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Drosophila center divider Gene Is Expressed in CNS Midline Cells and Encodes a Developmentally Regulated Protein Kinase Orthologous to Human TESK1

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ABSTRACT

The *Drosophila center divider* gene (*cdi*) was isolated in an enhancer trap screen undertaken to identify genes involved in embryonic central nervous system (CNS) midline cell development. Three independent lines with P-element insertions at 91F were analyzed that all showed prominent β-galactosidase expression in the CNS midline precursor cells and other cell types. Null mutations were created by imprecise P-element excision and shown to be larval lethal, although no severe CNS defects were observed in mutant embryos. The DNA surrounding the sites of insertion was cloned and found to contain a transcription unit that was dynamically expressed in a pattern corresponding to the enhancer trap line β-galactosidase expression. Sequencing of cDNA clones revealed that the *cdi* gene encodes a 1140-amino acid protein that is an ortholog of the mammalian testis-specific TESK1 protein kinase. This serine/threonine kinase is distinct from other protein kinases because of sequence differences in the residues conferring substrate specificity. The unique sequence is conserved in Cdi, suggesting that Cdi/TESK1 represents a novel class of signaling proteins.

INTRODUCTION

The function of certain proteins during embryonic development is tightly regulated by post-translational modification. Often, cell–cell interactions or extracellular morphogens trigger signal transduction cascades that lead to those alterations. Most prominent in post-translational modification during cellular signaling is protein phosphorylation. There exist a large variety of tyrosine kinases and serine/threonine kinases that catalyze protein phosphorylation, and these enzymes can be classified into subgroups on the basis of their sequence identity and functional properties.

In this paper, we describe the *Drosophila center divider* (*cdi*) gene, which encodes a protein highly related to a novel mammalian serine/threonine protein kinase. Both genes are expressed in a tissue-specific manner. Many of the kinases that function in vertebrates carry out similar roles in invertebrates. Entire signaling pathways involving phosphorylation cascades are conserved between human, *Drosophila, C. elegans*, and yeast. Different systems have unique advantages in understanding the role of these proteins in development and cell function. *Drosophila*, for example, offers the possibility of sophisticated cellular, genetic, and molecular techniques, coupled with a highly developed background in embryology.

Isolation of *cdi* emanated from two enhancer trap screens designed to identify genes that either are targets of the Single-minded (Sim) regulatory protein that controls CNS midline cell development and transcription (Crews, 1998) and plays novel roles in midline cell development (Crews *et al*., 1992) or are expressed in the developing CNS, including midline cells (Mlodzik *et al*., 1990). The CNS midline cells (Crews, 1998) constitute a small but developmentally important group of cells that separate the lateral cells within the CNS ganglia. At gastrulation, eight midline precursor cells (mesectoderm) are present. These cells divide and differentiate into 22 to 26 neurons and glia that constitute the CNS midline (Bossing and Technau, 1994). Besides forming functional glia, interneurons, and motoneurons, the midline cells use signaling to control ventral epidermal formation, proper development of adjacent lateral neuroblasts, muscle cell migration, and mesodermal development (Crews, 1998).

The Sim protein is a basic helix-loop-helix–PAS transcrip-

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tion factor (Nambu *et al*., 1991) that controls transcription and development of the CNS midline lineage. Ectodermal expression of *sim* is restricted to the CNS midline cells (Thomas *et al*., 1998). In *sim*-mutant embryos, none of the characteristic CNS midline developmental events occur, and this failure is accompanied by an absence of expression of genes that normally are transcribed in the midline (Thomas *et al*., 1988; Nambu *et al*., 1990, 1991). Instead, the midline cells of *sim*-mutant embryos adopt a lateral CNS fate (Chang *et al*., 1993; Mellerick and Nirenberg, 1995; Xiao *et al*., 1996). One important goal in understanding how master regulatory proteins control cell lineage development is identification of the genes that are transcriptional targets of these regulatory proteins. Additional insight into lineage-specific development follows through understanding of the role of the target genes.

Enhancer trap screens are useful for this purpose, because they identify genes on the basis of expression patterns (O'Kane and Gehring, 1987). For example, Sim initially functions around the time of gastrulation (Thomas *et al*., 1988; Nambu *et al*., 1991), and target genes can be identified from their expression in the CNS midline cells at this time (Klämbt *et al*., 1991; Crews *et al*., 1992). Genetic confirmation of the dependency of target gene expression on *sim* function requires showing that expression is absent in the CNS midline cells in *sim*-mutant embryos (Nambu *et al*., 1990). Once targets are identified, it is a relatively straightforward procedure to carry out molecular analysis to demonstrate that Sim directly controls their transcription and to study the function of the target gene by isolating mutations.

