis of a particular disc. For example, the eye develops in a fairly distinct manner from the legs or the wing. The developmental timing orchestrated by the morphogenetic furrow (and reflected by *hairy* expression) does not appear to have a counterpart in the discs of the body appendages. Second, we may consider the overall role of *hairy* that is common to each disc, e.g., its apparent general function in regulating the spatial pattern of PNS de-

Figure 7. The relationship between the *hairy* spatial pattern and compartmental boundaries defined by *en* expression. Pupal leg discs were double-labeled with *hairy* antibody and a monoclonal antibody to the *en* gene product (gift of N. Patel). (a) *hairy* expression in the tarsus of a pair of pupal prothoracic leg discs (6 hr APF). Two lines of *hairy* expression are visible along the lateral region of the right leg (1,2), and one line is visible in the leg at the upper left (arrow). (b) *en* expression in leg shown in a. Note that line 1 in a cuts through the middle of the *en*-labeled region of the right leg, whereas line 2 abuts the *en* pattern. The deposition of DAB shown in a quenches the rhodamine fluorescence signal in b, accounting for the dark shadows on the image. Note that there is no *en* expression in the area stained by *hairy* in the left leg (arrow); this *hairy* line is in the anterior compartment of the leg. (c,d) Another prothoracic leg disc 6 hr APF. This view highlights three femur–tibia lines of *hairy* expression across a more proximal portion of the disc. By double labeling with *en* antibody in d, it can be seen that line 1 lies entirely within the *en* pattern, line 2 abuts it, and line 3 is outside of it. Bars, 50 μ m.

velopment. We will discuss these points in the context of each disc's particular pattern of development.

The eye

The *hairy* protein expression in the eye disc was unexpected because no *hairy* mutants or clones of mutant cells have been shown to affect eye development. The anterior position of *hairy* expression with respect to the morphogenetic furrow is also unusual in that most eye antigens described previously have been restricted to the cells posterior to the furrow (Zipursky et al. 1984; Banerjee et al. 1987; Tomlinson et al. 1987). Because the line of *hairy* protein moves anteriorly as the furrow passes across the eye, it is clear that each set of photoreceptor cells transiently express *hairy* before they pack basally in the furrow, differentiate, and move to the apical region of the epithelium (for a review, see Tomlinson 1988). There is a relationship between *hairy* expression and cell behavior; loss of *hairy* expression is associated with the commitment to differentiate into an ommatidial cell type. We are attempting to investigate further

hairy's function in the eye by examining the behavior of *hairy*⁻ clones induced during larval development.

Whatever the precise molecular function of *hairy* may be in the eye, we point out that it must be different than when *hairy* is expressed in transverse stripes of nuclei around the cellular blastoderm embryo. During early embryogenesis, *hairy* is a regulator of the pair-rule genes *ftz* (Howard and Ingham 1986; Carroll and Scott 1986), *eve* (Frasch and Levine 1987) and *runt* (Ingham and Gergen 1988). No larval function for these pair-rule genes has ever been indicated, and we have confirmed that *ftz* and *eve* are not expressed in larval or pupal tissues (data not shown). Thus, the regulatory functions of these pair-rule genes diverge in later development, as well as in the embryonic central nervous system (Doe et al. 1988a).

The wing and leg

The wing and leg *hairy* patterns emerge most clearly during the first 6 hr of pupation. The expression in the wing disc is most germane to the interpretation of the

hairy mutant phenotype, as most previous studies have focused on the ectopic bristle phenotype on the notum and wing blade. We observed expression of *hairy* in both the notum and along the preveins of L2 and L3. These are also the positions that are most affected by mutations in the *hairy* locus or by a reduction in *hairy* dosage (Botas et al. 1982; Ingham et al. 1985).

