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Stripe-specific regulation of pair-rule genes by hopscotch, a putative Jak family tyrosine kinase in Drosophila

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We describe the characterization of the Drosophila gene, hopscotch (hop), which is required maternally for the establishment of the normal array of embryonic segments. In hop embryos, although expression of the gap genes appears normal, there are defects in the expression patterns of the pair-rule genes even-skipped, runt, and fushi tarazu, as well as the segment-polarity genes engrailed and wingless. We demonstrate that the effect of hop on the expression of these genes is stripe-specific. The hop gene encodes a putative nonreceptor tyrosine kinase of the Janus kinase family, based on an internal duplication of the catalytic domain. We present a model in which the Hop tyrosine kinase is involved in the control of pair-rule gene transcription in a stripe-specific manner. Our results provide the first evidence for stripe-specific regulation of pair-rule genes by a tyrosine kinase.

[Key Words: Drosophila; nonreceptor tyrosine kinase; pair-rule genes; pattern formation; segmentation]

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The basic segmental body pattern of Drosophila melanogaster is established during embryogenesis. Detailed genetic and molecular analyses have shown that the process of segmentation results from a dynamic pattern of gene interactions controlled in a precise way both spatially and temporally [for review, see Ingham 1988]. Generation of the proper array of embryonic segments involves a complex interplay between maternal and zygotic gene products. In a hierarchical model for the activities of these genes, the maternal effect genes act initially to set up the axial polarity of the egg [Meinhardt 1986, Nüsslein-Volhard et al. 1987]. Subsequent zygotic gene expression and combinatorial interactions between the resulting gene products subdivide the embryo into successively smaller domains [Nüsslein-Volhard and Wieschaus 1980], which then become uniquely specified by the products of the homeotic genes [Lewis 1978].

Systematic screens for mutants with altered patterns of the embryonic cuticle have identified a number of genes involved in segmentation [for review, see Akam 1987]. Maternal genes set up the anteroposterior and dorsoventral coordinates of the embryo [for review, see Nüsslein-Volhard et al. 1987, Manseau and Schupbach 1989, St. Johnston and Nüsslein-Volhard 1992]. Mutations in these genes delete pattern elements from restricted regions of the embryo by altering the coordinates along the anteroposterior axis. In these mutant embryos, structures deleted from one region of the embryo are replaced by structures from adjacent regions. The zygotic segmentation genes determine the proper number and orientation of segments and fall into three classes [Nüsslein-Volhard and Wieschaus 1980]: Mutations in gap genes delete multiple adjacent segments, those in pair-rule genes delete each alternate segment, and those in segment-polarity genes delete part of each segment, often replacing the missing region with a mirror-image duplication of the remainder of the segment.

Three maternal systems (anterior, posterior and terminal) have been defined that control pattern along the anteroposterior axis [for review, see St. Johnston and Nüsslein-Volhard 1992]. However, mutations exist with specific maternal effects on embryonic segmentation that do not belong to any one of these classes [Perrimon et al. 1989]. One such gene is hopscotch [hop], an X-linked larval/pupal zygotic lethal mutation [Perrimon and Ma­ howald 1986]. When analyzed in homozyous germ-line clones, hop embryos show specific segmentation defects. The most prominent aspect of this phenotype consists of the deletion of the fifth abdominal segment and the posterior mid-ventral portion of the fourth abdominal segment.

Given its unique maternal effect phenotype, we have undertaken a phenotypic and molecular analysis of hop. Analysis of the expression patterns of zygotic segmentation genes in hop germ-line clone-derived embryos indicates that the initial defect appears to be at the level of expression of specific stripes of the pair-rule genes. The cuticular defects observed in hop embryos can be accounted for by the subsequent misexpression of the segment-polarity genes engrailed (en) and wingless (wg). We have cloned the hop locus and show that hop encodes a nonreceptor tyrosine kinase with a structure similar to
that of the Janus [Jak] family of tyrosine kinases. Hop is thus the first example of a maternally provided nonreceptor tyrosine kinase involved in segmentation of the Drosophila embryo.