In this paper, we describe the initial characterization of the *cdi* gene on the basis of its prominent CNS midline cell expression and dependence on *sim* function (Nambu *et al*., 1990). The gene is dynamically expressed in a number of cell types in addition to the CNS midline cells. Sequence analysis indicates that *cdi* is a *Drosophila* ortholog of a human testis-specific serine/threonine kinase gene. The cytologic location of *cdi* is at 91F on the third chromosome. Deletion mutations encompassing the *cdi* gene were created, and homozygous mutant individuals did not survive. However, mutant embryos did not show severe CNS defects.

MATERIALS AND METHODS

Identification of enhancer trap lines

The BA01 enhancer trap line was identified in a large-scale screen carried out at UCLA designed to identify genes expressed in the CNS midline cells (Crews *et al*., 1992). The P element used for enhancer detection was P[*lacZ*; ry$^+$]A30 (O'Kane and Gehring, 1987). It contains *lacZ* coupled to the P-element promoter; *lacZ* is fused to the transposase nuclear localization sequence, resulting in nuclear β-galactosidase expression. The 87 and 242 lines were isolated by Yasushi Hiromi and Corey Goodman on the basis of CNS midline cell staining and were kindly provided to us. The 87 line used a P-element promoter, and β-galactosidase was localized to nuclei. The 242 line utilized the FZ vector, which has a *fushi tarazu* (*ftz*) promoter and results in cytoplasmic β-galactosidase (Jacobs *et al*., 1989). Both vectors contain *ry*$^+$ insertion selection markers.

Polytene chromosome and embryonic whole-mount in situ *hybridization*

The cytologic location of the *cdi* gene was identified by chromosomal *in situ* hybridization according to Langer-Safer *et al*. (1982). Polytene chromosome squashes from wild-type larvae were hybridized with a *cdi* genomic clone DNA probe, and squashes from enhancer trap larvae were hybridized with a *lacZ* probe. Salivary glands were dissected from third instar larvae, fixed, and hybridized with biotin-dUTP-labeled DNA fragments at 42°C. Hybridization was detected using horseradish peroxidase (HRP)-conjugated streptavidin and diaminobenzidine (DAB).

Embryo whole-mount *in situ* hybridization experiments were performed according to Tautz and Pfeiffle (1989). Hybridizations were carried out at 48°C using digoxygenin (DIG)-labeled DNA probes by hexamer-primed synthesis according to the manufacturer (Boehringer-Mannheim). Identification of the *cdi* transcription unit by *in situ* hybridization utilized genomic probes derived from DNA fragments d (2.5 kb) and g (2.1 kb) (see Fig. 2 below). Detailed examination of *cdi* transcription in wildtype and *cdi*^{C35} mutant embryos utilized a 4.8-kb *Hin*dIII fragment from the pC1 *cdi* cDNA clone. Hybridization was identified by incubating hybridized and washed embryos for 2 h with anti-DIG–alkaline phosphatase (1:2000), followed by an X-phosphate/PBT reaction. Embryos were examined and photographed as whole mounts or dissected filets in 80% glycerol/phosphate buffered saline.

Immunostaining of embryos

Embryos were collected, fixed, and stained using standard procedures (Patel *et al*., 1987). Enhancer trap line embryos were stained with a monoclonal antibody (Mab) against β-galactosidase (Promega) that was used at a dilution of 1:200. The CNS of mutant *cdi* embryos was examined by staining with Mabs BP102, BP104, BP106, anti-Engrailed, and 22C10 (all kindly provided by Corey Goodman) and anti-HRP. The *cdi* homozygous mutant embryos were identified by the absence of *lacZ ftz* stripe staining attributable to the presence of P[*ftz-lacZ*] on the TM3 balancer chromosome (Nambu *et al*., 1990). Stained whole-mount embryos were mounted in 100% methylsalicylate and examined and photographed with a Zeiss Axiophot microscope.

Inverse polymerase chain reaction of P-element contiguous genomic DNA

Inverse polymerase chain reaction (PCR) (Ochman *et al*., 1988) was used to clone the genomic DNA adjacent to the 87 and 242 P elements. Pairs of primer sequences corresponding to each of the P-element ends were used for PCR. Aliquots of 5 μg of genomic DNA from each strain were digested with a variety of enzymes, and the DNA was ligated overnight at 15°C in 400 μl of ligation buffer to circularize the DNA. After phenol extraction and precipitation, the DNA was resuspended in water, denatured, and added to a mix containing buffer, nucleotides, oligonucleotide primers, and *Taq* polymerase (Cetus), at the concentrations recommended, to a final volume of 50 μl. The solution was overlaid with 100 μl of mineral oil and subjected to 40 rounds of PCR under the following conditions. For

the first 10 rounds, the steps included: (1) denaturation at 94°C for 45 sec; (2) annealing at 55°C for 1 min; and (3) extension at 72°C for 2 min. For the remaining 30 cycles, the steps were: (1) denaturation at 94°C for 45 sec; (2) annealing at 62°C for 1 min; and (3) extension at 72°C for 2 min. After chloroform extraction to remove the mineral oil, the DNA was analyzed on 1.5% agarose gels. The primers used are listed below. Each primer contained 18 to 20 bp of P-element sequence with one or two restriction sites added at the ends for cloning (in parentheses in sequences below). The *Hin*dIII site (underlined) in primer C is within the P-element sequence:

