rhomboid, a gene required for dorsoventral axis establishment and peripheral nervous system development in Drosophila melanogaster

Ethan Bier, Lily Y. Jan, and Yuh Nung Jan
Howard Hughes Medical Institute and the Departments of Physiology and Biochemistry, University of California, San Francisco, California 9414 USA

rhomboid (rho) belongs to a group of four genes involved in the elaboration of positional information at a ventrolateral level in the Drosophila embryo. Mutations at any of these four loci also lead to a variety of other phenotypes, including reduction in the number of stretch receptor organs (chordotonal organs) in the peripheral nervous system (PNS). We have cloned rho with the aid of a lacZ-bearing P-element inserted into the rho gene. In the early blastoderm stage, a putative rho transcript is expressed in ventrolateral strips corresponding to the domain of activity of the rho gene on the embryonic fate map. Later expression of the transcript correlates with regions of the embryo that are disrupted in rho mutants and includes a cell that may be the precursor for the missing stretch receptor organs. We hypothesize that rho acts very early in differentiation pathways to specify the identities of domains and isolated precursor cells. Sequence analysis suggests that this transcript codes for a trans-membrane protein.

[Key Words: rhomboid; dorsoventral axis; spitz group; peripheral nervous system; lacZ; Drosophila melanogaster]

Received October 2, 1989; revised version accepted November 20, 1989.

Positional information along the dorsoventral axis of developing Drosophila embryos is specified by genes expressed maternally in the egg [Anderson et al. 1985a,b], as well as by genes that become active in the zygotic embryo [Jürgens et al. 1984; Nüsslein-Volhard et al. 1984]. The former group of maternal-effect genes is assumed to generate a dorsoventral gradient of morphogen, which is read by the latter group of zygotic genes [Anderson 1987]. The result of this genetic network may be the definition of a series of longitudinal strips of different dorsoventral positional cues. By the cellular blastoderm stage, cells of the Drosophila embryo have acquired specific fate according to their positions, so that different regions of the cellular blastoderm will give rise to different structures in a predictable manner, as defined by a fate map [Lohs-Schardin et al. 1979; Campos-Ortega and Hartenstein 1985]. Thus, the domains of activity of zygotic genes specifying position at the blastoderm stage may correspond to those regions of the Drosophila fate map, which give rise to tissues affected in the respective mutants. So far, molecular analysis of cloned genes of this type has been consistent with this expectation. For instance, the twist gene is required for defining the most ventral portion of the fate map, and twist is expressed in the most ventral region of the blastoderm [Thissell et al. 1988]. Similarly, the longitudinal strips of the fate map requiring the activities of single-minded [sim], zerknäult [zen], and decapentaplegic [dpp] all correlate with the regions of the blastoderm in which these genes are expressed [Doyle et al. 1986; St. Johnson and Gelbart 1987; Crews et al. 1988; Thomas et al. 1988].

Among the zygotic dorsoventral group of genes, those that specify positional information for cells situated in the ventrolateral domain, between the domains defined by sim and zen, are particularly relevant for neural development because both the central nervous system (CNS) and the ventral portion of the peripheral nervous system (PNS) arise from this region. These loci have not yet been characterized molecularly and include rhomboid (rho), Star (S), spitz (spi), and pointed (pnt) [Jürgens et al. 1984; Nüsslein-Volhard et al. 1984; Mayer and Nüsslein-Volhard 1988]. The domain of the embryonic fate map affected by these genes is thought to be a longitudinal strip just dorsal to the mesectodermal domain defined by sim [Mayer and Nüsslein-Volhard 1988]. Mutations in any of these genes lead to loss of ventral cuticular structures. In the CNS the spacing between the two longitudinal fiber tracks is also abnormal, it is much reduced in S, spi, and rho mutants but widened in pnt. These phenotypes have been interpreted as ventral midline defects [Mayer and Nüsslein-Volhard 1988; R.
Jacobs, pers. comm.). Besides structures derived from the ventrolateral regions of the fate map, these mutations eliminate the first row of dendrites in abdominal segments. The first row of dendrites is thought to arise from the segment boundary. Defects in head development and dorsal closure are also observed in mutants for these genes. This last phenotype is seen in rho, S, and pnt, but not in spi, mutants. Finally, S and spi are required for formation of the germ line, as shown in studies involving pole-cell transplantation. In contrast, no maternal role for pnt or rho was observed. This has been taken as an indication that pnt and rho play specific roles in specifying positional information in the embryo, as opposed to providing a more general cell function [Mayer and Nüsslein-Volhard 1988].

Here, we report further phenotypic analysis, as well as molecular characterization of the rho locus. The variety of apparently unrelated structures altered by the rho mutation correlates well with the complex and dynamic expression pattern of the rho transcript. Thus, rho appears to act early in embryonic development to establish position along the dorsoventral axis and then again later to specify the fate of neuronal precursor cells. We propose that rho acts very early in a variety of developmental contexts to specify either the fate of cells in which it is expressed or the fate of cells in the immediate vicinity.

Results

New alleles of rho

Besides the original rho allele rho<sup>7M~3</sup>, a new allele has been generated by insertion of a lacZ-containing P element, P-lacW [Bier et al. 1989]. The insertion is at 62A on the third chromosome and disrupts the rho gene, because it fails to complement rho<sup>7M~3</sup>. Revertants that appear to have resulted from precise excision of P-lacW sequences [see Methods], on the other hand, do complement rho<sup>7M~3</sup> and are viable. An imprecise excision that removes several hundred base pairs of DNA on one side of the P-lacW insertion has a cutoxic phenotype similar to rho<sup>7M~3</sup> and does not complement this mutation. Mutant embryos homozygous for the P-lacW insertion are similar to rho<sup>7M~3</sup> with regard to the PNS phenotype. They exhibit a cuticular defect that is less severe than that of rho<sup>7M~3</sup> or the deletion mutant, but they do not show any obvious CNS phenotype. Thus, the P-lacW insertion appears to have induced a weaker rho allele than rho<sup>7M~3</sup>. This new allele is designated rho<sup>lacZ</sup> and the deletion mutant derived from rho<sup>lacZ</sup> is called rho<sup>del1</sup>.

PNS phenotype of rho

The embryonic PNS in abdominal segments is made up of three major clusters of neurons and support cells located at ventral, lateral, and dorsal levels. One type of sensory organ [chordotonal organ] is thought to be a stretch receptor. Chordotonal organs are present in both the lateral and ventral clusters of each abdominal segment (Fig. 1C). In rho, as well as in S, spi, and pnt mutant embryos, two of the five lateral chordotonal organs are missing [Jan et al. 1986]. In the ventral cluster, the chordotonal organ with the neuronal dendrite pointing ventrally is also frequently missing in rho mutants. Chordotonal organs in the thoracic segments of rho mutant embryos, however, are normal.

