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Genes Dev. 1994 8: 300-312
Access the most recent version at doi:10.1101/gad.8.3.300

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]

Received October 13, 1993; revised version accepted December 9, 1993.

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 Mahowald 1986). When analyzed in homozygous 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 (hop^{lof}). *hop* is required for zygotic viability because hemi- and homozygous hop^{lof} 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 tissues are reduced in size, thus implying a zygotic role for *hop* in cellular proliferation. In addition to its zygotic function, *hop* is also required maternally because embryos derived from females lacking germ-line *hop* activity, referred to as *hop* embryos, die with very characteristic segmentation defects. The severity of the defects observed in *hop* embryos is dependent on the paternal contribution. The primary cuticular defect associated with both null or unrescued *hop* embryos, as well as rescued embryos, consists 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).

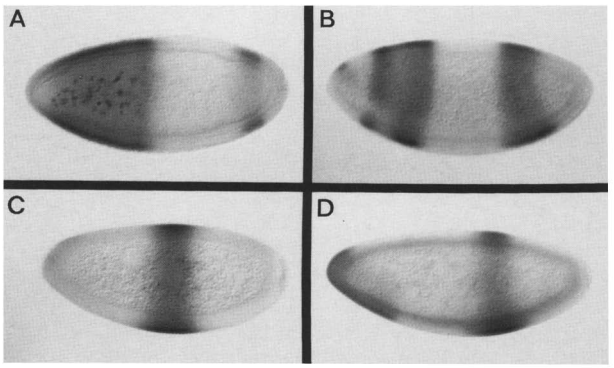


Figure 1. Gap gene protein expression in *hop* embryos. In each case, the pattern of expression appears identical to that of wild-type. (*A*) *hb* expression in a late cycle 14 embryo, showing the anterior and posterior domains, both of which have retracted from the poles. (*B*) *gt* expression in a late cycle 14 embryo, showing the broad domains of both anterior and posterior expression. (*C*) *Kr* expression in a mid-cycle 14 embryo, showing the central domain as well as the faint anterior domain. (*D*) *kni* expression in a late cycle 14 embryo, showing both the anterior and posterior domains. Embryos are oriented with anterior to the *left* and dorsal at the *top*; stages are according to Campos-Ortega and Hartenstein (1985).

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.

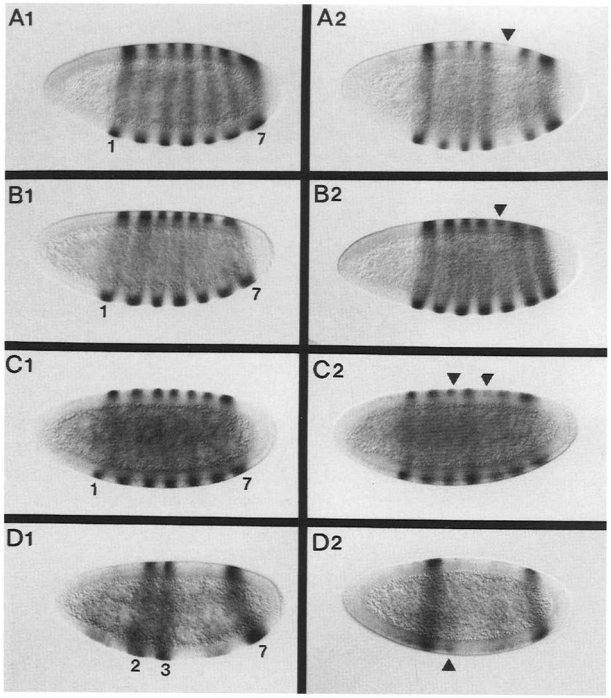


Figure 2. Pair–rule gene RNA expression in wild-type and *hop* embryos at cellular blastoderm. The pattern of *run* expression in wild-type (A1) and *hop* (A2) embryos is shown. The arrowhead in A2 indicates the almost complete loss of the fifth stripe of *run* expression, which is the most consistent defect in the *run* pattern seen in *hop* embryos. The remaining stripes, with the exception of the first and seventh, are also variably affected in this embryo. Note that the defects are more pronounced dorsally than ventrally. In addition, the borders of the stripes appear to be maintained in their proper positions. B1 and B2 show the expression pattern of *ftz* in a wild-type and *hop* embryo, respectively. Unlike the defects seen in the expression of *run*, the defect in *ftz* expression is much more subtle, and involves primarily a slight decrease in the dorsal expression of the fifth stripe (arrowhead in B2). In addition, the sixth stripe appears slightly wider at its posterior edge, such that the spacing between the sixth and seventh stripes is decreased from that in wild type. In *hop* embryos at later stages, the fifth stripe is almost completely missing (data not shown). We see the same pattern of defects in *ftz* protein expression in *hop* embryos when analyzed by anti-*ftz* antibody staining (data not shown). *eve* expression in a wild-type and *hop* embryo is shown in C1 and C2, respectively. The arrowheads in C2 indicate the third and fifth stripes, which show a decrease in intensity relative to wild type. In *hop* embryos, the fifth *eve* stripe is usually defective to a greater degree than the third stripe. In this embryo there is also a defect in the sixth stripe of expression. As with *run*, the defects are more pronounced dorsally than ventrally. We see the same pattern of defects in *eve* protein expression in *hop* embryos when analyzed by anti-*eve* antibody staining (data not shown). *lacZ* expression driven by the reporter gene construct *eve* 5.2/*lacZ* is shown in a wild-type (D1) and a *hop* (D2) embryo. In wild-type, *lacZ* expression is driven by a 5.2-kb fragment of the *eve* promoter in the pattern of the second, third, and seventh *eve* stripes. In *hop*, the *lacZ* expression corresponding to *eve* stripe three is almost completely missing (arrowhead). Embryos are oriented with anterior to the *left* and dorsal at the *top*.