Although there is a correlation between the phenotype of *hairy* mutants and the spatial distribution of *hairy* protein, we must explain why these sites are more sensitive to a reduction of *hairy*⁺ activity than the eye or leg. There are three reasonable explanations: (1) These sites may express a more critical balance of *hairy* to bristle-determining gene products (e.g., *achaete*); (2) *hairy* may have a different function in the eye and leg than in the wing; or (3) the more trivial possibility is that these sites yield a phenotype that is scored more easily. We strongly favor the first explanation because there is no doubt that wild-type wing and notal cells naturally express lower levels of *hairy* protein than the eye or leg cells do. If *hairy*⁺ is in balance with opposing gene functions, such as *achaete*⁺, it follows that even a slight reduction in *hairy*⁺ activity could produce a phenotype in places where expression is required, but is naturally low (e.g., the wing). We observe high and near wild-type levels of *hairy* protein in eye and leg discs from *h¹/h¹* larvae and pupae; either those discs express sufficient *hairy* to carry out its complete function or, as mentioned above, perhaps *hairy* has a different function in the legs and eyes.

The high-level expression of *hairy* in the longitudinal rows of pupal leg cells was also surprising, as there is no clearly discernible phenotype of *h¹/h¹* adult legs. In addition, the even spacing of these lines suggests that *hairy* is respecting the spatial coordinates of some regulatory system that subdivides the legs into smaller developmental units. It has been noted that the leg bristles are largely organized into longitudinal rows (for a review, see Held and Bryant 1984). The rows of bristles and the rows of *hairy* protein probably reflect the same underlying system of positional information; however, what is the relationship between them? Unfortunately, because of adverse changes in the properties of leg discs by 12 hr APF and later (secretion of cuticle, swelling with hemolymph), we were unable to pursue the analysis of *hairy* expression at the time of bristle determination. However, based on the exclusion of *hairy* from those leg neurons present by 4–8 hr APF, we would expect that either *hairy* expression must be lost by the time of bristle determination or that the *hairy* stripes run parallel to the rows of cells that give rise to bristles. It is possible that the four lines of *hairy* expression demarcate four of the eight stripes of naked cuticle separating the eight bristle rows that can be seen, for example, in the basitarsus (first tarsal segment) of the adult second leg. We are attempting further developmental studies to resolve this point.

Relationship between hairy expression, the cell cycle, sensory neuron differentiation, and overt morphology

The observed pattern of *hairy* expression in imaginal

discs suggests a possible relationship between the spatial pattern of DNA replication and *hairy* protein distribution. Several previous studies have indicated a relationship between changes in cell cycles and the position of morphological boundaries. In the eye disc, cells exist in a pattern of asynchronous divisions far ahead of the morphogenetic furrow, undergo little DNA synthesis, six to eight cell rows just anterior to and including the furrow, and resume replicative activity posterior to the furrow. Cells posterior to the furrow differentiate into the ommatidial precursors, whereas cells anterior to the furrow are yet to be recruited into these structures. (The preceding descriptions were based on Ready et al. 1976; and Tomlinson 1985). The expression of *hairy* appears to be coupled to these events in that the nonreplicating cells anterior to the furrow are those that are accumulating *hairy*, whereas the adjacent basally localized differentiating cells in the furrow have lost all traces of *hairy* protein.

In the wing disc, Schubiger and Palka (1987) have described the distinct spatial patterns of S-phase nuclei and have shown that different fields of cells enter an extended G₂ phase at different times of development. A zone of nonproliferating cells runs down the midline of the third-instar wing disc and corresponds to the rows of cells that will make up the L3 vein. *hairy* protein accumulates in these cells during the first several hours APF. By 3–4 hr APF, the nuclei of the wing blade are no longer replicating DNA and most have a tetraploid DNA content (Fain and Stevens 1982; Graves and Schubiger 1982), reflecting that they are in G₂ phase and have not undergone mitosis. During this period, morphogenetic movements occur for disc eversion, and neurons differentiate and extend their axons which are essentially the preveins of the wing. By 12 hr APF, the L3 cells and other veins re-enter S phase, whereas most intervein cells are still quiescent. Schubiger and Palka (1987) proposed that the step out of S phase is the first sign of cells beginning their commitment to participate in differentiative events in the wing, which for the midline (L3) cells involves formation of the L3 prevein, guidance of axons, and eventually the elaboration of the adult vein pattern. Because vein formation is normal in *hairy*[−] mitotic clones (Ingham et al. 1985) and the three principal dorsal L3 sensory neurons are born before the *hairy* pattern is elaborated (Fig. 6), we do not believe that *hairy* expression is driving vein formation but that it is somehow responding to this process.