Each chordotonal organ comprises four cells, a neuron, a scolopale cell that wraps around the dendrite of the neuron, and a cap cell and ligament cell that attach to the epidermis. The four cells of a chordotonal organ are derived from a single precursor [Bodmer et al. 1989]. Three of the four cells can be identified independently, as antibodies against horseradish peroxidase [anti-HRP] label the neuron and scolopale cell [Jan and Jan 1982, Bodmer et al. 1987]. The monoclonal antibody mAb44C11 labels neuronal nuclei [Bier et al. 1988], and the transformant line A18 expresses lacZ (β-gal) in the neuron, the scolopale cell, and the cap cell [Ghysen and O’Kane 1989]. For those chordotonal organs absent in rho mutants, three identifiable cells are removed [Fig. 1A–C]. Because the lacZ expression that results from the A18 insertion normally appears early, most likely in precursors of chordotonal organs as well, the total absence of such expression corresponding to chordotonal organs missing in rho mutants suggests that this gene acts early in the formation of chordotonal organs. Moreover, occasionally only one chordotonal organ is missing in a lateral cluster of the rho mutant embryo. In such cases, all identifiable cells of the four remaining chordotonal organs are present. The apparent all-or-none effect of the rho mutation on cells of a chordotonal organ again suggests that the mutation affects the precursor of the chordotonal organ.

To see whether rho mutations remove specific chordotonal organs, we used mAb49C4, which stains only four of the five chordotonal organs in wild-type embryos. The most anterior chordotonal organ does not express the mAb49C4 antigen [Bodmer et al. 1987]. In rho mutants, three mAb49C4-positive cells are seen [not shown], suggesting that one of the missing chordotonal organs is the most anterior one.

To test whether all four genes involved in specifying the same ventrolateral domain of the blastoderm also affect the same chordotonal organs, we examined the embryonic phenotype of double mutants. The PNS phenotype for double mutants of both rho and pnt is the same as for rho or pnt alone. The same phenotype is also seen in double mutants of rho and S or rho and spi. Thus, all of these genes appear to function in the formation of the same chordotonal organs.

Besides chordotonal organs, there are two other major classes of sensory cells in the PNS, namely neurons with multiple dendrites [md] and cells belonging to external sensory [es] organs. These cell types can be identified individually, using a variety of antibodies and lacZ marker lines [Bodmer et al. 1987; Bier et al. 1989; Uemura et al. 1989]. In abdominal segments, chordotonal organs seem to be the only PNS cell type deleted by the rho mutation; no effect on es organs or md neurons has been
found. In the thorax and head, on the other hand, es organs that are innervated by multiple neurons (e.g., Keilin's organs and the antennal maxillary complex) also show substantial reduction in cell number [not shown; see also Mayer and Nüsslein-Volhard 1988].

Muscle phenotype

The muscle pattern during mid-embryogenesis (10–11 hr) is easily seen by staining with mAb6D5 [Caudy et al. 1988]. In rho mutants, this pattern is abnormal. In thoracic segments, the muscle pattern is severely altered and the remaining fibers do not seem to follow a regular pattern. In abdominal segments, it appears that only a particular subset of muscles is affected and the altered pattern is regular from segment to segment (Fig. 1D–F). The dorsolateral longitudinal muscle and the dorsal oblique muscles, both with attachment sites within a dorsolateral strip of the embryo, are missing. This loss of muscles leads to a dorsolateral gap in the abdominal muscle pattern. No muscle fibers running along the dorsoventral axis are missing, however. The dorsal longitudinal muscle mass, which is found near the dorsal midline (lightly stained by mAb6D5), contains muscles that are unaffected by the rho mutation and may also contain some of the muscle fibers missing from the normal dorsolateral position in rho mutant embryos. In the ventral region, certain muscles are also missing [Fig. 1E,F].

Some muscles are retained in mutant embryos, although the site to which they attach is altered. For example, in Figure 1D and F, muscles that normally attach to the site marked by an asterisk [a] join at the more ventral position marked by an asterisk [a'] in rho embryos. This shift is accompanied by a change in the relative length of fibers that run along the dorsoventral axis at the segment boundary, suggesting that the location of this attachment site determines the shape and course of the fibers that attach to it. In the ventral region, one of the ventral oblique fibers that crosses the segment boundary and becomes fastened at the site marked b is found at a much more ventral location in rho mutant embryos. Often, these fibers take an abnormal dorsoventral path and attach to the ventral midline (labeled b'), as do the corresponding fibers from the opposite hemise-
ment. In general, muscles in ventral and lateral regions are displaced ventrally in rho mutant embryos, possibly as a secondary consequence of the loss of ventral epidermal structures. These observations suggest again that there is an intimate dependence of muscle development on the position of epidermal cues such as attachment sites. Because the fibers that are missing in the mutant are generally those with two segment-boundary attachment sites, and fibers running along the dorsoventral axis are retained, it is possible that defects at segment boundaries, as well as dorsoventral patterning, lead to loss of fibers or displacement of certain muscle attachment sites.

**Molecular analysis of the rho locus**

Genomic DNA flanking the P-lacW insertion in rho^{act} was isolated by plasmid rescue [Pirrotta 1986]. The rescued DNA fragments hybridize to the 62A region on the polytene chromosome and have the same restriction map (Fig. 2A) as that of the genomic DNA determined by Southern analysis [not shown]. On Northern blots, the genomic DNA fragment [2.8 kb] at the 5' end of the P-transposase--lacZ fusion gene detects two bands (2.5 and 2.9 kb) during all stages of development, with the exception of larval stages (Fig. 2B). Further Northern analysis showed that the 2.5- and 2.9-kb transcripts are present throughout embryogenesis but become more abundant after 12 hr. In contrast, no message was seen in ovaries, even with long exposure [not shown]. The genomic DNA on the other side of P-lacW [3.4 kb] gave only weak and diffuse signals on Northern blots of embryonic RNA. These weak signals may arise from some repetitive sequence in this region. When a probe including this fragment was used to screen a genomic library, many faintly positive plaques were seen in addition to the few strongly positive ones.

To better define the transcription unit near the P-lacW insertion of rho^{act}, we isolated three cDNA clones that hybridize to the 5' genomic fragment. Two of these are ~2.5 kb long. They map back to a 4.7-kb genomic region on Southern blots of both cloned and genomic DNA. When one of these cDNAs is used as a probe, on Northern blots the same transcripts are labeled as those that are labeled with the probe made from the rescued genomic DNA. A comparison between the sequence of the cDNAs and that of genomic DNA shows that this genomic region is interrupted by two introns (Fig. 2A).

To determine whether this transcription unit corresponds to the rho gene, we transformed flies with an 8.5-kb genomic fragment that includes the coding region for the 2.5-kb cDNA and 2.2 kb at its 5' end (Fig. 2A; Rubin and Spradling 1982). In contrast to control rho mutant stocks in which the mutation is 100% embryonic lethal,
Northern analysis suggests that this fragment contains only one full transcription unit, this transcription unit most likely corresponds to the rho gene.

Sequence analysis of the 2.5-kb cDNAs reveals a single long open reading frame coding for a protein of 355 residues [Fig. 3A]. In rhoact, the P-lacW insertion is 39 bp upstream from sequences corresponding to the 5' end of the longer of the two cDNAs. The P-lacW insertion site is flanked by a consensus cap site sequence [25 bp downstream] and three potential TATA box sequences [the first of which is immediately upstream]. Thus, the P-lacW insertion most likely reduces rho gene activity by separating coding sequences from the promoter. In the deletion mutant rhoact, the entire exon coding for much of the 5'-untranslated sequence of this rho transcript is eliminated [Fig. 2 legend]. This deletion may reduce rho activity even further than the original rhoact insertion, leading to the more severe cuticular phenotype.