(A) (*Xba*I) CGCTCTAGAATTCACTCGCACTTATTGCA (*Eco*RI);
(B) (*Eco*RI) CCAGAATTCTAACCCTTAGCATGTCCG-TG (*Eco*RI);
(C) (*Eco*RI) CACGAATTCATACTTCGGTAAGCTTCGGC (*Hin*dIII);
(D) (*Bam*HI) TAAGGATCCAATGCGTCGTTTAGAGCA-GC (*Bam*HI).

The sizes of the PCR products were checked by carrying out Southern blotting on genomic DNA restricted with the same enzymes and hybridized to P-element termini probes. The expected size of the bands on the Southern blot is the length of the corresponding PCR product plus the length of the vector sequence between the primers. *Sau*3A was the only enzyme tried that gave amplified products for both 87 and 242.

Isolation of cdi *genomic and cDNA clones*

Genomic clones containing the *cdi* gene were obtained by screening a wildtype Oregon-R λJ1 genomic library (constructed by S. T. C.) with a ^{32}P-hexamer-labeled probe derived from the inverse PCR hybridization probes described above. The *cdi* cDNA clones were isolated from a *Drosophila* 4- to 8-h embryonic pNB40 plasmid cDNA library (Brown and Kafatos, 1988) (kindly provided by N. Brown) by hybridizing with *cdi* genomic clone restriction fragment g. This probe was shown by Northern blot and *in situ* hybridization analyses to contain *cdi* transcribed sequences. Screening was carried out with a hexamer-primed ^{32}P-labeled probe.

Northern blot analysis

Poly(A)$^+$ RNA was isolated from 3-h timed collections (25°C) of embryos, as well as from larvae, pupae, and adults. Aliquots of RNA (2 μg) were electrophoresed, transferred to Nytran, and hybridized with ^{32}P-labeled probes. The probes derived from *cdi* genomic DNA fragments were labeled by hexamer-primed labeling, and RNA probes derived from a *cdi* cDNA clone were generated by SP6 *in vitro* transcription.

DNA sequencing and analysis

Nested sets of deletions were prepared from genomic and cDNA subclones in the pBluescript SK$^+$ vector (Stratagene) with the Erase-a-base system (Promega). The cDNA and genomic clones were sequenced on an Applied Biosystems automated DNA Sequenator. Both strands were sequenced using reverse, universal, T3, T7, and custom primers. Analysis of DNA sequences was done with UWGCG software package and GeneWorks (IntelliGenetics). Protein sequence relatedness searches were done using BLAST on the GenBank combined protein database. The relatedness tree comparing different protein kinase domains was carried out using the GeneWorks Unweighted Pair Group Method with Arithmetic Mean Tree program.

Generation of cdi *deletion mutants by imprecise P-element excision*

Deletions of the *cdi* gene were generated by P[ry^+; Δ2-3]99B transposase-mediated imprecise excision of the 87 and 242 P[ry^+; lacZ] lines (Robertson *et al.*, 1988). Deletions in 91F that result in lethality were identified by crossing individual *ry*/TM3 *Sb* p^p *ry e* males, which have presumably lost the P element, with *Df(3R)bxd*^{110} (91D2-92A2) *e*/TM6B *e* females and scoring for the absence of *ry*/*Df(3R)bxd*^{110} flies. This lethal class of flies can be distinguished by the absence of individuals that are both Sb^+ and have the wing phenotype of *Df(3R)bxd*^{110}. The lethal lines were maintained as balanced stocks over TM3 P[ry^+; *ftz-lacZ*] *ry*.

Complementation analysis of cdi *mutations*

Twenty-four *cdi* excision lines were tested for complementation *inter se* and also for complementation with six lethal mutants obtained from Marc Muskavitch that map left of *Delta* in 91F (Alton *et al.*, 1988). These mutants are *(3)91Fa*^{BE40}, *l(3)91Fb*^{BE50}, *l(3)91Fc*^{BE60}, *l(3)91Fd*^{BE70}, *l(3)91Fe*^{BE80}, and *l(3)91Ff*^{BE90}.