Results

hop is a novel maternal segmentation gene

Hop is an X-linked locus that maps to chromosomal bands 10B6-8 (Perrimon and Mahowald 1986). Among the 28 hop mutations that we have characterized, 27 behave as strong loss-of-function mutations [hopmut]. hop is required for zygotic viability because hemi- and homozygous hopmut animals die as late larvae or early pupae (Perrimon and Mahowald 1986). The dead larvae have a normal cuticle pattern, but all larval diploid imaginal embryos, as well as rescued embryos, consist of a deletion of the fifth abdominal denticle belt and the posterior mid-ventral portion of the fourth abdominal denticle belt (Perrimon and Mahowald 1986). Additional defects in the thoracic segments and the head and tail regions, as well as fusions of the sixth and seventh abdominal segments, can be seen in hop null embryos. The cuticular defects that result from a lack of maternal hop activity do not resemble any of the phenotypes associated with the three maternal systems that operate along the anteroposterior axis because these defects cannot be associated with a general rearrangement of the body plan (see introductory section).

hop activity is required for normal pair-rule gene expression

Reasoning that the generation of the hop phenotype should be reflected in altered expression of one or more of the zygotic segmentation genes, the expression patterns of a number of these genes were analyzed in hop embryos. No distinction was made between null and rescued hop embryos in these experiments. Our analysis begins with the gap genes, which are the first zygotic segmentation genes to be expressed. We found that the expression patterns of the gap genes hunchback [hb] (Tautz 1988), giant [gt] (Kraut and Levine 1991), Krüppel [Kr] (Gaul et al. 1987), and knirps [kni] (Rothe et al. 1989) are not affected in hop embryos (Fig. 1). Although the expression patterns of the terminal gap genes tailless [tll] and huckebein [hkb] (Pignoni et al. 1990, Weigel et al. 1990) were not analyzed, we believe that they are normal because we do not see any alteration in either the number of fushi tarazu [ftz] stripes or the position of the posteriormost ftz stripe (see below). Mutations in tll and hkb alter the expression of the posterior stripe of ftz (Weigel et al. 1990).

We then analyzed the expression of the three pair-rule genes runt [run] (Gergen and Butler 1988, Kania et al. 1990), ftz [Hafen et al. 1984], and even-skipped [eve] (Macdonald et al. 1986). Figure 2 shows the expression patterns of these genes in wild-type and hop embryos. Unlike the case for the gap genes, the expression of all three pair-rule genes is abnormal in hop embryos relative to wild type.

The most notable defect in run expression in hop embryos is the almost complete loss of the fifth stripe of expression (Fig. 2, cf. A1 and A2). In addition, the remaining stripes (with the exception of the first and seventh) are also defective to varying degrees, with the dorsal region of the stripe affected more severely than the ventral region. Despite the almost complete loss of the fifth stripe, the borders of the fourth and sixth stripes appear to be maintained in their normal positions. In addition, the spacing between the remaining stripes also appears to be normal.

Unlike the case for run, the defect in ftz expression in hop embryos is more subtle and consists mainly of a slight decrease in expression of the fifth stripe (Fig. 2, cf. B1 and B2). As with run, this effect is more pronounced dorsally than ventrally. This defect in the fifth ftz stripe becomes more pronounced with time, such that hop embryos at later stages show an almost complete loss of this stripe (data not shown). In addition to the effect on stripe five, there appears to be an increase in the width of the sixth stripe toward the posterior of the embryo, such that the spacing between the sixth and seventh stripes is slightly decreased.
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The expression pattern of eve in odd-numbered parasegments is completely complementary to that of ftz in even-numbered parasegments at the blastoderm stage (Lawrence and Johnston 1989). The most prominent defect in eve expression in hop embryos is a decrease in the fifth stripe, with the defect more pronounced dorsally than ventrally (Fig. 2, cf. C1 and C2). There are also defects in the expression of additional eve stripes, most notably the third and sixth stripes. As was seen for run expression, the decrease in eve expression in particular stripes does not appear to affect the borders of the stripes or to change the spacing between stripes.