One final point to consider concerns the relationship between *hairy* expression and the commitment to differentiate a neuron. In the eye, the sequence of events is clear: *hairy* is lost before differentiation is manifest. If we assume that *hairy* is behaving as a genetic regulatory protein, then *hairy* could be involved in repressing (even transiently) that commitment. In the wing, it appears that some neurons are specified before *hairy* even starts to accumulate to higher levels, yet ectopic bristles will appear if *hairy* function is reduced or lost. Perhaps, in the wing and legs, *hairy* serves more of a maintenance function to prevent epithelial cells from differentiating into neurons, whereas in the eye, *hairy* may be required

for the synchronization of the wave of ommatidial differentiation. One of the more promising avenues for resolving the regulatory function of *hairy* in neuronal determination would be to pursue the basis for its interaction with *achaete* (Botas et al. 1982). A better understanding of the genes driving neuronal determination in imaginal discs could yield helpful clues to the relationship between *hairy* function and cell behavior.

Methods

Stocks

Wild-type *Drosophila melanogaster* (Oregon R) was raised at 18–20°C. To select individuals of specific ages, the following staging methods were used: For second-instar larvae, animals were collected 50–65 hr after egg deposition; for middle to late third-instar larvae, wandering-stage animals were collected off bottle walls; for pupae, white-stage prepupae (0-hr puparium formation) were collected and aged for controlled time periods on moist filter paper at 18°C.

Fixation and antibody staining

The overall technique is very similar to that described by Brower (1987). Larval or pupal heads were pulled or cut off, and extraneous tissues such as trachea, fat body, and salivary glands were removed so that penetration of the reagents was more uniform.

The tissues were fixed for 20 min in 0.1 M PIPES (pH 6.9), 2 mM MgSO₄, 1 mM EGTA, and 1.0% NP-40, containing 1% formaldehyde (vol/vol). After fixation, the tissue was blocked for 1–8 hr at 4°C in 50 mM Tris-HCl (pH 7.4), 150 mM NaCl, and 0.5% NP-40, containing 5 mg/ml bovine serum albumin (BSA). All antibody incubations and washes were in this buffer at 4°C, except the BSA concentration was reduced to 1 mg/ml. All transfers were done by moving the heads from well to well with forceps.

After blocking, the tissues (three to four larval or pupal heads) were incubated overnight in 200 µl of antibody solutions in individual wells of 48-well tissue-culture dishes. Affinity-purified rabbit anti-*hairy* antibody (Carroll et al. 1988) was used at 3 µg/ml, whereas monoclonal antibody 22C10 supernatant was used at a dilution of 1 : 200 for rhodamine double-labeling experiments and at 1 : 1500–1 : 3000 for peroxidase-based double-labeling experiments. Monoclonal anti-*en* antibody [gift of Nipam Patel] was diluted 1 : 10. The heads were washed with four changes of the incubation buffer over 90 min and then incubated in the appropriate secondary antibodies for 2 hr. These include biotinylated goat anti-rabbit IgG (Vector) or biotinylated goat anti-mouse IgG (Vector) at 4 µg/ml, or rhodamine-conjugated goat anti-mouse IgG (Boehringer-Mannheim) at 1 : 100. The heads were washed four times over 60 min and incubated with streptavidin–horseradish peroxidase conjugate (BRL) at 1 : 150 for 1 hr. (The specific activity of this last reagent is critical to the sensitivity and clarity of the *hairy* signal and diminishes significantly over several months of storage at 4°C.) The heads were washed again with four changes of buffer over 60 min and then rinsed twice with 0.1 M Tris-HCl (pH 6.8). The peroxidase signal was then developed in 0.1 M Tris-HCl (pH 6.8), 0.5 mg/ml diaminobenzidine (DAB), containing 0.03% Co²⁺ and Ni²⁺, and 0.02% H₂O₂. If 4,6-diamidino-2-phenyl-indole (DAPI) counterstaining [Carroll et al. 1988] was desired, then heads were returned to wash buffer and incubated for 15 min with 1 µg/ml DAPI before being placed in the mounting solutions.