Southern blot analysis of cdi *deletion mutant breakpoints*

Mapping the breakpoints of excision deletion strains was carried out by Southern analysis. Genomic DNA from P-element excision strains was digested with a variety of restriction enzymes, blotted, and hybridized with ^{32}P-labeled probes corresponding to P-element and genomic DNA fragments. Two P-element probes, one from each end of the element, were used to determine whether the P element was completely excised. Three adjacent genomic DNA *Eco*RI fragments, as well as 0.4-kb *Nae*I-*Xho*I and 1.1-kb *Nru*I-*Eco*RI fragments, were used to map the extent of the deletions.

Determination of the cdi *lethal period*

Flies with *cdi*^{A1}, *cdi*^{C35}, *cdi*^{S59}, and *cdi*^{T13} *cdi* mutant chromosomes balanced over TM3 flies were crossed with *Df(3R)bxd*^{110}/+ flies. Embryo progeny were placed on grids overlying grape juice agar in petri dishes, and the numbers that hatched were counted. The larvae that hatched were seeded into vials, and the numbers that pupated and eclosed were counted.

RESULTS

Three enhancer trap lines with expression in the CNS midline cells have insertions at 91F

An enhancer trap screen was carried out to identify genes expressed in the CNS midline cells that both were potential tar-

gets of the Sim master regulatory protein and functioned in CNS midline cell development. The BA01 enhancer trap line displayed strong CNS midline precursor-cell expression (Fig. 1A). *In situ* hybridization of a *lacZ* probe with *Drosophila* polytene chromosomes identified the site of insertion as 91F on the right arm of the third chromosome (data not shown). Two additional enhancer trap lines, 87 and 242, that exhibited strong CNS midline staining were identified by Yasushi Hiromi and Corey Goodman and also mapped to 91F (Fig. 1B, C). The β-galactosidase staining patterns of the BA01 and 87 lines were similar and involved a variety of cell types, whereas in the 242 line, staining was seen mainly in the CNS midline cells. All three lines were fully viable when the P-element chromosomes were homozygous.

Initial β-galactosidase expression was seen in the midline precursor cells at the end of embryonic embryonic stage 9 in all three lines (Fig. 1D). This point in development is soon after initial expression of the *sim* gene, suggesting that the enhancer trap gene is a direct target of Sim. Expression of β-galactosidase continued in the midline precursor cells as they differentiated into mature midline neurons and glia (Fig. 1E, F) and remained detectable in all of the midline neurons and glia through the end of embryogenesis. The BA01 and 87 lines showed β-galactosidase expression at detectable levels in many cells in the embryo, but expression was enhanced in several cell types. These cells included anterior and posterior midgut, proventriculus, hindgut, and visceral mesoderm (Fig. 1D–H). Most cells of the epidermis is also stained (Fig. 1I).

Isolation and identification of the cdi *gene*

Hybridization probes containing genomic sequences adjacent to the sites of the 87 and 242 P-element insertions were generated by inverse PCR. These probes were used to screen a *Drosophila* genomic library, and overlapping clones representing 27 kb of contiguous genomic DNA were isolated (Fig. 2). The clones were restriction mapped and the DNA sequence obtained containing the region corresponding to the 5′ end of the longest cDNA clone (see below) and adjacent flanking sequences. The DNA sequence was also obtained adjacent to each