**Segment-polarity gene expression is abnormal in hop embryos**

In addition to defects in pair-rule gene expression, hop embryos also show defects in the expression of the segment-polarity genes en and wg, both of which are normally expressed in individual parasegments (Fjose et al. 1985; Kornberg et al. 1985; Baker 1987). The most pronounced and consistent defect in hop embryos is the almost complete loss of the tenth stripe of en expression and the ninth stripe of wg expression (data not shown). Almost all of the lateral staining in these stripes is missing, with residual staining only in those cells at the midline of the stripes. In addition, the remnants of these stripes have shifted posteriorly. These two stripes correspond to parasegments 9 (wg) and 10 (en), which is the region where we detect defects in the expression of the fifth stripes of both eve and ftz. The cuticle defect in this region of hop embryos is thus an accurate reflection of the misexpression of both en and wg.

**The effect of hop on eve stripe three expression is mediated through a 500-bp element of the eve promoter**

To determine whether the effect of hop on pair-rule gene expression is stripe specific, stripe-specific reporter gene constructs for the eve promoter (Goto et al. 1989; Harding et al. 1989) were employed. Because a stripe-specific element for eve stripe five has not yet been identified, the expression of the third eve stripe as driven by an eve promoter–lacZ fusion construct was analyzed. The construct used for analysis (eve5.2/lacZ) (Goto et al. 1989) contains ~5.2 kb of DNA from the proximal eve promoter fused to β-galactosidase, such that β-gal is expressed in the normal positions of the second, third, and seventh eve stripes. Stripes two and seven, which are not affected in hop embryos, thus act as internal controls for changes in stripe three expression. The wild-type pattern of β-gal expression of this construct is seen in Figure 2D1, whereas the pattern seen in hop embryos is shown in Figure 2D2. In hop embryos the β-gal expression corresponding to the second and seventh eve stripes is normal while that corresponding to the third stripe is almost completely missing. A similar result was obtained using a construct containing only a 500-bp fragment from the eve promoter [M. Levine and S. Small, pers. comm.], which drives the expression of only stripe 3.
[data not shown]. These results indicate that the effect of hop on eve stripe 3 expression is mediated through those factors that normally activate this particular promoter element for stripe 3 expression.

Cloning of the hop locus

To clone the hop locus, a chromosomal walk in the 10B6-8 region of the X chromosome was initiated using as an entry point a bacteriophage from the distal end of a walk generated in the cloning of the discs-large (dlg) locus [Woods and Bryant 1989]. With this phage as a starting point, overlapping phage and cosmid clones were isolated that encompass ~60 kb of wild-type chromosomal DNA spanning the distal breakpoints of Df(1)DA622 and Df(1)N71 (Fig. 3A). Using these cloned phages as probes, Southern blots of genomic DNA from several deficiencies and a number of hop alleles were analyzed (Fig. 3B). Hybridization with phage 1-2 enabled us to determine that we had crossed the distal breakpoint of Df(1)DA622, a deficiency that delimits hop from the next proximal lethal locus dlv. The next overlapping phage in the distal direction (phage 2-3) detects a small deficiency of ~300 bp in genomic DNA of the X-ray-induced allele hopC111, which was further mapped to a 3.5-kb SalI fragment. This same phage also detects alterations relative to wild type in the DNA of the chromosomal rearrangement GA118 that delimits hop from the next distal lethal lo-