The predicted rho protein has a molecular weight of 39,356 and is most likely an integral membrane protein. The predicted number of membrane-spanning regions ranges from three to seven, depending on the algorithm used in the identification of hydrophobic segments [Fig. 3B]. No hydrophobic signal sequence is present at the amino terminus, suggesting that this end may be cytoplasmic. A PEST sequence postulated to be associated with proteins of short half-life [Rogers et al. 1986] is found 45 amino acids from the amino terminus. In the 3'-untranslated portion of the cDNA, 14 copies of the sequence ATTATA are found. This motif has been shown to cause rapid mRNA turnover and to lower translation efficiency [Shaw and Kamen 1986; Kruys et al. 1989]. Thus, both the rho message and protein may be turned over rapidly. In the amino-terminal region there is a stretch of alternating positive and negative charges, similar to a sequence that is proposed to form an α helix in the c-fos and N-myc leucine zipper domains [Kohl et al. 1986; Landschulz et al. 1988]. A similar sequence is also found and predicted to be cytoplasmic in the gene product of the maternal-effect Toll gene [Hashimoto et al. 1988; Fig. 3B].

In summary, the site of P-lacW insertion in rhoact is likely to be in or downstream from the promoter of a transcription unit. The genomic fragment that includes this transcription unit rescues much of the rho embryonic mutant phenotype. Moreover, as described below, the complex and dynamic expression pattern of this transcription unit corresponds closely to those parts of the embryo affected by rho mutations. Therefore, this transcription unit is most likely the rho locus.

Expression patterns of rho

Expression of rho transcripts is dynamic and complex. The earliest expression is seen at the cellular blastoderm stage [Fig. 5A,B]. In cross sections [Fig. 5A] this expression appears in two longitudinal ventrolateral domains seven to eight cells wide, with irregular boundaries both dorsally and ventrally. These domains are separated by
Figure 3. Primary sequence analysis of the 2.5-kb rho cDNAs. (A) DNA sequence of the 2.5-kb cDNAs. Opposite strands of two independently isolated 2.5-kb cDNAs were sequenced. Both cDNAs yielded the same sequence. The cDNA sequence agrees with the sequence of genomic DNA, the latter also contains two introns. The first intron is located between nucleotides 339 and 340. The donor and acceptor sequences are, respectively, AAG/gtaag and ttcctctttcctccag/AAT. The second intron is found between nucleotides 2561 and 2562. The sequence GTACGT TT is found 25 bp downstream of the P-lacW insertion site and matches closely the mRNA cap site consensus ATCA(G/T)T(C/T) for non-heat-shock insect genes (Hultmark et al. 1986). Three potential TATA box sequences are found 6, 29, and 72 bp upstream of the 5′-untranslated region, and the underlined segments in this region of the protein are predicted as possible membrane-spanning regions by only one of the programs.

(B) Schematic representation of the putative rho protein. Marked structures correspond to those described in A. Solid boxes represent potential membrane-spanning regions. Numbers beneath the boxes refer to the programs that predict the segment to be trans-membrane: [1] Eisenberg et al., [2] Rao and Argos, [3] Klein et al., [4] Ralph et al. Segments four and six are predicted by all four programs. The program of Ralph et al., run by T. Smith, suggests that segment two may form a β sheet. The box filled with dashed lines denotes the charged domain containing the EKEKE sequence; the stippled box is the region containing the PEST motif.
Table 1. Partial rescue of rho phenotype by the 8.3-kb fragment

|                | rho genotype |                         |                         | rho1°/rho zM~ |
|----------------|--------------|--------------------------|--------------------------|----------------|
| A. Embryonic lethality | rho1°/rho zM~ |                         |                         | rho1°/rho zM~ |
| % unhatched embryos | ± RynA-3/RynA-3 | 25                       | 15                       | 139            |
| total number tested | N           | 1418                     |                          |                |
| B. Length of first denticle band | rho genotype |                         |                         | rho1°/rho zM~ |
| % of wild type | ± RynA-3/RynA-3 | 51 ± 6                  | 81 ± 6                  | 81             |
| total number tested | N           | 86                       |                          |                |
| fourth abdominal segment | % of wild type | 57 ± 5                  | 78 ± 5                  | 81             |

To get an estimate of the rho function provided by the 8.3-kb genomic fragment within the RynA-3 P element on the X chromosome, we examined the effect of this RynA-3 insertion on the rho mutant phenotype, by measuring (A) the percentage of unhatched embryos from heterozygous parents and (B) the length of the first row of denticles in the third and fourth abdominal segments as normalized to that of wild-type embryos [mean ± S.D. of the mean (μ)]. Details of the crosses are provided in Methods. The significance of the difference in embryonic lethality in a rho1°/rho zM~ background was estimated by z = (y/μ - p)/√p[N(1 - p)], where y/μ is the observed fraction of unhatched embryos and p = 0.25. For rho1°/rho zM~ embryos, p < 0.001 for the observed partial rescue of lethality. rho1°/rho zM~ is also completely embryonic lethal (24%, N = 164). In addition, all cuticles from rho zM~/rho1°, rho1°/ rho zM~ and rho1°/rho1° dead embryos show defects in denticle belt morphology. Dead embryos of the RynA-3/RynA-3, rho1°/rho1° genotype show a range of phenotypes but, overall, are clearly intermediate between rho1°/rho1° and wild type. This effect has been quantitatively addressed by measuring the length of the first row of denticles in abdominal segments 3 and 4 in wild type, in rho1°/rho1°, and in RynA-3/RynA-3, rho1°/rho1°. For rescue of the cuticle phenotype, probability values (p) were calculated, using z = (y1 - y2)/√y1[N1 + s2/N2], where x is the length of the first row of denticles, d is the width of the embryo in segment 3 and 4, and P_y/μ-wild-type = 0.43 for segment 3 and 0.46 for segment 4, s2 = (μx[N2 - X]\(2/N - 1)), where N is the sample size and X is the mean. The significance of differences in length of the first row of denticles in rho1°/rho1° mutants with versus without the RynA-3 element is p < 0.001 for segments 3 and 4. rho1°/rho zM~ or rho1°/rho zM~ embryos generally lack the first row of denticles. Many embryos of these genotypes that also carry RynA-3 have distinct first rows. This effect is difficult to quantitate because there are also many embryos for which it is not obvious whether the first row is present, as denticle bands often interdigitate and follow irregular paths. The extent of rescue of the cuticular phenotype in rho mutant embryos carrying RynA-3 is an underestimate of the true average effect, as the 40% of transformant embryos that hatch were not scored. In fact, examination of dead first instar larvae on the grape plates revealed that the hatching larvae were rescued more completely than unhatched embryos on the same plate.

an unlabeled ventral strip that is 13–15 cells wide. Shortly thereafter, expression appears in a dorsal strip as well, leading to signals in three approximately evenly spaced circumferential locations [Fig. 5C]. Subsequently, the ventrolateral domains of expression become narrower and periodically modulated in intensity along the anterior–posterior axis. During this time, the ventrolateral strips become delimited by a sharp boundary ventrally [Fig. 5D]. Horizontal sections and whole-mount preparations also reveal two stripes of expression in the head, running perpendicularly to the long axis of the embryo [Fig. 5F]. As mesoderm invagination begins, the label is moved ventrally and becomes progressively restricted to a single row of cells on each side of the embryo. These two rows meet at the ventral midline as the ventral furrow closes [Fig. 5E,G]. Upon closure of the ventral furrow the labeled cells interdigitate to form a single mesectodermal strip along the ventral midline [Fig. 5H]. This expression remains until germ-band retraction.