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 (*eve*5.2/*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 phage 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 *dlg*. 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 hop^{C111}, which was further mapped to a 3.5-kb *Sal*I 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-

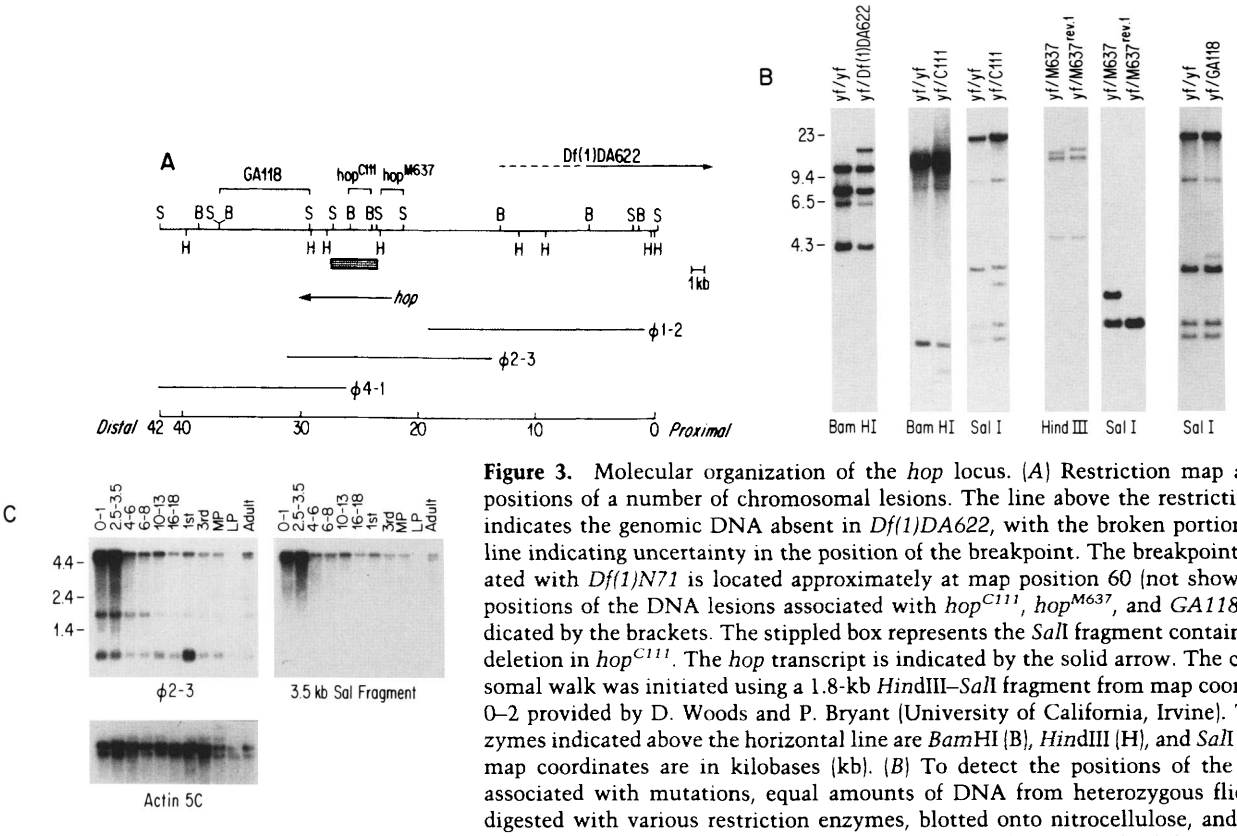


Figure 3. Molecular organization of the *hop* locus. (*A*) Restriction map and the positions of a number of chromosomal lesions. The line above the restriction map indicates the genomic DNA absent in *Df(1)DA622*, with the broken portion of the line indicating uncertainty in the position of the breakpoint. The breakpoint associated with *Df(1)N71* is located approximately at map position 60 (not shown). The positions of the DNA lesions associated with hop^{C111}, hop^{M637}, and *GA118* are indicated by the brackets. The stippled box represents the *Sal*I fragment containing the deletion in hop^{C111}. The *hop* transcript is indicated by the solid arrow. The chromosomal walk was initiated using a 1.8-kb *Hin*dIII–*Sal*I 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 *Bam*HI (B), *Hin*dIII (H), and *Sal*I (S). The map coordinates are in kilobases (kb). (*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 hop^{C111}. This lesion was initially mapped to a 3.5-kb *Sal*I fragment and subsequently mapped to a 1.8-kb *Bam*HI fragment within this *Sal*I fragment. The next two panels indicate alterations in hop^{M637} relative to the viable dysgenically-induced revertant $hop^{M637rev.1}$. 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 *Sal*I 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 *Sal*I 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 *Sal*I 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 quantitate the amounts of RNA present.

cus *dishevelled* (*dsh*) (Fig. 3B). We have used subclones of this phage to map additional alterations in genomic DNA of other X-ray- and gamma-ray-induced alleles of *hop* (data not shown), as well as the site of insertion of a P-element-induced allele (hop^{M637}). Comparison of the genomic DNA of hop^{M637} to that of a viable dysgenically induced revertant of *M637* ($M637^{rev.1}$) allowed us to position the site of insertion of the P element within the 2.3-kb *Sal*I fragment from phage 2-3 (Fig. 3B). All of the alterations detected in five other X-ray- and gamma-ray-induced *hop* alleles fall within the same genomic fragments to which we have mapped the putative *hop* cDNA (see below).

hop *is expressed throughout development*

A developmental Northern analysis using phage 2-3 detects four transcripts of ~5.4, 5.1, 1.8, and 0.9 kb in size (Fig. 3C). The 3.5-kb *Sal*I genomic fragment from phage 2-3 that detects a small deletion in genomic DNA of hop^{C111} 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 presyncitial blastoderm while the zygotic lethality characterized by small imaginal diploid structures indicates a requirement during larval/pupal development.