Figure 3. Molecular organization of the hop locus. (A) Restriction map and the positions of a number of chromosomal lesions. The line above the restriction map indicates the genomic DNA absent in Df(1)DA622, with the broken portion of the line indicating uncertainty in the position of the breakpoint. The breakpoint associated with Df(1)N71 is located approximately at map position 60 (not shown). The positions of the DNA lesions associated with hopC111, hopM637, and GA118 are indicated by the brackets. The stippled box represents the SalI fragment containing the deletion in hopC111. The hop transcript is indicated by the solid arrow. The chromosomal walk was initiated using a 1.8-kb HindIII–SalI fragment from map coordinates 0–2 provided by D. Woods and P. Bryant [University of California, Irvine]. The enzymes indicated above the horizontal line are BamHI [B], HindIII [H], and SalI [S]. The map coordinates are in kilobases [kbl]. (B) To detect the positions of the lesions associated with mutations, equal amounts of DNA from heterozygous flies were digested with various restriction enzymes, blotted onto nitrocellulose, and probed with either entire phage or phage subclones from the walk. The left-most panel, probed with phage 1-2, shows the alteration associated with Df(1)DA622. The restriction fragments that are missing in Df(1)DA622 have an intensity half as great as those from homozygous yellow forked (yf) control DNA. The next two panels, hybridized with phage 2-3, show the alteration associated with hopC111. This lesion was initially mapped to a 3.5-kb SalI fragment and subsequently mapped to a 1.8-kb BamHI fragment within this SalI fragment. The next two panels indicate alterations in hopM637 relative to the viable dysgenically-induced revertant hopM637rev+. The left-most of these two panels was hybridized with phage 2-3, whereas the right-most one was hybridized with the 2.3-kb SalI fragment from phage 2-3. The final right-most panel, hybridized with phage 2-3, indicates the alteration associated with the rearrangement breakpoint GA118. These data (and other restriction data not shown) were used to map the positions of Df(1)DA622, GA118, and the hop alleles C111 and M637. (C) Developmental Northern analysis of the hop locus. A Northern blot of poly(A)+ RNA of different developmental stages was hybridized with phage 2-3 and the 3.5-kb SalI genomic fragment. In this exposure the 5.4- and 5.1-kb transcripts are not easily resolved from each other, with a lighter exposure these two transcripts are easily distinguishable from each other. Phage 2-3 detects two additional transcripts of 1.8 and 0.9 kb in size that are not detected with either the 3.5-kb SalI fragment or the hop cDNA. We believe that the larger of these two transcripts lies distal to hop because it is also detected by restriction fragments derived from phage 4-1. As yet, we do not know the origin of the 0.9-kb transcript. The embryonic stages are denoted in hours after egg deposition, the other stages are first (1st) and third (3rd) instar larvae, 24- to 96-hr pupae (MP), 96- to 120-hr pupae (LP), and adult. The lower panel shows the same Northern blot hybridized with an actin 5C probe to quantify the amounts of RNA present.

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hop is expressed throughout development

A developmental Northern analysis using phase 2-3 detects four transcripts of ~5.4, 5.1, 1.8, and 0.9 kb in phase [Fig. 3C]. The 3.5-kb SalI genomic fragment from phase 2-3 that detects a small deletion in genomic DNA of hopC114 hybridizes to only the 5.4- and 5.1-kb transcripts. The larger of these two transcripts is present at all developmental stages analyzed, whereas the smaller transcript is strictly maternal. This temporal pattern of expression is consistent with the known developmental defects associated with hop, because the maternal effect predicts expression during oogenesis and presynaptic blastoderm while the zygotic lethality characterized by hop alleles fall within the same genomic fragments to which we have mapped the putative hop cDNA (see below).

hop encodes a putative nonreceptor tyrosine kinase

We determined the nucleotide sequence of 5-1 (5068 bp) [Fig. 4B]. The conceptualized open reading frame begins with an ATG at position 620 and extends 3531 nucleotides to position 4150, encoding a putative protein of 1177 amino acids [Fig. 4B]. The nucleotides immediately upstream of the putative initiation codon (TCTC) are not a good match to the consensus for Drosophila translation start sites [C/A][C/A][C/A][C/A] [Cavener 1987]; however, there is another potential in-frame initiation codon at position 812 with a much better match to the consensus sequence (CAAC). A 3'-untranslated region of 911 nucleotides ends in a tract of 16 As, 25 bp downstream of the consensus polyadenylation recognition sequence AATAAA [Brinster et al. 1985]. Nucleotide sequencing of genomic DNA corresponding to the cDNA sequence shows that hop is comprised of 10 exons, ranging in size from 161 to 1675 nucleotides, and extending over ~7.5 kb [Fig. 4A].

A search of the available data bases indicates that hop encodes a protein with significant homology at its carboxyl terminus to the catalytic domain of tyrosine kinases [Fig. 5A]. A hydrophobicity analysis shows no obvious signal sequence or membrane-spanning domain, suggesting that Hop is a cytoplasmic protein rather than a transmembrane receptor protein. However, there is a short sequence [KKAKRR, amino acids 315–320] that resembles previously described nuclear localization signals [Richardson et al. 1986; Wychowski et al. 1986; Lyons et al. 1987], suggesting that Hop may translocate to the nucleus.