For mounting, the tissues were rinsed twice with 50 mM Tris-HCl (pH 8.8) and placed in 50 mM Tris-HCl (pH 8.8), containing 10% glycerol (vol/vol) overnight. Imaginal tissues were dissected from the heads and mounted on polylysine-coated slides. Bright-field photography was normally done with Kodak Technical Pan film (ASA 50), and fluorescence photography was done with Kodak Tri-X film (ASA 400). Where images of DAPI, rhodamine, and DAB triply labeled tissues were desired, we used the faster Tri-X film to record the bright-field pictures as well.

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References

- Akam, M.E. 1987. The molecular basis for metameric pattern in the *Drosophila* embryo. *Developmental* **101**: 1–22.
- Akam, M.E. and A. Martinez-Arias. 1985. The distribution of *Ultrabithorax* transcripts in *Drosophila* embryos. *EMBO J.* **4**: 1689–1700.
- Anderson, K.V. and C. Nüsslein-Volhard. 1986. Dorsal-group genes of *Drosophila*. *Symp. Soc. Dev. Biol.* **44**: 177–194.
- Banerjee, V., P.J. Renfranz, D.R. Hinton, B.A. Rabin, and S. Benzer. 1987. The *sevenless* protein is expressed apically in cell membranes of the developing *Drosophila* retina; It is not restricted to cell R7. *Cell* **51**: 151–158.
- Bienz, M., G. Saari, G. Tremml, J. Muller, B. Zust, and P.A. Lawrence. 1988. Differential regulation of the *Ultrabithorax* expression in two germ layers of *Drosophila*. *Cell* **53**: 567–576.
- Botas, J., J. Moscoso del Prado, and A. Garcia-Bellido. 1982. Gene dose titration analysis in the search of trans-regulatory genes in *Drosophila*. *EMBO J.* **1**: 307–310.
- Boulet, A.M. and M.P. Scott. 1988. Control elements of the P2 promoter of the *Antennapedia* gene. *Genes Dev.* **2**: 1600–1614.
- Brower, D.L. 1987. *Ultrabithorax* gene expression in *Drosophila* imaginal disc and larval nervous system. *Development* **101**: 83–92.
- Bryant, P.J. 1975. Pattern formation in the imaginal wing disc of *Drosophila melanogaster*: Fate map, regeneration and duplication. *J. Exp. Zool.* **193**: 49–78.
- . 1978. Pattern formation in imaginal discs. In *Genetics and biology of Drosophila* (ed. M. Ashburner and T.R.F. Wright), vol. 2c, pp. 229–335. Academic Press, New York.
- Campos-Ortega, J.A. and A. Hofbauer. 1977. Cell clones and pattern formation: On the lineage of photoreceptor cells in the compound eye of *Drosophila*. *Wilhelm Roux's Arch.* **181**: 227–245.
- Carroll, S.B. and M.P. Scott. 1985. Localization of the *fushi tarazu* gene product during *Drosophila* embryogenesis. *Cell* **43**: 47–57.