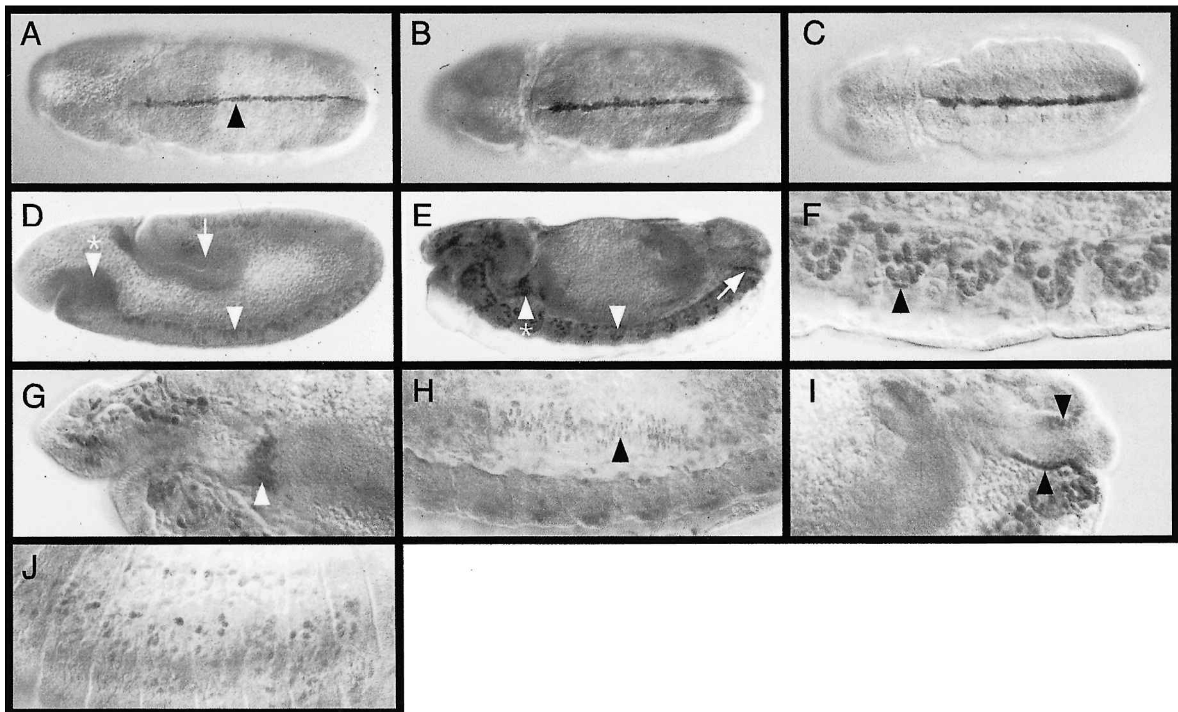

FIG. 1. Embryonic expression patterns of *cdi* enhancer trap lines. The expression of *lacZ* in the BA01, 87, and 242 P[*lacZ*] enhancer trap lines was revealed by staining whole-mount embryos with an antibody against β-galactosidase followed by HRP/DAB histochemistry. In all panels, anterior is to the left. Dorsal is at the top for sagittal views. **A**. Dorsal view of a stage 10 BA01 embryo showing midline precursor cell expression (arrowhead). **B**. Dorsal view of a stage 11 87 embryo showing midline precursor cell expression. **C**. Dorsal view of a stage 11 242 embryo showing midline precursor cell expression. **D**. Sagittal view of a stage 10 BA01 embryo showing *lacZ* expression in the CNS midline precursor cells (arrowhead), anterior midgut (arrowhead with asterisk), and posterior midgut (arrow). **E**. Sagittal view of a stage 15 BA01 embryo showing *lacZ* expression in the proventriculus (arrowhead with asterisk), CNS midline neurons and glia (arrowhead), and hindgut (arrow). **F.** High-magnification sagittal view of a stage 13 BA01 embryo showing staining in the differentiating midline neurons (arrowhead) and glia. **G**. Sagittal view of a stage 13 BA01 embryo at higher magnification showing β-galactosidase staining in the proventriculus (arrowhead). **H**. Sagittal view of a stage 13 BA01 embryo showing *lacZ* expression in the visceral mesoderm (arrowhead). **I**. Sagittal view of a stage 13 BA01 embryo showing *lacZ* expression in the hindgut (arrowheads). **J**. Sagittal view of a stage 13 BA01 embryo showing *lacZ* expression in the epidermal cells. Most cells stain, but some stain more intensely.