The putative catalytic domain of Hop shows the highest similarity to the catalytic domains of Elk, Jak1, and Fes [Fig. 5A] with the degree of identity ranging from 38% [Fes] to 42% [Elk]. Elk encodes a rat brain-specific receptor tyrosine kinase belonging to the eph subfamily [Lhotak et al. 1991]. Jak1 is the prototype of the Jak (or Janus kinase) subfamily of nonreceptor tyrosine kinases and was originally isolated from a murine hematopoietic cell line [Wilks et al. 1991]. Fes is the transforming protein of the Gardner–Arnstein and Snyder–Theilin strains of feline sarcoma virus [Hampe et al. 1982]. Within the catalytic domain, Hop shows a greater degree of identity in those regions that mediate nucleotide binding [subdomains I and VII] and distinguish tyrosine kinase catalytic domains from those of serine–threonine kinases [subdomains VI and VIII] [Hanks et al. 1988].

Hop belongs to the Janus family of nonreceptor tyrosine kinases

In addition to a putative tyrosine kinase catalytic domain, the deduced Hop protein contains a region of internal homology such that residues 528–864 show 22% identity to residues 864–1177 within the kinase domain. This internal homology resembles that found in members of the Jak family of tyrosine kinases. This family consists of the nonreceptor tyrosine kinases Jak1 [Wilks et al. 1991], Jak2 [Harpur et al. 1992], and Tyk2 [Firmbach-Kraft et al. 1990], all of which contain a second kinase-related domain in addition to a more canonical tyrosine kinase domain. Like Hop, members of the Jak family lack SH2 and SH3 domains, one or both of which are found in all other nonreceptor tyrosine kinases described to date [Koch et al. 1991].

The two kinase domains of Jak family members show varying degrees of homology both within and between individual proteins. Figure 5B shows a schematic diagram comparing Hop with the other members of the Jak family. Although the canonical kinase domain of Hop is
39% identical to those of Jak1, Jak2, and Tyk2, the kinase-like domain of Hop is 27% identical to that of Jak1 but only 21% identical to that of Tyk2 and 24% identical to that of Jak2. In the carboxy-terminal half of the protein Hop would thus be somewhat more closely related to Jak1 than to either Jak2 or Tyk2. However, across the length of the entire protein Hop shows the highest degree of identity to Jak2 (27%) (see Fig. 5B).

Discussion

The zygotic lethal mutation hop of Drosophila shows a specific defect in segmentation when analyzed in embryos derived from homozygous germ-line clones. The major defect involves the fourth and fifth abdominal segments, although other regions of the embryo can be variably affected. We have analyzed the expression of a number of zygotic segmentation genes in hop embryos. Although expression of the gap genes appears normal, we find defects in the expression of specific stripes of pair-rule genes. We have cloned hop and show that it is a nonreceptor tyrosine kinase belonging to the Janus family of tyrosine kinases. Hop is thus the first maternally provided nonreceptor tyrosine kinase involved in embryonic segmentation in Drosophila.

Segmentation gene expression in hop embryos

The analysis of the expression patterns of segmentation genes in embryos mutant for other patterning genes has been a fruitful approach for determining both the hierarchy of expression and the cross-regulatory interactions between various gene products. In our analysis of zygotic segmentation gene expression in hop embryos, we have found that the protein expression patterns of the gap genes hb, gt, Kr, and kni appear to be normal. It is possible that one or more gap gene proteins are not posttranscriptionally regulated in hop embryos. However, we do not believe this to be the case, because altered activity of one or more gap genes should be reflected in changes in the spatial domains of other segmentation genes (Jäckle et al. 1992 and references therein). In contrast to the expression of the gap genes, the expression patterns of several of the pair-rule and segment-polarity genes are abnormal in hop embryos. The pair-rule genes run, eve, and ftz all show defects in levels of expression in hop embryos. In each case, the defects are confined to one, or a subset, of the normal seven stripes. In no case are all of the stripes affected. In addition, the positions and borders of both the affected and unaffected stripes are maintained normally in hop embryos (with the possible exception of the posterior border of the sixth ftz stripe). The fact that the positions and borders of pair-rule gene expression are normal in hop embryos is a further indication that the activities of the gap genes are unaffected in these embryos, because the striped domains of pair-rule gene expression are normally set up by the activities of the gap genes (for review, see Pankratz and Jäckle 1990).