The 3.5-kb *Sal*I genomic fragment from phage 2-3 was used as a probe to isolate clones representing the larger zygotic mRNA from a 9- to 12-hr embryonic cDNA library. The longest clone (designated 5-1) was selected for further analysis. A developmental Northern analysis with 5-1 as probe detects both the 5.4- and 5.1-kb transcripts (data not shown), indicating that they are related to each other. In situ hybridization to whole-mount embryos with 5-1 as probe indicates that *hop* transcripts are present uniformly throughout the embryo from blastoderm through germ-band retraction (data not shown).

To demonstrate that *hop* function is encoded by 5-1, the entire open reading frame from this cDNA was cloned into the heat-inducible P-element transformation vector pCasPeR (Thummel et al. 1988). Basal levels of expression from an autosomal transformant carrying this construct provided significant rescue of the *hop* germline clone phenotype (data not shown).

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)AA(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 A's, 25 bp downstream of the consensus polyadenylation recognition sequence AATAAA (Birnstiel 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).

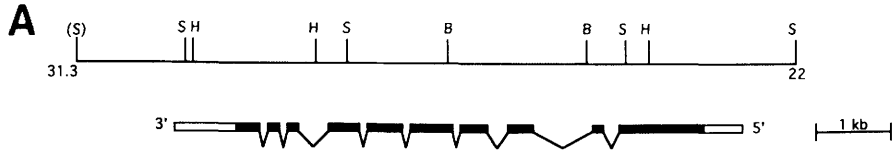
A search of the available data bases indicates that *hop* encodes a protein with significant homology at its carboxyl terminus to the catalytic domain of tyrosine kinases (Fig. 5A). A hydrophobicity analysis shows no obvious signal sequence or membrane-spanning domain, suggesting that Hop is a cytoplasmic 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



B

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1    gaattcCTGTCCGATGAATTTCACTGATATTCGTGGCTTGTATTGTAGCTTCGCAATTGCAGCTTCGTTTCGACAGCAGGCCCACTTTCAGATCGAAGGCGTCTATAATCGCCATCCAGTCCCGTCGCCCTG  132
133  TTCCCACAACGCCCCACAAATTTCGGGATCTAGCTGGCGTTTCGGGTGTCTAATTTCGGACCAGTGCACTGTGATTTCCGATTTCCAGTTTACTTTGCTGACGGATCGCCCAAGTGGCGAAGTGGTTCTTGC  264
265  TCGGAAAGTTGGCGCTTGCTAAAAGAAGGAGCAGTCCAGGTGGTTCCCCGCCAGTGGCATCAATGGAACTCGCGCTGGAGTTTGGGGCAACCTTGCAACTGCAAGGGTAAGAGCATCGCCGATTCCAGGAGC  396
397  ATTCGCAGTAAGCCCAAAAATAGCAACTCAAGTCAGTCGCGGTGGTCATTGTTATTGGCAAGAAGAAGAGGAAACGGATAGAACGCGGCAAATTCCTAGGAAAGCTCCCATGATCTAGCTTCAGTGCGGCCG  528

                                                                                                                                    ====
529  CGTAAGGAAACTGCTCCAGCCAAGGAACTGGGCTTGCAGGAGCAGCAGATAGCACCCAGCCCACAAGGGACCACTCACCGACAATCCTCTCATGGCCCTGGCCAACGGGGGAGAGGATAGAATGGACGACAG  660
                                                                                                                                    M  A  L  A  N  G  G  E  D  R  M  D  D  S

661  CAGCAGCGGACGCACTTCGCTGGCGGACAGCGCCAGCCTGACGAACTCCTCGCTGCGCAGCGGCACCTCCTCGCAGAGCATCCACACCAACGATGGCACAATCCGTGTGTTCAACTTCACCACCGGCGAATT  792
     S  S  G  R  T  S  L  A  D  S  A  S  L  T  N  S  S  L  R  S  G  T  S  S  Q  S  I  H  T  N  D  G  T  I  R  V  F  N  F  T  T  G  E  F

                ====
793  TGAGCGTTTCCATCCCAACATGCTGTGCGAGGAGATCTGCAATACGATGTGCCGCCAGCTGGGCATTGCACCAATTGCCCAGCTGCTCTACGGAATACGGGAGCACTCGACATCGCGACGTCCCTCGCCGCT  924
     E  R  F  H  P  N  M  L  C  E  E  I  C  N  T  M  C  R  Q  L  G  I  A  P  I  A  Q  L  L  Y  G  I  R  E  H  S  T  S  R  R  P  S  P  L

925  GGTGCGTTTGGATCTGACGTGGTGCCTGCCCGGCGAGCGACTCAACTGCCAGCTGGTCTATTGCTTTCGGATGCGTTTCCGTGTGCCCGAATTGGATAGCCAGCTGGAGTTGATCGATGGACGGAGCCACAA  1056
     V  R  L  D  L  T  W  C  L  P  G  E  R  L  N  C  Q  L  V  Y  C  F  R  M  R  F  R  V  P  E  L  D  S  Q  L  E  L  I  D  G  R  S  H  K

1057 GTTCCTCTACCGCCAAATGCGCTACGATATGCGGACCGAGCAAATCCCCGGAGATTCGCTATCCGGAGCACAAGGACAAATCAACGGGACTGGCCGTGATGGATATGCTAATCGACGATCAGGAACAGTCGGA  1188
     F  L  Y  R  Q  M  R  Y  D  M  R  T  E  Q  I  P  E  I  R  Y  P  E  H  K  D  K  S  T  G  L  A  V  M  D  M  L  I  D  D  Q  E  Q  S  E