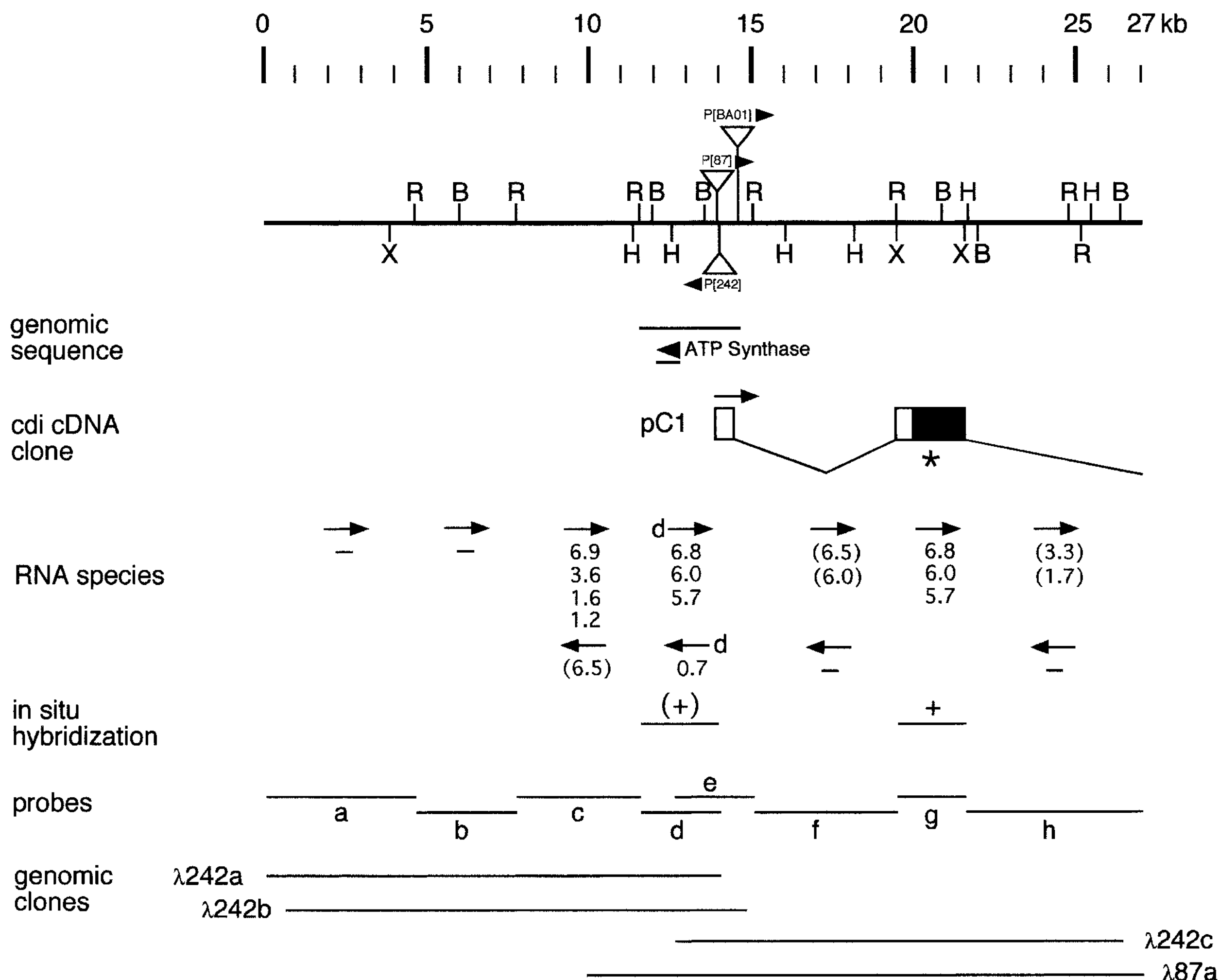

FIG. 2. Structure of the *cdi* gene. Representation of 27 kb of genomic DNA containing the *cdi* gene is shown at the top, along with the sites of the P[87], P[242], and P[BA01] P-element insertions and restriction map. The orientations (5′ > 3′) of the P elements are indicated by the direction of the arrowheads. P[87] and P[242] reside within the 5′-UTR of exon 1 of the *cdi* mRNA sequence, and P[BA01] lies within intron 1. Enzyme sites are *Bam*HI (B), *Eco*RI (E), *Hin*dIII (H), and *Xba*I (X). The extent of the genomic DNA sequenced is indicated by a line. The extent and orientation of the ATP synthase chain D ORF that lies 5′ to the *cdi* transcription unit is indicated, as is the partial genomic correspondence of the pC1 *cdi* cDNA clone. The orientation of *cdi* transcription is shown by an arrow. The location of exon 1 was determined by sequence comparison between genomic DNA and pC1. The location of additional genomic sequences corresponding to pC1 was determined by hybridization of the cDNA clone with a restriction digest of genomic clones. The extent of the cDNA clone by hybridization with fragment g is incompletely defined. Thus, more than one exon is possible in this interval, and the extent of the coding sequence is unclear. The size of the pC1 cDNA clone indicates that additional genomic sequence encoding pC1 exist 3′ to the genomic DNA analyzed. The asterisk indicates this uncertainty. Untranslated regions of pC1 are indicated by unfilled blocks; the filled region indicates the presence of coding sequence. The connecting lines between the blocks indicate intronic sequences. Seven restriction fragments (a–d, f–h) were used to make strand-specific probes for Northern analysis of embryonic and postembryonic RNA. The RNA species that each probe detected are shown by size below the arrows that indicate the orientation of the probe. Not all probes were used in both directions. (−) indicates no detectable bands of hybridization. RNA sizes enclosed by brackets indicate that the signal was weak. The d next to two arrows indicates that these probes correspond to fragment d and not fragment e. Fragments d and g were used for whole-mount *in situ* hybridization experiments. Positive hybridization is indicated by a +, and brackets denote weak hybridization. The four genomic clones (λ87a, λ242a, λ242b, λ242c) that cover the *cdi* genomic region are shown.

P element, allowing the insertion sites to be placed along the map. The P[87] and P[242] insertions were found to lie within the genomic DNA corresponding to the 5′-untranslated region (UTR) of the *cdi* transcript and P[BA01] to lie within intron 1.