Although the mesectoderm is labeled, strong but very transient expression is seen in cells in each abdominal hemisegment at the location of the invaginating tracheal

Figure 4. rho function is supplied by an 8.3-kb genomic frag-
Figure 5. Pattern of expression of rho RNA. (A) A section of an embryo at the early cellular blastoderm stage hybridized with a ^3S-labeled RNA probe, revealing two ventrolateral signals. (B) Whole-mount embryo at the same stage hybridized with a digoxigenin-labeled DNA probe. (C) Soon after the stage depicted in A and B, the signal is localized in three nearly symmetrical locations in transverse sections. Two of the domains correspond to ventrolateral positions, and the third marks the dorsal aspect of the embryo. (D) At about the same stage as in C, the longitudinal domains in whole-mount embryos become modulated along the anterior–posterior axis in a pair-rule-like fashion. At this point the ventral margins of the ventrolateral domains become defined by a single sharp row of cells. (E) As gastrulation begins, the ventral furrow forms, so that the ventrolateral label is displaced ventrally. In addition, the domain of expression has narrowed considerably, as shown on this section. (F) A dorsal view at the same stage as in D. (G) When the ventral furrow has invaginated almost completely, the ventral label becomes restricted to two single rows of cells on opposite sides of the presumptive mesoderm. (H) As ventral closure takes place, rho-expressing cells from the two halves of the embryo meet to form the mesectodermal cells along the ventral midline. From their location, these cells should also express sim (Crews et al. 1988, Thomas et al. 1988). Note the neuroblasts (nb) in contact with these cells. (I) As germ-band extension proceeds, the mesectoderm continues to label. In each abdominal hemisegment, transient but strong expression is also seen in cells that will form the tracheal pit and in a single large cell that is in a dorsal and posterior position relative to the tracheal pit—most likely the precursor of chordotonal organs. (J) During germ-band retraction, cells in the CNS begin to label. (K and L) As germ-band retraction is completed, cells forming the anterior-most row in each abdominal segment label both ventrally and dorsally. In the thoracic segments, this expression is restricted to the dorsal region. Abbreviations in this and subsequent figures are (A) anterior; (Ab) abdominal segments; (am) anterior midgut invagination; (cf) cephalic furrow; (CHO Prec.) chordotonal organ precursor; (D) dorsal; (dml) dorsal midline; (H) head; (me) mesectoderm; (ms) mesoderm; (mu) muscle; (nb) neuroblasts; (P) posterior; (seg-B) segment boundary; (T) thoracic segments; (tp) tracheal pit; (V) ventral; (vml) ventral midline.
pit and in a single large cell that is dorsal and posterior to the pit [Fig. 5l]. The single large cell that expresses rho may be a chordotonal organ precursor, as its location relative to the tracheal pit corresponds well to that of the first chordotonal precursor revealed by the A18 lacZ marker line [Fig. 6A,B]. In slightly older embryos, two labeled cells can be seen at this location. Thus, the first labeled cell may divide to give these two cells or, alternatively, another potential precursor may arise de novo. Several other isolated cells in the periphery label less intensely and transiently during this time. The identity of these cells is currently unknown, but some are in the correct locations to be precursors for the ventral chordotonal organ missing in rho mutants, or to be progenitors of the Keilin’s organs.

When germ-band retraction begins, rho expression in tracheal pits and along the ventral midline fades rapidly and cells in the CNS begin to label in a segmentally repeated pattern [Fig. 5l]. Upon completion of germ-band retraction, segmentally repeated stripes emerge [Fig. 5K,L], which are one to two cells wide in the ectoderm at the anterior border of each segment. These ectodermal stripes are seen dorsally (only one cell wide) in both thoracic and abdominal segments [Fig. 5L] but are seen ventrally only in the abdominal segments [Fig. 5K]. A small lateral gap separates the dorsal and ventral stripes in abdominal segments. In addition, a single row of cells at the dorsal margin of the epidermis express rho shortly before dorsal closure. Light staining is also seen in two clusters of epidermal cells in the middle of each abdominal hemisegment. One cluster is found laterally and the other dorsally.

In collections of embryos from heterozygous rho<sup>act</sup> flies, approximately one-quarter of the blastoderm-stage embryos show only weak and patchy rho expression in the ventrolateral domains. At the germ-band-extended stage, weakly staining embryos are also seen, in which midline expression is clumpy and irregular. This weak and patchy expression pattern is never seen in collections of wild-type embryos. Thus, the P-lacW insertion in rho<sup>act</sup> mutant embryos seems to decrease rho expression during early stages of embryogenesis.

**Figure 6.** Correlation of the single rho-expressing cell with the chordotonal precursor. (A) A high magnification view of the embryo shown in Fig. 5l. (B) The chordotonal precursors that express lacZ in the A18 line [Ghysen and O’Kane 1989] are shown in A18 lines (Ghysen and O’Kane 1989). The position of the first chordotonal precursor relative to the tracheal pit is very similar to the position of the single rho-expressing cell relative to the tracheal pit. These cells are also similar in size, noticeably larger than the surrounding cells. Vertical dashed lines mark the approximate locations of future segment boundaries.

**lacZ expression pattern in rho<sup>act</sup> embryos**

The pattern of lacZ expression derived from the P-lacW insert in rho<sup>act</sup> after blastoderm formation is similar to the expression pattern of the rho transcript as described below. The P-element transposase–lacZ fusion gene is transcribed from the weak transposase promoter. O’Kane and Gehring [1987] have shown that a similar fusion gene gives rise to different expression patterns at different insertion sites. In some cases, the lacZ expression pattern is consistent with that of the gene near the insertion site [Bellen et al. 1989; Bier et al. 1989; Wilson et al. 1989]. This also appears to be the case for the P-lacW insertion into rho.

lacZ staining in heterozygous [rho<sup>act</sup>/+] embryos parallels that of the rho transcript along the ventral midline [Fig. 7A], in the CNS [Fig. 7B], and at segment boundaries [Fig. 7C,D]. The blastoderm pattern of rho expression and the transient expression in the tracheal pit and a single cell of each abdominal hemisegment, however, are not mimicked by detectable lacZ expression. Because the orientation of transcription of rho is opposite that of the P-element transposase–lacZ fusion gene and their respective promoters are >10 kb apart, it is possible that both the reduced level of early expression of rho RNA in rho<sup>act</sup> mutants and the lack of early lacZ staining are attributable to a differential ability of distinct rho promoter elements to function across the P-lacW insertion.

In addition to strong lacZ expression in the most anterior row of cells in each abdominal segment during the late stage (10–12 hr) of rho expression, much lighter staining is also transiently visible ventrally in what is likely to be the most posterior row of cells of the preceding segment. Strings of staining cells are also found internally [Fig. 7D], in the proventriculus (where the esophagus meets the midgut), in a loop in the hindgut, in rings at the base of the posterior spiracles, and just above the pharynx in two parallel rows that run in an anterior–posterior direction. The same pattern of internal expression has been detected in whole-mount embryos hybridized with rho cDNA, although the label is less intense than in superficial tissues, probably because of limited penetration of the cDNA probe. A common aspect of both ectodermal and internal expression at this later stage is that the arrangements of labeled cells are
primarily in linear arrays rather than in sheets or clusters.

The lacZ expression in homozygous rho\textsuperscript{act} mutants is very similar to that in heterozygous embryos. The only exception is that instead of a single row of strongly expressing cells located ventrally in the most anterior row of each segment, mutants have two strongly labeled rows that are irregularly arranged in the anterior-most part of each abdominal segment [Fig. 7F].