1189 GGATCAGCAGGCGATGCGCTCCATCGAGAAGCTTTACAAACTGTATTTGCCACCGAGCTTGTGGCGCGCCCACAGCTTCTTTGTGGGCTCCAAGATACGTGAGGTTTTCCGCAGTCTAAAAGCGAATTCGCT  1320
     D  Q  Q  A  M  R  S  I  E  K  L  Y  K  L  Y  L  P  P  S  L  W  R  A  H  S  F  F  V  G  S  K  I  R  E  V  F  R  S  L  K  A  N  S  L

1321 GAGTGTGGAGCGTTTGAAGTGGCACTATGTGCATCAGGTATCCCATTTGGCGCCCACCTATATGACCGAACAGTTCACCTGCACCGTTCAGTATCTGCCCAACGAGGAGGTGGCCCGCGGTAGCGGACCCAT  1452
     S  V  E  R  L  K  W  H  Y  V  H  Q  V  S  H  L  A  P  T  Y  M  T  E  Q  F  T  C  T  V  Q  Y  L  P  N  E  E  V  A  R  G  S  G  P  I

1453 CGGCACCAGTCTGGCCCACTCGACGTCGACGCTGGCCAGTTCTGGATCGACCAACACTCTGTCCACGCTCACCACCAACACCAATTCCGTTGCACTCGGCGGCAGTGGCAAGAAGGCCAAACGACGTTCCAC  1584
     G  T  S  L  A  H  S  T  S  T  L  A  S  S  G  S  T  N  T  L  S  T  L  T  T  N  T  N  S  V  A  L  G  G  S  G  K  K  A  K  R  R  S  T
                                                                                                                                    *  *  *  *  *  *

1585 CAGCGGTGGAATCGATGTCTATGTGCGAGTCTTTCCACACGATTCCCTGGAGCCTGGACTCAAGGTGGCCAGGGTCACCTCCGAGGCCACACTCAAGTGGATCCTTGTTGGCGCCGTGGAAGGAATTGTTAT  1716
     S  G  G  I  D  V  Y  V  R  V  F  P  H  D  S  L  E  P  G  L  K  V  A  R  V  T  S  E  A  T  L  K  W  I  L  V  G  A  V  E  G  I  V  M

1717 GATCTCCAAAATCAACGACACCTCTGTGCGGCTGGAAATCGTTGGTCTGCCCAAGGGCTACGAGATGCAGTTTCAGACGGAGAAGGAGATGAAGTCCTTCATCTCCTACCTGGGCATCTACATCAGATTGTC  1848
     I  S  K  I  N  D  T  S  V  R  L  E  I  V  G  L  P  K  G  Y  E  M  Q  F  Q  T  E  K  E  M  K  S  F  I  S  Y  L  G  I  Y  I  R  L  S

1849 GAGCAAATGGATGCAGGATCTGTGCCATTCGTACCGCACGCCCTCCCTGGAGGAGCTGAGCTCCCTCCACTGCCATGGTCCAATCGGCGGTGCCTACTCGCTGATGAAGCTGCACGAGAACGGTGACAAGTG  1980
     S  K  W  M  Q  D  L  C  H  S  Y  R  T  P  S  L  E  E  L  S  S  L  H  C  H  G  P  I  G  G  A  Y  S  L  M  K  L  H  E  N  G  D  K  C

1981 CGGCAGCTATATTGTGCGTGAATGCGATCGAGAATACAACATATACTACATCGATATCAACACCAAAATCATGGCCAAGAAAACCGATCAGGAACGCTGCAAGACGGAGACATTTAGGATCGTGCGCAAGGA  2112
     G  S  Y  I  V  R  E  C  D  R  E  Y  N  I  Y  Y  I  D  I  N  T  K  I  M  A  K  K  T  D  Q  E  R  C  K  T  E  T  F  R  I  V  R  K  D

2113 CTCGCAATGGAAGCTAAGCTACAACAACGGCGAGCATGTACTAAACTCACTGCACGAAGTGGCGCACATCATTCAAGCGGATAGTCCCGATCGCTATCGCATACCCGCCTCCAAATATGACAAGCCGCCGCT  2244
     S  Q  W  K  L  S  Y  N  N  G  E  H  V  L  N  S  L  H  E  V  A  H  I  I  Q  A  D  S  P  D  R  Y  R  I  P  A  S  K  Y  D  K  P  P  L

2245 GCTGCTACTGCTGCTGCCAAAGAACCTCAAGGCCAAAAAGACTGACCTCCAGCTGAGCGAGGCGGAGCTGCAGCGCCGCAATCCGCAGATCTTCAATCCCCGAACGGATCTGCAGTGGTATCCAGATTCGAT  2376
     L  L  L  L  L  P  K  N  L  K  A  K  K  T  D  L  Q  L  S  E  A  E  L  Q  R  R  N  P  Q  I  F  N  P  R  T  D  L  Q  W  Y  P  D  S  I

2377 ATCGCTTAGCGACGATGCCATGATGTTTACGATGCGCGGCGATTGGATCCAACAGAGTCCCGTCAAGGATGTGTCCGTGACGATGAAAATGCTAAAGAGCGATGGCAATTTCATGGAGTTCTTTCGCTTGGC  2508
     S  L  S  D  D  A  M  M  F  T  M  R  G  D  W  I  Q  Q  S  P  V  K  D  V  S  V  T  M  K  M  L  K  S  D  G  N  F  M  E  F  F  R  L  A