To identify transcription units within the cloned region, seven fragments (a–d; f–h; Fig. 2) that span nearly the entire length of the cloned DNA were used. Strand-specific RNA probes were synthesized from each of the seven fragments and hybridized with Northern blots containing poly(A)$^+$ RNA isolated from embryos at 3-h intervals and from larvae, pupae, and

adults. Probes derived from fragments a and b, corresponding to the rightward direction (the 5′ > 3′ orientation of *cdi*; see below), did not detect a transcript, and the RNA probes derived from fragment c detected several bands in the rightward orientation and a faint band in the other orientation. Probe d showed strong hybridization with three species (6.8, 6.0, 5.7 kb) in the rightward orientation. There was also a 0.7-kb transcript in the leftward orientation.

The three rightward fragment d transcripts were present throughout embryonic and postembryonic development (Fig. 3). The 5.7-kb transcript was particularly abundant in 0- to 3-h RNA, suggesting that this transcript is maternally transmitted. The other two transcripts were absent at this time but appeared at 3 to 6 h after fertilization. The three transcripts were present together at all other embryonic stages, as well as in the larvae, pupae, and adult stages. The 6.8-kb and 6.0-kb transcripts peaked at mid-embryogenesis (9–12 h) and gradually declined, along with the 5.7-kb transcript, as development proceeded. Nevertheless, they were all expressed at significant levels. Probe g hybridized with the same set of RNAs as probe d, while probe f, which mapped between probes d and g, showed weak hybridization with the 6.5- and 6.0-kb species. Probe h detected weak 3.3- and 1.7-kb RNA species.

Given their proximity to the P-element insertions, the transcripts detected by probes d and g might be encoded by the gene that directs *lacZ* expression in the midline cells. This idea was tested by *in situ* hybridization with probes d and g. Probe g gave a pattern of hybridization similar to the β-galactosidase expression observed for the BA01 and 87 enhancer trap lines (Fig. 4). Expression within the CNS midline cells, proventriculus, and hindgut and broad, low-level staining throughout the embryo were apparent. Probe d did not show specific hybridization (e.g., CNS midline and proventriculus) but rather uniform, low-level hybridization (data not shown). This result is not surprising, as this probe has only 140 bp of overlap with the longest cDNA clone, and the bands detected by Northern blotting were weaker than those observed with probe g. These results indicate that there is a gene adjacent to the P-element insertions that is transcribed in a manner similar to the *lacZ* expression pattern of the P-element insertions.

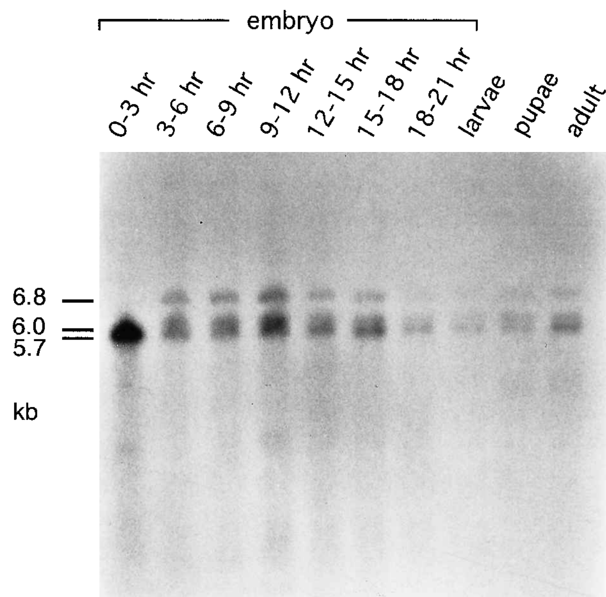

FIG. 3. Northern analysis reveals multiple *cdi* transcripts. Embryonic poly(A)$^+$ RNA isolated from embryonic and postembryonic stages was electrophoresed and transferred to a Nytran membrane. The blot was hybridized with ^{32}P-labeled *cdi* genomic clone probe d and subjected to autoradiography. Three transcripts, 5.7, 6.0, and 6.8 kb, were detected. Embryonic RNA was isolated from 3-h collections covering all of embryogenesis (0–21 h at 25°C). RNA from larvae, pupae, and adults was also analyzed.

Isolation of cdi *cDNA clones*

The probe g fragment was used to screen a *Drosophila* cDNA library constructed from embryonic 4- to 8-h RNA. Ten clones