Discussion

The rho locus has been cloned by plasmid rescue, using the P-lacW insertion in the rho\textsuperscript{act} mutant. An 8.3-kb genomic piece of DNA that includes much of the rho transcription unit shows significant rho function in embryos. In the rho\textsuperscript{act} mutant, only genomic sequences from the rho transcription unit are deleted, and the mutant phenotype is even stronger than that of rho\textsuperscript{lac}, from which it was derived. The P-lacW insertion in rho\textsuperscript{act} is most likely in the promoter of the rho gene and leads to reduced and patchy rho expression in rho\textsuperscript{act} mutant embryos. The rho\textsuperscript{act} insertion induces lacZ expression in patterns that closely resemble the rho expression profile after 5 hr of embryogenesis. As discussed below, the complex expression pattern of rho recapitulates the many facets of the rho phenotype.

Correlation between rho expression and mutant phenotypes

Early rho expression in ventrolateral strips at the cellular blastoderm correlates with the region of the embryonic fate map predicted to be the domain of rho activity [Mayer and Nüsslein-Volhard 1988]. As judged from the expression of rho in mesectodermal cells, it appears that the domain of rho expression may eventually include or overlap with the domain expressing sim. Because mutations in rho and sim appear to affect separate adjacent longitudinal domains of cuticle [Mayer and Nüsslein-Volhard 1988], the precise domains in which these two genes are initially expressed will be important to determine. There are other features of blastoderm expression that correlate with additional aspects of the cuticular phenotype. Thus, a dorsal strip of expression is seen in the blastoderm, and dorsal closure is defective in rho mutant embryos. There is also early expression in the head region, and mutant embryos exhibit head defects resulting in a pointed head skeleton.

Late rho expression, as revealed by in situ hybridization and by lacZ fusion gene expression, correlates with other aspects of the rho phenotype. For instance, expression is seen along the ventral midline in the mesectoderm and later in cells along the midline of the CNS. In rho mutants, the CNS defects seem localized mostly to a region near the ventral midline [Mayer and Nüsslein-Volhard 1988; R. Jacobs, pers. comm.]. At the germ-band extended stage, rho is expressed in tracheal pits. This may correlate with the observed frequent breaks in major tracheal trunks and general disorganization of finer tracheal branches in rho mutant embryos. Although the lacZ pattern closely mimics features of rho expression, it does not reproduce the entire pattern of rho expression, a point worth noting with regard to the use of pattern detecting P-element vectors.

In the PNS of mutant rho embryos, two of the five lateral chordotonal organs are missing in abdominal segments. One of the chordotonal organs removed in the mutant is the most anterior one, which is the first to form in the lateral cluster of a wild-type embryo [Bodmer et al. 1989]. Most likely, its precursor also fails to form or to differentiate, as no constituents of this chordotonal organ can be detected with markers that label chordotonal organs and their precursors. At about the same time that the first chordotonal organ precursors divide (5 hr after fertilization; Bodmer et al. 

Figure 7. *lacZ* expression pattern in heterozygous rho\textsuperscript{act}/+ embryos (A-E) or homozygous rho\textsuperscript{act} mutant embryos (F). (A) A ventral view of an embryo at germ-band extension in which cells in the mesectoderm along the ventral midline are labeled. (B) Following germ-band extension, cells in the CNS begin to label. Eventually, clusters of cells throughout the CNS label, including a cluster of about four cells that lies along the ventral surface of each segment of the CNS, as well as a set of segmentally repeated cells on the dorsal aspect of the mature CNS. (C–E) After germ-band retraction, the *lacZ* expression pattern becomes complex. (C) Strong staining is seen in the anterior-most row of cells in the ventral region. (D) The staining of the anterior-most row of cells is seen in both abdominal and thoracic segments dorsally. This expression is visible only after germ-band retraction is complete. (E) Notable expression in the pharynx (ph), cells forming a loop in the hindgut (hg), which starts at one posterior spiracle and ends at the other, and rings of cells at the bases of the posterior spiracles (ps). (F) *lacZ* expression in rho\textsuperscript{act}/rho\textsuperscript{act} mutant embryos differs from the pattern observed in rho\textsuperscript{act}/+ embryos, in that there are two rows of strongly labeled ventral cells instead of one. The nuclear localization of the *lacZ* fusion gene product is likely to be a consequence of the nuclear localization of P-element transposase to which *lacZ* is fused.
1989, Ghysen and O’Kane 1989], rho is transiently expressed in a single cell and then in two cells located in a dorsal and posterior position relative to the tracheal pit in each abdominal hemisegment. These cells are very near, if not at, the site where the first chordotonal precursors arise (Bodmer et al. 1989; Ghysen and O’Kane 1989). The large size of the initial single cell also suggests that it may be a PNS precursor, as cells surrounding the large β-gal-expressing cell in the lacZ marker line are consistently smaller than the precursor cell. Thus, the deletion of the lateral chordotonal organs may be the result of a requirement of some function that rho normally provides for the specification of the precursor cell[s]. The absence of two chordotonal organs in rho mutants may imply that the earliest chordotonal precursor gives rise to two chordotonal organs. This notion is consistent with the observation that the five lateral chordotonal organs may derive from only two primary precursors (Ghysen and O’Kane 1989; V. Hartenstein, pers. comm.). Alternatively, the two precursors for the two missing chordotonal organs may arise independently, one after the other.

After 10 hr of embryonic development, rho expression is primarily in linear arrays of cells in the endoderm and ectoderm. The ectodermal expression includes the most anterior row of cells in each abdominal segment and, transiently, the most posterior row of cells of the previous segment. In the rho lacZ mutant, two irregular rows of ventral cells at the anterior border of each segment express lacZ. This alteration of the normal expression pattern may be related to the lack of the first row of denticles (normally provided by the most posterior row of cells in the previous segment). The affected cells may either normally express rho or be adjacent to cells that do so. Because cells at the dorsal margin of the epidermis also express rho briefly during this period, the defective dorsal closure in rho mutants may also result from a failure of these cells to act as boundary cells.

A number of factors may underlie the muscle defects in rho mutant embryos. The apparent absence or disarray of some longitudinal and oblique muscles may reflect defects at the segment boundaries, as these muscles have segment boundary attachment sites in wild-type embryos. Evidence for an intimate dependence of the muscle pattern on the location of attachment sites in rho mutant embryos is consistent with this interpretation. Alternatively, muscle defects may be secondary to other ectodermal or mesectodermal abnormalities. The ventral displacement of the ventral and lateral portions of the remaining muscle pattern may be the result of a corresponding shift of epidermal cues that result from loss of more ventral positional information [as evident from cuticular defects]. rho may also play a direct role in muscle development, as some muscles appear to be missing entirely and mesodermal cells are adjacent to rho-expressing cells of the mesectoderm early in development.

No gross phenotypes have been associated with any of the internal tissues expressing rho. We note, however, that virtually all rho expression is in linear arrays of cells, often at known boundaries. The rows of rho-expressing cells at the segment boundaries, at the dorsal margin in the proventriculus, and at the base of the posterior spiracles mark obvious morphological boundaries. rho expression along the ventral midline in the mesectoderm also marks a boundary, namely that between the two halves of the embryo and between mesoderm and neuroectoderm. It is possible that the labeling of cells in the hindgut and above the pharynx also corresponds to some unknown boundaries.