2509 ACAGACCTGGAGCCTCATCCAGTCGCCGCAGTTCCTCAAGCTGTACGGCCTAACGCTCGCCGATCCGTACACAATGGTCATGGAGTATTCGCGGTATGGTCCGCTCAATAAGTTCCTGCACTCGATGCCCAA  2640
     Q  T  W  S  L  I  Q  S  P  Q  F  L  K  L  Y  G  L  T  L  A  D  P  Y  T  M  V  M  E  Y  S  R  Y  G  P  L  N  K  F  L  H  S  M  P  N

2641 TGTAACACTGCATTGTCTACTGGATCTGATGCATGGATTGGTGCGCGGCATGCACTATTTGGAGGACAACAAGATTATCCACAATTACATACGATGCAGTAATCTGTACGTGACCAAGTACGATCCGAATTC  2772
     V  T  L  H  C  L  L  D  L  M  H  G  L  V  R  G  M  H  Y  L  E  D  N  K  I  I  H  N  Y  I  R  C  S  N  L  Y  V  T  K  Y  D  P  N  S

2773 GTACGTGCTAGACGCGAAAATTAGCGATCCCGGCTATCCGCGTCCCTACCGAGAATCCGACTCGCCATGGATACCTGTTAAGTATTATCGCAATTTCGCAAGCAGCCAAAACGGATCAGTTTGCCCAGCTGTG  2904
     Y  V  L  D  A  K  I  S  D  P  G  Y  P  R  P  Y  R  E  S  D  S  P  W  I  P  V  K  Y  Y  R  N  L  Q  A  A  K  T  D  Q  F  A  Q  L  W

2905 GGCCTTCGCTACCACCATATACGAGATCTTCTCGCGCTGCAAGGAAGATCTGAGCACACTGCGCCAGGAGCAGCTACTCAGGCAGAAAAATCTCGATGGCAACATACTCAAGATGCTCGATCAGGACATCTG  3036
     A  F  A  T  T  I  Y  E  I  F  S  R  C  K  E  D  L  S  T  L  R  Q  E  Q  L  L  R  Q  K  N  L  D  G  N  I  L  K  M  L  D  Q  D  I  C

3037 TCCGGCGCCGATATTCGAGACAATCATGGATGGATGGTCCGATGATGAGACCAAGCGCTTCAGTCACCACGACATTTTCTCGCGTCTCAACACGATCAAGGCGGAAATTCTGCCCAATTATATGCCGCCACC  3168
     P  A  P  I  F  E  T  I  M  D  G  W  S  D  D  E  T  K  R  F  S  H  H  D  I  F  S  R  L  N  T  I  K  A  E  I  L  P  N  Y  M  P  P  P

3169 CGAGATCGCAACGAATGGAACTGGAGACGAGACCGTGATCGATAGAAGCGACATACCCTTCCTACCCTTTCCGCGTTCGAATATGTTGATGGTCATACCGCTGACCAGCGAATGTCGAGTGATCTACAACAT  3300
     E  I  A  T  N  G  T  G  D  E  T  V  I  D  R  S  D  I  P  F  L  P  F  P  R  S  N  M  L  M  V  I  P  L  T  S  E  C  R  V  I  Y  N  M

3301 GGAGAACATGATCGGGCGCGGTCATTATGGCACTGTCTACAAGGGTCATCTGGAGTTCAACGACAAGGATCAGCCGCGCGAGCAGGTTGCCATCAAGATGCTGAACACCATGCAGGTGTCGACCGATTTTCA  3432
     E  N  M  I  G  R  G  H  Y  G  T  V  Y  K  G  H  L  E  F  N  D  K  D  Q  P  R  E  Q  V  A  I  K  M  L  N  T  M  Q  V  S  T  D  F  H

3433 TCGCGAGATCGGCATCATGCGCACCCTCAGTCATCCGAACATAGTCAAGTTCAAGTATTGGGCTGAGAAGTCGCACTGCATCATTATGGAGTATCTGCAATCTGGTTCCTTCGACATTTACCTGCGCTTCAC  3564
     R  E  I  G  I  M  R  R  T  L  S  H  P  N  I  V  K  F  K  Y  W  A  E  K  S  H  C  I  I  M  E  Y  L  Q  S  G  S  F  D  I  Y  L  R  F  T

3565 GGCACCCAATCTCAATAATCCACGGCTCGTCAGCTTCGCTTTAGATATTGCAAATGGCATGAAATACTTGTCCGACATGGGACTCATCCACCGCGACCTGGCCGCCCGCAACATTTGGTGGACCACAACGG  3696
     A  P  N  L  N  N  P  R  L  V  S  F  A  L  D  I  A  N  G  M  K  Y  L  S  D  M  G  L  I  H  R  D  L  A  A  R  N  I  L  V  D  H  N  G

3697 CGATGGTGACTGCGTCAAAATCTCAGACTTCGGCCTGGCGCAATTCGCCAATTCCGATGGATATTACTATGCGAAAAGCAAGCGTGATATTCCCATTAGATGGTACTCTCCGGAGGCCATCTCCACCTGCAG  3828
     D  G  D  C  V  K  I  S  D  F  G  L  A  Q  F  A  N  S  D  G  Y  Y  Y  A  K  S  K  R  D  I  P  I  R  W  Y  S  P  E  A  I  S  T  C  R

3829 ATTCTCATCGTATTCGGATGTTTGGAGCTATGGCGTGACGCTCTTCGAAATGTTTTCGCGCGGCGAGGAGCCCAATTTGGTGCCGATCCAGACATCGCAGGAGGATTTCCTCAATCGCCTTCAGAGCGGCGA  3960
     F  S  S  Y  S  D  V  W  S  Y  G  V  T  L  F  E  M  F  S  R  G  E  E  P  N  L  V  P  I  Q  T  S  Q  E  D  F  L  N  R  L  Q  S  G  E