The effect of hop on the expression of the eve stripe-specific reporter constructs may enable a dissection of these regulatory components functioning through the elements that are directly or indirectly affected by the loss of maternal hop gene product. In this regard it would be of interest to identify the factors involved in directing proper expression of eve stripe three as mediated through these promoter elements; one or more of these factors would then be candidates for direct or indirect targets of hop. Stripe-specific elements for run would allow a similar analysis of the role of hop in activating specific stripes of run expression (P. Gergen, pers. comm.).

The segment-polarity genes en and wg are required for patterning of both the denticles and the region of naked cuticle in each segment (for review, see Peifer and Bejsovec 1992). Their expression patterns are initiated in the early embryo by the activity of a number of the earlier-acting pair-rule genes (DiNardo and O’Farrell 1987; Ingham et al. 1988). Because en and wg are required for intrasegmental patterning, any cuticular defect should be reflected in abnormal expression of these genes. In hop embryos both en and wg show defects in expression (data not shown). This is not unexpected and is consistent with both the cuticular defects and the misexpression of pair-rule genes in hop embryos. Because pair-rule genes are required for initiating the 14-stripe complement of en expression in the posterior compartment of each segment, defects in their expression would be expected to generate abnormal expression of en. This...
Within particular segments and subsequent defects in expression in this parasegment is defective. This may be attributable to the fact that run expression in this parasegment is also abnormal. Because eve negatively regulates run, which negatively regulates en, the lower levels of expression of both these gene products in parasegment 9 would lead to normal en expression (Manoukian and Krause 1993).

Hop as a nonreceptor tyrosine kinase

The putative Hop protein has significant homology at its carboxyl terminus to the catalytic domain of tyrosine kinases. More specifically, Hop is a new member of the Janus family of nonreceptor tyrosine kinases. Several members of this family have recently been shown to be involved in mediating signal transduction through the interferon α, erythropoietin, and growth hormone receptors (Velasquez et al. 1992; Witthuhn et al. 1993; Argetsinger et al. 1993). As a tyrosine kinase, Hop could act in a signal transduction pathway whose ultimate function is the correct expression of particular stripes of pair-rule genes. In this regard, another member of the Jak family of nonreceptor tyrosine kinases has been implicated in mediating signal transduction through the interferon α, erythropoietin, and growth hormone receptors (Velasquez et al. 1992). The proteins comprising this complex have been shown to require tyrosine phosphorylation for activity and may interact directly with an interferon α-induced cytoplasmic tyrosine kinase via interactions.
Mechanism of Hop function in the early embryo

The hop cuticular phenotype does not allow the characterization of hop as a member of any of the known segmentation gene classes. The aperiodicity of the segmental defects suggests that the hop gene product may only be required in specific regions of the embryo and thus act in a segment-specific manner. Because expression of hop mRNA is ubiquitous throughout the embryo, it is possible that the maternal hop product has as one of its downstream target genes a zygotic segmentation gene whose expression is localized. Lack of activation of this spatially localized target gene in hop embryos would then lead to localized defects in the segmentation pattern. All of the zygotic segmentation genes so far analyzed show spatially restricted patterns of expression (Akam 1987; Ingham 1988), with the regions of cuticle that are defective in mutant embryos showing a close correspondence with the relevant expression domains. In this respect the phenotype of the zygotic lethal mutation unpaired [upd] is of considerable interest (Gergen and Wieschaus 1986). upd embryos die with a segmentation defect extremely similar to that seen in hop embryos. One could thus imagine that upd may be a target of hop. However, any possible interaction between hop and upd remains to be determined, as upd has not yet been characterized at the molecular level.