It is interesting that three potential TATA box motifs are found upstream of the 5’ end of transcribed rho sequences. Whether different start sites may be used for different components of the complex rho expression pattern remains to be determined.

In summary, during early blastoderm stages, rho may act to divide the embryo along the dorsoventral axis into longitudinal strips. Anterior–posterior information may also be integrated at this stage, because double segment modulation of the dorsoventral domain is seen shortly before ventral furrow formation. rho may subsequently function to define the first chordotonal precursor cells and still later to specify boundaries, including those defining details of the anterior–posterior axis. Thus, rho provides a striking example where a single zygotic gene is involved in interpreting both dorsoventral and anterior–posterior cues. The hypothesis that rho first functions to set up dorsoventral pattern in the blastoderm and then is subsequently required for unrelated developmental choices, such as sensory organ specification, is similar to the proposal that the segmentation genes fushi tarazu (ftz) and even-skipped (eve) are required early to elaborate anterior–posterior pattern and later to specify cells in the CNS (Doe et al. 1988a,b). Together, these data lend support to the idea that primary patterning genes are, in fact, reused later during nervous system development.

Potential function of the rho protein

The rapid RNA turnover, as evident from the dynamic rho RNA expression pattern, together with the PEST sequence found in the predicted protein sequence, suggests that the rho protein may be only transiently found in rho-expressing cells. The prediction of an integral membrane protein may imply a cell interaction role for rho. It may act as part of a receiving pathway, because most, if not all, rho phenotypes can be associated with rho expression in cells that are affected by the mutation. It is also possible that rho acts as a signal to adjacent cells. The other gene products with which rho interacts are unknown; however, there are several obvious candidates. The zygotic dorsoventral pattern must be connected in some way to the maternal-effect loci required for establishing dorsoventral cues. Another obvious set of genes with which rho may interact include zygotic genes active in neighboring longitudinal stripes, such as sim or dpp. The unexpected finding that rho and sim may be expressed in the same cells in the mesectoderm makes interaction between these two genes an inter-
esting possibility. There may be interactions between rho and other genes within the same ventralateral group as rho (i.e., S, spi, and pnt). It is worth noting in this context that double mutants of rho with pnt, but not with S or spi, have a stronger CNS phenotype than either single mutant alone, although the PNS phenotype of double and single mutants is the same (E. Bier, unpubl.). Finally, rho and other members of the ventralateral group may also interact with those genes that determine the formation of neuroblasts (e.g., the neurogenic loci). The neurogenic loci are required for the development of epidermis in the ventralateral neurogenic region, loss-of-function mutations in these genes lead to hypertrophy of neurons at the expense of epidermis (Lehmann et al. 1983). The neurogenic region overlaps the domain of action of the ventralateral genes. Interestingly, a set of transcripts within one complex neurogenic locus [Enhancer of split] [Knust et al. 1987]) are expressed during blastoderm stages in a pattern very similar to that of rho. The identification of gene products with which the putative rho protein interacts will be an important goal for future studies.

Methods

Fly stocks

All genetic markers and chromosome balancers used are described in Lindsley and Grell [1968]. The A2-3 stock was obtained from W. Engels, and the rho^TM43 allele was obtained from C. Nüsslein-Volhard.

Reversion of the lethality of the rho^jac insertion

The rho^jac insert was remobilized in the presence of a transposase source [the A2-3 chromosome [for genetic details, see Robertson et al. 1988]]. Revertants were selected by the loss or reduced expression of the P-element marker gene white. Southern blot analysis revealed that those revertants that are still homozygous-lethal retain P-element sequences. In the rho^jac mutant, a 5.0-kb segment of DNA has been deleted that includes the lacZ gene in P-lacW and 0.5-0.8 kb of flanking genomic sequences at the 5' end of the transposase—lacZ fusion gene. The white gene is still functional, although the eye color in this mutant is reduced relative to that in rho^jac flies. A significant proportion (60%) of the revertants, however, are homozygous viable and fully complement rho^TM43, unlike rho^jac. No P-element remnants were seen among the fully viable revertants. This demonstrates that the P-element insertion is the cause of the lethality and of the rho^jac mutation.

Transformation of flies with rho genomic sequences

The 8.3-kb genomic HindIII fragment (Fig. 2A) was inserted into pW8 [Klemenz et al. 1987]. Because pW8 has no HindIII site available for cloning, the genomic fragment was first subcloned into the HindIII site of the Bluescript polyn linker, flanked by XbaI and KpnI sites. This fragment was then inserted into pW8. The resulting plasmid pWRyn was introduced into the germ line of transposase (A2-3)-expressing flies by standard injection methods [Rubin and Spradling 1982]. Nine independent transformants were obtained, and one of these was analyzed in detail. Attempts to construct P elements that contain larger pieces of genomic DNA with additional sequences at the 5' end have been unsuccessful thus far.

Testing pWRyn transformants for rho function

A transformant (RynA-3) with pWRyn on the X chromosome was made homozygous [hemizygous] in rho^jac or rho^TM43 genetic backgrounds. This insertion partially rescued rho to produce viable larvae that did not, however, survive to adulthood. RynA-3/RynA-3, rho/TM3 flies were outcrossed to flies homozygous or hemizygous for RynA-3 and rho/+, progeny homozygous or hemizygous for RynA-3 were crossed to each other. Embryos were then collected on grape plates with a small amount of yeast. Embryonic lethality was scored after 36 hr at 25°C by counting the number of unhatched embryos and the number of chorions left by emerging larvae. The sum of these closely approximates the total number of eggs deposited.

Cuticles from unhatched 36-hr embryos were prepared by dechorionating embryos in 50% bleach (5.25% sodium hypochlorite), washing them in 0.7% NaCl and 0.1% Triton X-100 [NaCl/Triton], fixing in acetic acid and glycerol (4:1) at 65°C for 1 hr, and rinsing in NaCl/Triton. Embryos were then dried down on slides and mounted in Hoyer's medium. The cuticles were examined using a 20× objective lens with Nomarski optics and a micrometer scale in the eyepiece. The length of the first row of denticles in the third and fourth abdominal segments, as well as the width of the embryos between these segments, was tabulated for all embryos that were oriented with the ventral side either up or down.

Anti-β-gal staining of embryos

Embryos were dechorionated with bleach, rinsed with NaCl/Triton, and fixed with equal volumes of 4% formaldehyde in 0.1 M sodium phosphate [pH 7.2] and heptane. The embryos were then devitellinized with equal volumes of 90% methanol and heptane, rehydrated with 0.1 M sodium phosphate and 0.3% Triton X-100, and blocked with 2% bovine serum albumin. The antibody incubations and rinses were all done in the above buffer. The rabbit anti-β-gal (Cappel) was used at 1:2000, and the biotinylated goat anti-rabbit/Avidin D-HRP [Vector Laboratories, PK-4001], at 1:200. After reacting HRP with diaminobenzidine, the samples were dehydrated in ethanol, cleared in xylene, mounted on slides with Permount (Fisher), and examined under a microscope with Nomarski optics.