3961 ACGCTTAAATCGTCCGGCCAGTTGTCCCGATTTCATTTACGACCTGATGCAGCTGTGCTGGCATGCCACGCCCCGTTCGCGACCCAGTTTCGCGACCATTGTGGACATCATCACAAGGGAGGTGGCCACCAA  4092
     R  L  N  R  P  A  S  C  P  D  F  I  Y  D  L  M  Q  L  C  W  H  A  T  P  R  S  R  P  S  F  A  T  I  V  D  I  I  T  R  E  V  A  T  K

4093 GGTGACGCACCCGACAGATGGACACCAGTCGCCGCCGAATCAGCCGACGGATGCCGAGTGACTGTTGACAACTGAATGAAATCGATATACACACACAATTAGGCATAAGGTCTTTTAAGATGTTAGCATTTA  4224
     V  T  H  P  T  D  G  H  Q  S  P  P  N  Q  P  T  D  A  E

4225 AACTGTACGTTTTTAAAACAACACAAGCCAGACCCCAAAAACCACTTGACGCAATCCTTGGCTTAAAGCGCTATCGGAAGAATCACATTGACCCCCAACCAATCAATCATTCATCCATACCAAATCCGGATA  4356
4357 CACTTACGCCATCGCAAGCATTTTTGGATTCTCGCCAGATCTTAGTTTTATTTGATTGTGCTTACCACTGGCCACGCCCCCTACCTCCAATGTCATTAAGCCCACCCCTCCTCCCCCCTCTTGACAAAGCG  4488
4489 AAAGTCCACACTTTATATGATATATGTATATGTATAAACAAATTCTCAACCTACTTTGTGTTATACATTTTAGACAACTTTGTTAAATATTGTTAAGCTTGAGCTTCAATTTTTTTTTTTGTAATGCCCACAC  4620
4621 CACGCATAAACACTGAACTAAACTAAACTAAATTAATCATAACGTGTAAATAATAATTAAATTTAGTTAAGGGTTTAATCGCGAAACGGCAACACACGGTAATTTACAAAATGAATGGACGTCGACAGAATCG  4752
4753 AAAGGATGCTGCTGCGATCTGTTCTGTTCGGCGACTAAGTCGAACCGCCTAATCTACGCAAAAAAAAAAAGTAACATGTGCACATATAGTCGTAAGGTATGTCAATTAATGTTAATCAAATGCCATTTTAAC  4884
4885 CTACAATACTTCGAAACAGAAACGAATTATATATTTACAAGCTATACAAGCCACTATACAAGCCGGTATATGCACAACCACACACCCGCAGGCACAATAACTTATTATTGCTGTTAACGAGAAAGGATATTT  5016
5017 CCGAAATAAATCAATTTTTCTTACGTGTTTAAAAAAAAAAAAAAAGgaattc  5068
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Figure 4. *(See following page for legend.)*

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 post-transcriptionally 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 those regulatory components functioning through these 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

Figure 4. Molecular organization and sequence of the *hop* gene. (*A*) Molecular map of the genomic DNA encompassing the *hop* gene from the genomic walk coordinates 22 (proximal on the X chromosome) to 31.3 (distal on the X chromosome). Below the molecular map is a schematic diagram of the exon–intron structure of the genomic sequence corresponding to the cDNA sequence of the larger of the two *hop* transcripts. The sizes of the 10 exons in base pairs (reading from 5′ to 3′) are as follows: 1675; 161; 207; 320; 463; 350; 437; 182; 162; and 1099. Solid black indicates translated sequence; white indicates untranslated sequence. The direction of transcription is indicated. The enzymes indicated above the horizontal line are *Bam*HI (B), *Hin*dIII (H), and *Sal*I (S). The parentheses enclose a restriction site not present in genomic DNA but present at the junction of genomic and vector DNA in the particular walk phage used to generate this map. (*B*) Nucleotide and deduced amino acid (single-letter code) sequence of *hop*. Nine cDNAs were isolated from a 9- to 12-hr embryonic cDNA library made in λgt11 (Zinn et al. 1988). The sequence shown is that of the longest cDNA, designated 5-1 (5068 bp). Numbers refer to nucleotide position. Double dashed lines indicate the sequence flanking the potential translation start sites. Single dashed line indicates the putative polyadenylation signal. Asterisks indicate a putative nuclear localization signal. Two in-frame termination codons are indicated by the solid single lines. Lowercase sequence is the *Eco*RI site in the cloning vector. The G residue between the poly(A) tract and the 3′ *Eco*RI site is probably a cloning artifact introduced during the construction of the cDNA library.