Our results indicate that maternal hop product is required for the proper levels of expression of particular stripes of pair-rule genes. The maternal activity of hop is in contrast to that of the tramtrack gene, which has been shown to be a maternally provided repressor of pair-rule genes in the preblastoderm embryo (Brown and Wu 1993). We propose a model in which hop is required to activate a subset of the stripes of several pair-rule genes [Fig. 6]. It is known that activation of specific pair-rule stripes by the gap genes is attributable to different combinations of gap gene proteins acting on individual stripe-specific promoter elements upstream of the pair-rule genes (Fankrat et al. 1990; Stanoevich et al. 1991). For example, the expression of eve stripe two is attributable to a combination of overlapping activators and repressors, with the bcd and hb proteins mediating activation, whereas the Kr and gt proteins determine the borders of the stripe through repression (Small et al. 1991, 1992). In the case of eve stripe 3, the anterior and posterior borders are set through repression by the hb and kni proteins, respectively, whereas the stripe is activated by unknown activators, one or more of which may be gap gene proteins. The final result is the expression of eve to its wild-type levels in both stripes two and three (black boxes). In hop embryos, stripe two is unaffected by lack of Hop. Stripe three, however, is unable to be activated to wild-type levels in the absence of Hop (stippled box).

Figure 6. Model of Hop function. In wild type, expression of eve stripe 2 is regulated by overlapping activators [Bcd and Hb] and repressors [Gt and Kr]. Stripe 3 is negatively regulated through repression by Hb and Kni, and is activated by Hop and other unidentified activators, some of which may be gap gene products. The final result is the expression of eve to its wild-type levels in both stripes two and three (black boxes). In hop embryos, stripe two is unaffected by lack of Hop. Stripe three, however, is unable to be activated to wild-type levels in the absence of Hop (stippled box).
reflected in defects in pair-rule gene expression only in those stripes requiring the activity of the target protein. An alternative model postulates that hop is a component of a more general transcriptional activation system. In hop embryos, specific stripes of pair-rule gene expression may be more affected than others, reflecting the fact that other activators cannot compensate for lack of Hop function in these stripes. For example, eve stripe two would be unaffected by loss of Hop because of the presence of the strong activators Bcd and Hb, whereas expression of eve stripe 3 would be greatly diminished because of the lack of strong activators other than Hop. It will be of interest to determine both the activator[s] and the targets of hop in the early embryo, because identification of the components of this pathway may lead to novel insights into the process of embryonic segmentation in Drosophila and mechanisms of tissue-specific transcriptional regulation in eukaryotes.

Materials and methods

Genetic strains

hop has been mapped to polytene bands IGB6-8 on the X chromosome, between the distal breakpoints of Df(1)DA622 and Df(1)N71. Its meiotic map position of 34.61 places it between dsb at 34.50 and dig at 34.82. In this paper we only characterized the loss-of-function allele hop<sup>-1</sup>, an X-ray-induced allele generated by G. Lefevre [Perrimon and Mahowald 1986]. The molecular lesion associated with this allele is described in the text. The allele hop<sup>M637</sup> was isolated in a P-element-mediated mutagenesis for X-linked lethal mutations exhibiting melanotic tumor formation [Watson et al. 1989]. The lesion generating this allele was shown to be attributable to the insertion of a P element by reverting the mutation through a dysgenic cross with M strain females and recovering viable fertile males. We chose one of these revertants, designated M637<sup>rev-1</sup>, for molecular analysis [see text].

Production of germ-line clones

Females carrying germ-line clones of hop were generated by use of the FLP–DHS technique (Chou and Perrimon 1992). Virgin females of the genotype FM7/hop FRT<sup>101</sup> were mated with males of the genotype w ovo<sup>D1</sup> FRT<sup>101</sup>/; F38/F38. The resulting progeny were heat-shocked at 37°C for 2 hr at the third larval instar, and females of the genotype hop FRT<sup>101</sup>/w ovo<sup>D1</sup> FRT<sup>101</sup> F38/+ were examined for germ-line clones, as indicated by the presence of vitellogenic egg chambers. We chose one of these revertants, designated M637<sup>rev-1</sup>, for molecular analysis [see text].