In situ hybridization to RNA in sections or whole-mount embryos

In situ hybridization of 35S-labeled RNA probes to cryostat sections was performed according to Vaessen et al. [1987]. In situ hybridization to whole-mount embryos was done following the method of Tautz and Pfeifle [1989], using digoxigenin-labeled probes [Boehringer–Mannheim, 1093-657] synthesized according to the Boehringer–Mannheim protocol. Briefly, embryos were fixed in 1.6 ml of 0.1 M HEPES (pH 6.9), 2 mM MgSO4, 1 mM EGTA plus 0.4 ml 20% paraformaldehyde, and 8 ml heptane with vigorous shaking for 15 min. The embryos were then treated with 90% methanol, followed by PBS plus 4% paraformaldehyde, rinsed in PBS plus 0.1% Tween-20 (PBT), and digested for 5 min with 50-100 μg/ml nonpredigested proteinase K. Digestion was stopped by the addition of 2 μg/ml glycine in PBT. Following two washes in PBT, the embryos were refixed in PBS plus 4% paraformaldehyde for 20 min and then washed five times in PBT. Embryos were then slowly
exchanged from PBT into hybridization solution [50% formamide, 5 × SSC, 100 μg/ml sonicated salmon sperm DNA, 50 μg/ml heparin, 0.1% Tween-20] and incubated for 1 hr at 45°C. To approximately 100 μl of settled embryos, 50 μl of hybridization solution containing one-tenth of a probe (made from the Boehringer–Mannheim kit) was added after denaturation at 80°C for 10 min. Hybridization was carried out at 45°C overnight. The embryos were then exchanged into PBT over the course of six 20-min washes (all at 45°C). An alkaline phosphatase-conjugated antibody to digoxygenin, which was previously preabsorbed with fixed embryos, was then applied to embryos for 1 hr (1 : 2000 dilution of the Boehringer–Mannheim preparation). The unbound antibody was then washed off, and the alkaline phosphatase reaction was carried out with nitro blue tetrazolium [NBT] and stopped by washing in PBT. Embryos were cleared with xylene and mounted with Permount.

Molecular techniques
Plasmid rescue from minipreps of fly DNA was performed according to Pirrotta (1986). Other cloning techniques followed standard procedures, as in Maniatis et al. (1982). In situ hybridization to polytene chromosomes used the chromosome squash procedure of Gall and Pardue (1971) and a protocol of hybridization with biotinylated probes [Langer-Safer et al. 1982].

Acknowledgments
We thank C. Nusslein-Volhard for providing the rho743 allele, Rolf Bodmer and Volker Hartenstein for helpful comments about the rho phenotype, Susan Shepherd and Ellsworth Grell for advice with genetics, Robert Carretto and Sandra Barbel for considerable help with tissue in situ hybridization, Denise Muhlrad for help in determining features of the genomic sequence, Larry Ackerman for unfailing assistance with photography, Temple Smith for running his computer program to make predictions based on the deduced rho protein sequence, Ed Giniger for comments on the manuscript, Katherine Prewitt for patient preparation of the manuscript, and Kathryn Burton Bier for her assistance with injection of pWRyn and the isolation of transformant lines. L.Y.J. and Y.N.J. are Howard Hughes Medical Institute investigators.

References
Anderson, K.V., L. Bokia, and C. Nüsslein-Volhard. 1985a. Establishment of dorsal-ventral polarity in the Drosophila embryo: The induction of polarity by the Toll gene product. Cell 42: 791–798.
Anderson, K.V., G. Jürgens, and C. Nüsslein-Volhard. 1985b. Establishment of dorsal-ventral polarity in the Drosophila embryo: Genetic studies on the role of the Toll gene product. Cell 42: 779–789.
Anderson, L.V. 1987. Dorsal-ventral embryonic pattern genes in Drosophila. Trends Genet. 3: 91–97.
Bellen, H.J., C.J. O’Kane, C. Wilson, U. Grossniklaus, R. Pearson, and W.J. Gehring. 1989. P-element-mediated enhancer detection: A versatile method to study development in Drosophila. Genes Dev. 3: 1288–1300.
Bier, E., L. Ackerman, S. Barbel, L.Y. Jan, and Y.N. Jan. 1988. Identification and characterization of a neuron-specific nuclear antigen in Drosophila. Science 240: 913–916.
Bier, E., H. Vaessen, S. Shepherd, K. Lee, K. McCall, S. Barbel, L. Ackerman, R. Carretto, T. Uemura, E. Grell, L.Y. Jan, and Y.N. Jan. 1989. Searching for pattern and mutation in the Drosophila genome with a P-lacZ vector. Genes Dev. 3: 1273–1287.
Bodmer, R., S. Shepherd, J. Jack, L.Y. Jan, and Y.N. Jan. 1987. Transformation of sensory organs by mutations of the cut locus of D. melanogaster. Cell 51: 293–307.
Bodmer, R., R. Carretto, and Y.N. Jan. 1989. Neurogenesis of the peripheral nervous system in Drosophila melanogaster embryos: DNA replication patterns and cell lineages. Neuron 3: 21–32.
Campos-Ortega, J.A. and V. Hartenstein. 1985. The embryonic development of Drosophila melanogaster. Springer-Verlag, Berlin.
Caudy, M., E. Grell, C. Dambly-Chaudiere, A. Ghysen, L.Y. Jan, and Y.N. Jan. 1988. The maternal sex determination gene daughterless has zygotic activity necessary for the formation of peripheral neurons in Drosophila. Genes Dev. 2: 843–852.
Cavener, D.R. 1987. Comparison of the consensus sequence flanking translation start sites in Drosophila and vertebrates. Nucleic Acids Res. 15: 1353–1361.
Crews, S., J. Thomas, and C.S. Goodman. 1988. The Drosophila single-minded gene encodes a nuclear protein with sequence similarity to the per gene product. Cell 52: 143–151.
Crossley, A.C. 1978. The morphology and development of the Drosophila muscular system. In The genetics and biology of Drosophila. (ed. M. Ashburner and T.R.F. Wright), vol. 2b, pp. 499–560. Academic press, New York.
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.
Doyle, H., K. Harding, T. Hoey, and M. Levine. 1986. Transcripts encoded by a homeo box gene are restricted to dorsal tissues of Drosophila embryos. Nature 323: 76–79.
Eisenberg, D., L. Schwartz, M. Komaromy, and R. Wall. 1984. Analysis of membrane and surface protein sequences with the hydrophobic moment plot. J. Mol. Biol. 179: 125–142.
Gall, J.C. and M. L. Pardue. 1971. Nucleic acid hybridization in cytological preparations. Methods Enzymol. 21: 470–480.
Ghysen, A. and C. O’Kane. 1989. Neural enhancer-like elements as cell markers in Drosophila. Development 105: 35–52.
Hashimoto, C., K. Hudson, and K. Anderson. 1988. The Toll gene of Drosophila, required for dorsal-ventral embryonic polarity, appears to encode a transmembrane protein. Cell 52: 269–279.
Hultmark, D., R. Klemenez, and W. Gehring. 1986. Translational and transcriptional control elements in the untranslated leader of the heat shock gene hsp93. Cell 44: 429–438.
Jan, L.Y. and Y.N. Jan. 1982. Antibodies to horseradish peroxidase as specific neuronal markers in Drosophila and grasshopper embryos. Proc. Natl. Acad. Sci. 70: 2700–2704.
Jan, Y.N., R. Bodmer, A. Ghysen, C. Dambly-Chaudiere, and L.Y. Jan. 1986. Mutations affecting the peripheral nervous system in Drosophila embryos. Proceedings of the UCLA Symposium for Molecular Entomology. J. Cell. Biochem. 45–56.
Jürgens, G., E. Weischaus, C. Nüsslein-Volhard, and H. Kluding. 1984. Mutations affecting the pattern of the larval cuticle in Drosophila melanogaster. II. Zygotic loci on the third chromosome. Wilhelm Roux’s Arch. Dev. Biol. 193: 283–295.
Klein, P., M. Chenchis, and C. Delisi. 1985. The detection and
rhomboid