- Carroll, S.B. and M.P. Scott. 1986. Zytotically active genes that affect the spatial expression of the *fushi tarazu* segmentation gene during early *Drosophila* embryogenesis. *Cell* **45**: 113–126.
- Carroll, S.B., R.A. Laymon, M.A. McCutcheon, P.D. Riley, and M.P. Scott. 1986. The localization and regulation of *Antennapedia* protein expression in *Drosophila* embryos. *Cell* **47**: 113–122.
- Carroll, S.B., A. Laughon, and B.S. Thalley. 1988. Expression, function and regulation of the *hairy* segmentation protein in the *Drosophila* embryo. *Genes Dev.* **2**: 883–890.
- DiNardo, S., J.M. Kumer, J. Theis, and P.H. O'Farrell. 1985. Development of embryonic pattern in *D. melanogaster* as revealed by accumulation of the nuclear *engrailed* protein. *Cell* **43**: 59–69.
- Doe, C.Q., D. Smouse, and C.S. Goodman. 1988a. Control of neuronal fate by the *Drosophila* segmentation gene *even-skipped*. *Nature* **333**: 376–378.
- Doe, C.Q., Y. Hiromi, W.J. Gehring, and C.S. Goodman. 1988b. Expression and function of the segmentation gene *fushi tarazu* during *Drosophila* neurogenesis. *Science* **239**: 170–175.
- Fain, M.J. and B. Stevens. 1982. Alterations in the cell cycle of *Drosophila* imaginal disc cells precede metamorphosis. *Dev. Biol.* **92**: 247–258.
- Frasch, M. and M. Levine. 1987. Complementary patterns of *even-skipped* and *fushi tarazu* expression involve their differential regulation by a common set of segmentation genes in *Drosophila*. *Genes Dev.* **1**: 981–995.
- Frasch, M., T. Hoey, C. Rushlow, H. Doyle, and M. Levine. 1987. Characterization and localization of the *even-skipped* protein of *Drosophila*. *EMBO J.* **6**: 749–760.
- Fristrom, D. 1976. The mechanism of evagination of imaginal discs of *Drosophila melanogaster*. III. Evidence for cell rearrangement. *Dev. Biol.* **54**: 163–171.
- Fristrom, D. and J.W. Fristrom. 1975. The mechanism of evagination of imaginal discs of *Drosophila melanogaster*. I. General considerations. *Dev. Biol.* **43**: 1–23.
- Fujita, S.C., S.L. Zipursky, S. Benzer, A. Ferrus, and S.L. Shottwell. 1982. Monoclonal antibodies against the *Drosophila* nervous system. *Proc. Natl. Acad. Sci.* **79**: 7929–7933.
- Garcia-Bellido, A. and J.R. Merriam. 1971. Genetic analysis of cell heredity in imaginal discs of *Drosophila melanogaster*. *Proc. Natl. Acad. Sci.* **65**: 2222–2228.
- Ghysen, A. and C. Dambly-Chaudiere. 1988. From DNA to form: The *achaete-scute* complex. *Genes Dev.* **2**: 495–501.
- Graves, B.J. and G. Schubiger. 1982. Cell cycle changes during growth and differentiation of imaginal leg discs in *Drosophila melanogaster*. *Dev. Biol.* **93**: 104–110.
- Held, L.I., Jr., and P.J. Bryant. 1984. Cell interactions controlling the formation of bristle patterns in *Drosophila*. In *Pattern formation: A primer in developmental biology* (ed. G.M. Malacinski and S.V. Bryant), pp. 291–322. Macmillan, New York and London.
- Hiromi, Y., A. Kuroiwa, and W.J. Gehring. 1985. Control elements of the *Drosophila* segmentation gene *fushi tarazu*. *Cell* **43**: 603–613.
- Holmgren, R. 1984. Cloning sequences from the *hairy* gene of *Drosophila*. *EMBO J.* **3**: 569–573.
- Howard, K. and P. Ingham. 1986. Regulatory interactions between the segmentation genes *fushi tarazu*, *hairy*, and *engrailed* in the *Drosophila* blastoderm. *Cell* **44**: 949–957.