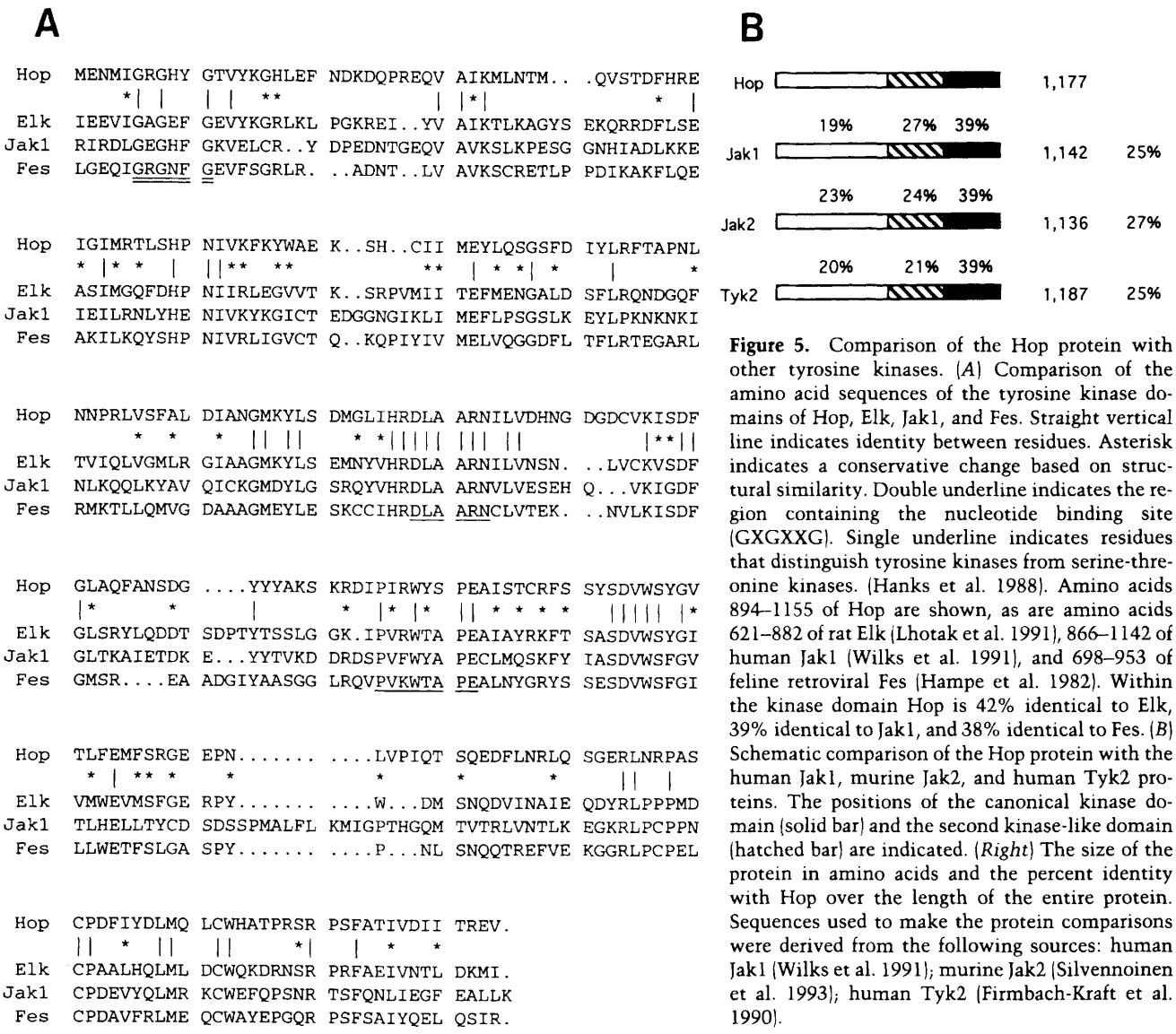

Figure 5. Comparison of the Hop protein with other tyrosine kinases. (*A*) Comparison of the amino acid sequences of the tyrosine kinase domains of Hop, Elk, Jak1, and Fes. Straight vertical line indicates identity between residues. Asterisk indicates a conservative change based on structural similarity. Double underline indicates the region containing the nucleotide binding site (GXGXXG). Single underline indicates residues that distinguish tyrosine kinases from serine-threonine kinases. (Hanks et al. 1988). Amino acids 894–1155 of Hop are shown, as are amino acids 621–882 of rat Elk (Lhotak et al. 1991), 866–1142 of human Jak1 (Wilks et al. 1991), and 698–953 of feline retroviral Fes (Hampe et al. 1982). Within the kinase domain Hop is 42% identical to Elk, 39% identical to Jak1, and 38% identical to Fes. (*B*) Schematic comparison of the Hop protein with the human Jak1, murine Jak2, and human Tyk2 proteins. The positions of the canonical kinase domain (solid bar) and the second kinase-like domain (hatched bar) are indicated. (*Right*) The size of the protein in amino acids and the percent identity with Hop over the length of the entire protein. Sequences used to make the protein comparisons were derived from the following sources: human Jak1 (Wilks et al. 1991); murine Jak2 (Silvennoinen et al. 1993); human Tyk2 (Firmbach-Kraft et al. 1990).

would lead to misspecification of pattern elements within particular segments and subsequent defects in the normal pattern of naked cuticle and denticles within the affected segments. In *hop* embryos, altered expression of the fifth *ftz* stripe precedes defects in the expression of *en* in parasegment 10. In parasegment 9, however, *en* expression appears normal, even though the expression of *eve* 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 transducing a signal from a cell-surface receptor to a transcription factor complex. The *Tyk2* gene has recently been shown to be a link between the interferon α receptor and the ISGF3α transcription factor complex (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

their SH2 and SH3 domains (Fu 1992). After phosphorylation, this complex then translocates to the nucleus, where it forms a functional transcriptional activation complex (ISGF3) by its association with an additional protein, ISGF3γ. The active ISGF3 complex then binds to the ISRE site, thus initiating transcription of interferon α-inducible genes (Levy et al. 1989; Kessler et al. 1990). In an analogous manner, Hop may phosphorylate and thus activate a component of a transcription factor complex that translocates to the nucleus and subsequently ensures the expression of particular stripes of pair–rule genes to their wild-type levels. A number of early-acting segmentation genes are phosphoproteins (Ollo and Maniatis 1987; Krause and Gehring 1989), so the involvement of protein kinases in the segmentation gene hierarchy is not without precedent.

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 (Pankratz et al. 1990; Stanojevic 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 (Stanojevic et al. 1989; M. Levine, pers. comm.). A good candidate for one of these activators is Hop, which may be present ubiquitously throughout the embryo.