classification of membrane-spanning proteins. Biochim. Biophys. Acta. 815: 468–476.
Klemenz, R., U. Wever, and W. Gehring. 1987. The white gene as a marker in a new P-element vector for gene transfer in Drosophila. Nucleic Acids Res. 15: 3947–3959.
Knust, E., K. Tietze, and J.A. Campos-Ortega. 1987. Molecular analysis of the neurogenic locus Enhancer of split of Drosophila melanogaster. EMBO J. 6: 4113–4123.
Kohl, N.E., E. Legony, R.A. DePinho, P.D. Nisen, R.K. Smith, C.E. Gee, and F.W. Alt. 1986. Human N-myc is closely related in organization and nucleotide sequence to C-myc. Nature 319: 73–77.
Kruys, V., O. Marinx, G. Shaw, J. Deschamps, and G. Huez. 1989. Translation blockade imposed by cytokine-derived UA-rich sequences. Science 245: 852–854.
Landschulz, W., P.F. Johnson, and S.L. McKnight. 1988. The leucine zipper: A hypothetical structure common to a new class of DNA binding proteins. Science 240: 1759–1764.
Langer-Safer, P.R., M. Levine, and D.C. War. 1982. Immunological methods for mapping genes on Drosophila polytene chromosomes. Proc. Natl. Acad. Sci. 79: 4381–4385.
Lemmann, 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.
Landschulz, W., P.F. Johnson, and S.L. McKnight. 1988. The leucine zipper: A hypothetical structure common to a new class of DNA binding proteins. Science 240: 1759–1764.
Langer-Safer, P.R., M. Levine, and D.C. War. 1982. Immunological methods for mapping genes on Drosophila polytene chromosomes. Proc. Natl. Acad. Sci. 79: 4381–4385.
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.
Lindsley, D.L. and E.H. Grell. 1968. Genetic variations in Drosophila melanogaster. Carnegie Institute of Washington, Washington, D.C.
Lohs-Schardin, M., C. Cremer, and C. Nüsslein-Volhard. 1979. A fate map for the larval epidermis of Drosophila melanogaster. Wilhelm Roux’s Arch. Dev. Biol. 192: 62–74.
Lindsley, D.L. and E.H. Grell. 1968. Genetic variations in Drosophila melanogaster. Carnegie Institute of Washington, Washington, D.C.
Lohs-Schardin, M., C. Cremer, and C. Nüsslein-Volhard. 1979. A fate map for the larval epidermis of Drosophila melanogaster: Localized cuticle defects following irradiation of the blastoderm with an ultraviolet laser microbeam. Dev. Biol. 73: 239–255.
Maniatis, T., E.F. Fritsch, and J. Sambrook. 1982. Molecular cloning: A laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.
Mayer, U. and C. Nüsslein-Volhard. 1988. A group of genes required for pattern formation in the ventral ectoderm of the Drosophila embryo. Genes Dev. 2: 1496–1511.
Mount, S. 1982. A catalogue of splice junction sequences. Nucleic Acids Res. 10: 459–472.
Nüsslein-Volhard, C., E. Weischaus, and H. Kluding. 1984. Mutations affecting the pattern of the larval cuticle in Drosophila melanogaster. I. Zygotic loci on the second chromosome. Wilhelm Roux’s Arch. Dev. Biol. 183: 267–282.
O’Kane, C. and W. Gehring. 1987. Detection in situ of genomic regulatory elements in Drosophila. Proc. Natl. Acad. Sci. 84: 9123–9127.
Pirrotta, V. 1986. Cloning Drosophila genes. In Drosophila, a practical approach (ed. D.R. Roberts), pp. 83–110. IRL press, Oxford, Washington, D.C.
Ralph, W.W., T. Webster, and T.F. Smith. 1987. A modified Chou-Fasman protein structure algorithm. Cabios 3: 211–216.
Rao, M.I.K. and P. Argos. 1986. A conformational preference parameter to predict helices in integral membrane proteins. Biochim. Biophys. Acta 869: 197–214.
Robertson, H.M., C.R. Preston, R.W. Phillips, D. Johnson-Schitz, W.K. Benz, and W.R. Lingels. 1988. A stable source of P-element transposase in Drosophila melanogaster. Genetics 118: 461–470.
Rogers, S., R. Wells, and M. Rechsteiner. 1986. Amino acid sequences common to rapidly degraded proteins: The PEST hypothesis. Science 234: 364–368.
Rubin, G.M. and A. Spradling. 1982. Genetic transformation of Drosophila with transposable element vectors. Science 218: 348–353.
St. Johnson, D.R. and W.M. Gelbart. 1987. decapentaplegic transcripts are localized along the dorsal-ventral axis of the Drosophila embryo. EMBO J. 6: 2785–2791.
Shaw, G. and R. Kamen. 1986. A conserved AU sequence from the 3’ untranslated region of GM-CSF mRNA mediates selective mRNA degradation. Cell 46: 659–667.
Tautz, D. and C. Pfeifle. 1989. A nonradioactive in situ hybridization method for the localization of specific RNAs in Drosophila embryos reveals a translational control of segmentation gene hunchback. Chromosoma 98: 81–85.
Thissee, B., C. Stroetzel, C. Gorostiza-Thisse and F. Perrin-Schmitt. 1988. Sequence of the twist gene and nuclear localization of its protein in endomesodermal cells of early Drosophila embryos. EMBO J. 7: 2175–2183.
Thomas, J., S. Crews, and C.S. Goodman. 1988. Molecular genetics of the single-minded locus: A gene involved in the development of the Drosophila nervous system. Cell 52: 133–141.
Uemura, T., S. Shepherd, L. Ackerman, L.Y. Jan, and Y.N. Jan. 1989. numb, a gene required in determination of cell fate during sensory organ formation in Drosophila embryos. Cell 58: 349–360.
Vaessin, H., K.A. Bremer, E. Knust, and J.A. Campos-Ortega. 1987. The neurogenic gene Delta of Drosophila melanogaster is expressed in neurogenic territories and encodes a putative transmembrane protein with EGF-like repeats. EMBO J. 6: 3431–3440.
Villares, R. and C.V. Cabrera. 1987. The achaete-scute gene complex of D. melanogaster: Conserved domains in a subset of genes required for neurogenesis and their homology to myc. Cell 50: 415–424.
Wilson, C., R. Pearson, H.J. Bellen, C.J. O’Kane, U. Grossniklaus, and W.J. Gehring. 1989. P-element-mediated enhancer detection: An efficient method for isolating and characterizing developmentally regulated genes in Drosophila. Genes Dev. 3: 1310–1313.
rhomboid, a gene required for dorsoventral axis establishment and peripheral nervous system development in Drosophila melanogaster.

E Bier, L Y Jan and Y N Jan

Genes Dev. 1990, 4:
Access the most recent version at doi:10.1101/gad.4.2.190

References
This article cites 48 articles, 13 of which can be accessed free at:
http://genesdev.cshlp.org/content/4/2/190.full.html#ref-list-1

License

Email Alerting Service
Receive free email alerts when new articles cite this article - sign up in the box at the top right corner of the article or click here.