- Howard, K., P. Ingham, and C. Rushlow. 1988. Region-specific alleles of the *Drosophila* segmentation gene *hairy*. *Genes Dev.* **2**: 1037–1046.
- Ingham, P.W. and J.P. Gergen. 1988. Interactions between the pair-rule genes *runt*, *hairy*, *even-skipped*, and *fushi tarazu* and the establishment of periodic pattern in the *Drosophila* embryo. *Dev. Suppl.* **104**: 51–60.
- Ingham, P., S.M. Pinchin, K.R. Howard, and D. Ish-Horowicz. 1985. Genetic analysis of the *hairy* gene in *Drosophila*. *Genetics* **111**: 463–486.
- Ish-Horowicz, D. and S.M. Pinchin. 1987. Pattern abnormalities induced by ectopic expression of the *Drosophila* *hairy* gene are associated with repression of *ftz* transcription. *Cell* **51**: 405–415.
- Ish-Horowicz, D., K.R. Howard, S.M. Pinchin, and P.W. Ingham. 1985. Molecular and genetic analysis of the *hairy* locus in *Drosophila*. *Cold Spring Harbor Symp. Quant. Biol.* **50**: 135–144.
- Kornberg, T., I. Siden, P. O'Farrell, and M. Simon. 1985. The *engrailed* locus of *Drosophila*: in situ localization of transcripts reveals compartment-specific expression. *Cell* **40**: 45–53.
- Lehmann, R., F. Jimenez, U. Dietrich, and J.A. Campos-Ortega. 1983. On the phenotype and development of mutants of early neurogenesis in *Drosophila melanogaster*. *Wilhelm Roux's Arch. Dev. Biol.* **192**: 62–74.
- Lewis, E.B. 1978. A gene complex controlling segmentation in *Drosophila*. *Nature* **276**: 565–570.
- Murray, M.A., M. Schibiger, and J. Palka. 1984. Neuron differentiation and axon growth in the developing wing of *Drosophila melanogaster*. *Dev. Biol.* **104**: 259–273.
- Nüsslein-Volhard, C., H.G. Frohnhofer, and R. Lehmann. 1987. Determination of anteroposterior polarity in the *Drosophila* embryo. *Science* **238**: 1675–1681.
- Peifer, M., F. Karch, and W. Bender. 1987. The bithorax complex: Control of segmental identity. *Genes Dev.* **1**: 891–898.
- Ready, D.F., T.E. Hanson, and S. Benzer. 1976. Development of the *Drosophila* retina, a neurocrystalline lattice. *Dev. Biol.* **53**: 217–240.
- Schubiger, M. and J. Palka. 1987. Changing spatial patterns of DNA replication in the developing wing of *Drosophila*. *Dev. Biol.* **123**: 145–153.
- Scott, M.P. and S.B. Carroll. 1987. The segmentation and homeotic gene network in early *Drosophila* development. *Cell* **51**: 689–698.
- Simpson, P. and G. Morata. 1980. The control of growth in the imaginal discs of *Drosophila*. In *Development and neurobiology of Drosophila* (ed. O. Sidding, P. Babu, L.M. Hall, and J.C. Hall), pp. 129–140. Plenum Press, New York and London.
- Tomlinson, A. 1985. The cellular dynamics of pattern formation in the eye of *Drosophila*. *J. Embryol. Exp. Morph.* **89**: 313–331.
- . 1988. Cellular interactions in the developing *Drosophila* eye. *Development* **104**: 183–193.
- Tomlinson, A., D.D.L. Bowtell, E. Hafen, and G.M. Rubin. 1987. Localization of the *sevenless* protein, a putative receptor for positional information in the eye imaginal disc of *Drosophila*. *Cell* **51**: 143–150.
- Zipursky, S.L., T.D. Venkatesh, D.B. Teplow, and S. Benzer. 1984. Neuronal development in the *Drosophila* retina: Monoclonal antibodies as molecular probes. *Cell* **36**: 15–21.

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