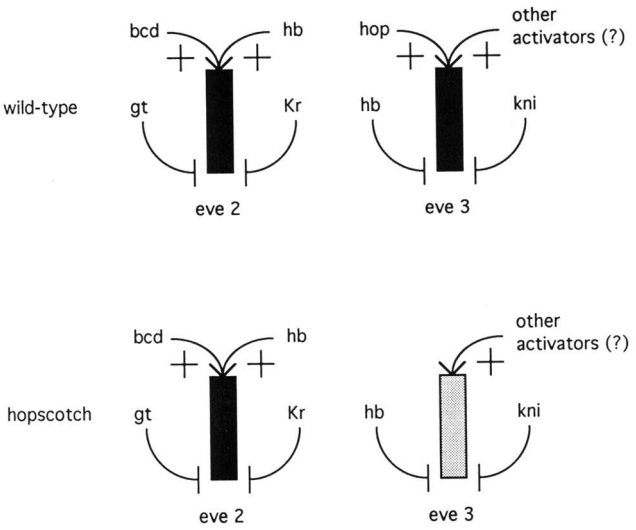

Figure 6. Model of Hop function. In wild type, expression of *eve* stripe 2 is regulated by overlapping activators (Bcd and Hb) and repressors (Gt and Kr). Stripe 3 is negatively regulated through repression by Hb and Kni, and is activated by Hop and other unidentified activators, some of which may be 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).

The mechanism by which *hop* acts as an activator of specific stripes of pair–rule gene expression remains to be determined. Like other members of the Jak family (i.e., Tyk2 and Jak2), Hop may be activated by its interaction with a membrane-bound receptor lacking a kinase domain. Such a receptor would require association with a cytoplasmic kinase to couple ligand binding to activation of a signal transduction pathway. The subsequent requirement for the function of Hop in specific regions of the embryo could then be mediated by its activation of a more spatially restricted downstream target gene, such as a transcription factor. This target gene would only be required for activation of a subset of pair–rule gene stripes. In this model the activity of the putative target gene is required to activate those pair–rule gene stripes for which the combinations or concentrations of gap gene proteins are not sufficient for stripe activation to wild-type levels, but are sufficient for determining the borders of stripe expression by repression. Loss of maternal *hop* product throughout the embryo would then be

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 10B6-8 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 *dsh* at 34.50 and *dlg* at 34.82. In this paper we only characterized the loss-of-function allele hop^{C111}, 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^{M637} was isolated in a P-element-mediated mutagenesis for X-linked lethal mutations exhibiting melanotic tumor formation (Watson et al. 1991). 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 $M637^{rev.1}$, for molecular analysis (see text).

Production of germ-line clones

Females carrying germ-line clones of *hop* were generated by use of the FLP–DFS technique (Chou and Perrimon 1992). Virgin females of the genotype $FM7/hop\ FRT^{101}$ were mated with males of the genotype $w\ ovo^{D1}\ FRT^{101}/Y$; *F38/F38*. The resulting progeny were heat-shocked at 37°C for 2 hr at the third larval instar, and females of the genotype $hop\ FRT^{101}/w\ ovo^{D1}\ FRT^{101}$; *F38/+* were examined for germ-line clones, as indicated by the presence of vitellogenic egg chambers.

In situ hybridizations and immunocytochemistry

In situ hybridization to whole-mount embryos using digoxygenin-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 (p572-B7; 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 (pED5′; the entire *run*-coding sequence cloned into pBSK+); *lacZ*-coding region (a 2.4-kb *Bam*HI–*Xba*I fragment from pC4BGal; 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.

Antibody staining to whole-mount embryos was performed as described in Smouse et al. (1988). All antibody detection was done with horseradish peroxidase using biotinylated secondary antibodies and the Vectastain Elite Kit (Vector Laboratories). For visualization, embryos were dehydrated through an ethanol series and mounted in methylsalicylate. Embryos were analyzed and photographed as above. Antibodies were as follows: rat anti-*hb* (from P. Macdonald) used at 1:800; rabbit anti-*gt* (from S. Carroll, University of Wisconsin, Madison) used at 1:500; rabbit anti-*Kr* (from S. Carroll) used at 1:200; rat anti-*kni* (from G. Struhl, Columbia University College of Physicians and Surgeons, New York) used at 1:200 after preabsorption against 8- to 20-hr-old embryos; mouse monoclonal anti-*en* (from R. Holmgren, Northwestern University, Evanston, IL) used at 1:1; rabbit anti-*wg* (from R. Nusse, Stanford University, CA) used at 1:500 after preabsorption against 6- to 16-hr-old embryos.

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 ^{32}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(A)$^+$ RNA per lane was fractionated on a 1% formaldehyde 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 λgt11 (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 *Eco*RI linkers, the three *Eco*RI 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 *Not*I–*Xba*I 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

were crossed to virgin females bearing germ-line clones of hop^{C111}.

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 *SalI* fragments of phage 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.

Acknowledgments

We thank D. Woods and P. Bryant for the phage clone that enabled us to begin our chromosomal walk; R. Blackman, R. Jones, and W. Gelbart for genomic DNA libraries; K. Zinn for the embryonic cDNA library; and K. Watson and R. Denell for hop^{M637}. In addition, we thank M. Levine, S. Carroll, H. Krause, P. Gergen, R. Lehmann, G. Struhl, P. Macdonald, R. Holmgren, and R. Nusse for various antibodies and DNAs. Special thanks go to S. Small and M. Levine for providing strains carrying the *eve* promoter–*lacZ* fusion constructs. We are especially grateful to D. Harrison for generating the germ-line transformants, to T.B. Chou for kindly providing the FLP-DFS stocks prior to publication, and to M. Levine and J. Ihle for helpful discussions. Finally, we acknowledge past and present members of the Perrimon laboratory for helpful suggestions during the course of this work. This work was supported by the Howard Hughes Medical Institute and a grant to N.P. from the National Institutes of Health.

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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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