In situ hybridizations and immunocytochemistry

In situ hybridization to whole-mount embryos using digoxigenin-labeled probes was performed according to Tautz and Pfeifle [1989], with minor modifications [U. Grossniklaus, pers. comm.]. Single-stranded antisense DNA probes were generated by use of PCR (N. Patel, pers. comm.) with the appropriate primers. Probes were prepared from plasmids containing the following sequences: eve cDNA [pS72B7, 0.9 kb of eve-coding sequence cloned into pGEM1], ftz cDNA [pGEMF3], the carboxy-terminal two-thirds of the ftz-coding sequence cloned into pGEM3], run cDNA [pTED5], the entire run-coding sequence cloned into pBSK+, lacZ-coding region [a 2.4-kb BamHI–XhoI fragment from pC48Gal; Thummel et al. 1988]. For visualization, embryos were dehydrated through an ethanol series and mounted in Euparal (Carolina Biological Supply). Embryos were analyzed and photographed on a Zeiss Axiophot microscope with Nomarski optics.

Control of pair-rule gene expression by a tyrosine kinase

Genomic DNA analysis

Overlapping phage were isolated from a Drosophila genomic library made in bacteriophage EMBL3 [Blackman et al. 1987]. Overlapping cosmids were isolated from a Drosophila genomic library made in pWE16 [Jones and Gelbart 1993]. Plaque and colony hybridizations, DNA purification and cloning, and Southern blot analysis were done as described in Sambrook et al. [1989]. Probe DNAs were <sup>32</sup>P-labeled using the random priming method [Feinberg and Vogelstein 1983].

RNA analysis

Northern blotting and probe preparation were carried out according to standard methods [Sambrook et al. 1989]. Approximately 5 µg of poly[<sup>A</sup>]<sup>+</sup> RNA per lane was fractionated on a 1% formamide agarose gel and transferred to nitrocellulose. Probe DNAs were labeled as above.

cDNA analysis

Putative hop cDNAs were isolated from a 9- to 12-hr Drosophila embryonic cDNA library made in agt11 [Zinn et al. 1988]. The cDNA insert from the largest isolate (designated 5-1) was subsequently subcloned into pBSK+ for further manipulations and analysis. Because this cDNA library was generated by use of EcoRI linkers, the three EcoRI fragments comprising this cDNA insert were hybridized to genomic DNA from the phage walk to ensure that they all derived from the same region of the genome. This was subsequently confirmed by comparison of the cDNA sequence to that of the appropriate genomic DNA subclones.

P-element-mediated germ-line transformation and rescue

A P-element transformation construct containing the hop open reading frame under the control of the hsp70 heat-inducible promoter was generated by cloning a 4.5-kb NotI–XhoI fragment from the hop cDNA into pCasPer [Thummel et al. 1988]. In addition to the entire open reading frame, this fragment also contains 100 bp of 5′-untranslated sequence and 900 bp of 3′-untranslated sequence. Germ-line transformants were generated using standard techniques [Spradling 1986]. One X-linked and one autosomal line were obtained. To rescue the germ-line clone phenotype, male transformants from the autosomal line
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were crossed to virgin females bearing germ-line clones of hop

DNA sequencing

DNA sequencing was carried out using the dideoxy chain termination method [Sanger et al. 1977] and Sequenase (U.S. Biochemical Corp.). For the cDNA sequence, templates were made for the first strand by generating nested exonuclease III deletions using the Erase-a-Base system (Promega). The second strand was sequenced by use of a combination of nested deletions and oligonucleotides to known sequence. Regions of sequence compression were resolved with deaza-guanosine and deaza-inosine in place of guanosine. The entire sequence of the cDNA was determined on both strands. For the genomic sequence, the Sall fragments of phase 2-3 that hybridized to 5-1 were subcloned into pBSK+. The ends of each genomic subclone were initially sequenced to determine their position and orientation relative to the sequence of the cDNA. The remainder of each genomic subclone was then sequenced, until the entire sequence of the cDNA could be accounted for, by use of oligonucleotides specific for the cDNA sequence and other oligonucleotides synthesized specifically to extend the sequence across intron-exon borders. The positions of the intron-exon boundaries were determined by comparison of the sequence of genomic DNA to that of the cDNA. DNA sequence analysis was carried out with the Wisconsin Genetics Computer Group sequence analysis programs (Devereux et al. 1984). GenBank and EMBL data bases were searched by use of the TFASTA, BLASTP, and WORDSEARCH programs. Alignments were generated using the BESTFIT program.

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Note added in proof

The nucleotide sequence data reported in this paper have been submitted to the GenBank data library under accession number L26975.

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