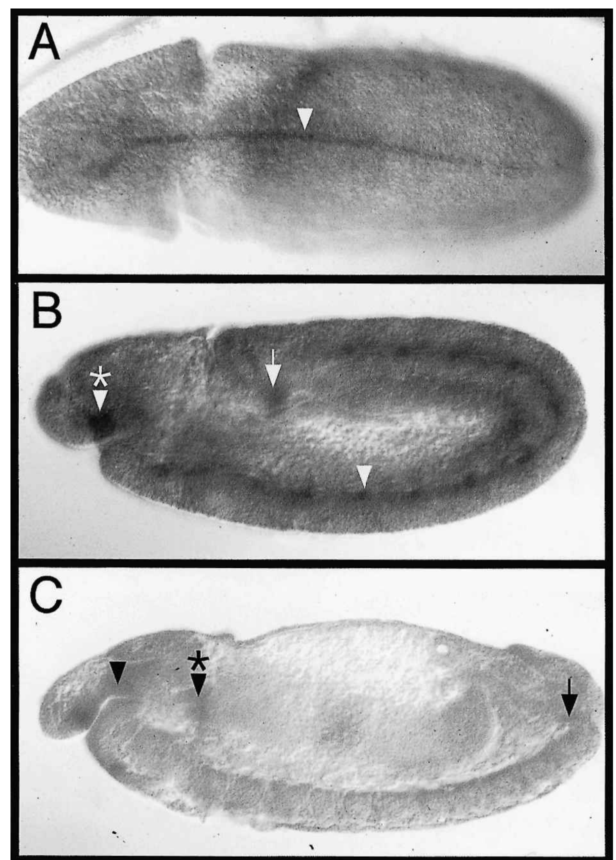

FIG. 4. *In situ* hybridization probes of the *cdi* genomic region reveal a transcription unit that is expressed similar to the pattern in the 91F enhancer trap lines. Whole-mount embryos were hybridized with DIG-labeled *cdi* genomic probe g, and hybridization was revealed by alkaline phosphatase/NBT histochemistry staining. **A**. Ventral view of a stage 10 embryo showing CNS midline cell expression (arrowhead). **B**. Sagittal view of a stage 11 embryo showing broad expression coupled with enhanced expression in the foregut (arrowhead with asterisks), CNS midline cells (arrowhead), and hindgut (arrow). **C**. Sagittal view of a stage 13 embryo showing enhanced expression in the foregut (arrowhead), proventriculus (arrowhead with asterisk) and hindgut (arrow).

were obtained, ranging in size from 5.8 to 0.93 kb. Restriction mapping of eight clones indicated that internal sites were conserved in all of the clones, suggesting that the clones differ only at their 5′ and 3′ ends (Fig. 5). Only the two longest clones, pC1 and pC2, also hybridized with probe d. Four of the clones (pC1, pC2, pC4, pC9) hybridized with probe e, suggesting that only these four clones contain sequences near the 5′ end. The RNA antisense probe of the pC1 cDNA revealed the same bands on a Northern blot as genomic fragment g, indicating that the cDNA clone corresponds to the same transcription unit (data not shown). Similarly, the *in situ* hybridization expression pattern observed with a 4.8-kb fragment derived from a *cdi* cDNA clone was the same as that observed with probe g (data not shown).

The pC1 cDNA clone was sequenced in its entirety (Fig. 6). The size of this clone, 5684 nt, was close to that of the 5.7-kb transcript observed on Northern blots (see Fig. 3). The genomic DNA surrounding the 5′ end of the pC1 cDNA clone was also sequenced (Figs. 2 and 6). The 5′ end of the cDNA clone lay 47 nt downstream of the sequence ATCGGTT, which closely resembles the consensus sequence for transcriptional start sites (ATCAG/TTC/T) (Hultmark *et al.*, 1986). The predicted initiator ATG was preceded by 1110 nt of 5′-UTR. The nucleotide sequence AAAC directly preceding the ATG at nt 1101 exactly matched the consensus sequence for *Drosophila* translational start sites (Cavener and Ray, 1991). The open reading frame (ORF) ended at nt position 4757. This sequence predicts a protein of 1219 amino acids with a molecular weight of 134 kD. Thirteen other ATG triplets preceded the one at nt 1101. None exactly matched the Cavener consensus sequence, and all were followed closely by in-frame termination codons. The 3′-UTR of pC1 was 927 nt. There was a polyadenylation signal residing 22 nt upstream of the poly(A) tract found at the 3′ end of the clone.

Five additional cDNA clones were analyzed by sequence and restriction cleavage analysis (see Fig. 5). Clone pC3 began 847 nt downstream of pC1. However, its 5′ end had the sequence ATCCGTT, which also closely resembles the eukaryotic transcription start site consensus sequence and could represent an alternative start site. The 3′ end of pC3 extended 400 nt beyond the 3′ end of pC1 and was polyadenylated. Thus, pC3 represents an mRNA with a longer 3′-UTR than pC1. The other cDNA clones were all shorter than pC1 at both the 5′ and 3′ ends. Internal restriction sites matched those of pC1. It is not clear if these clones were incompletely formed during cDNA clone synthesis or represent different mRNAs. In summary, our collection of cDNA clones differed at the 5′ and 3′ ends and did not provide evidence for differences in *cdi* proteins, although coding sequence differences cannot be ruled out by these data. How these *cdi* clones relate to the three *cdi* transcripts observed on Northern blots has not been directly examined.

The complete extent of the *cdi* gene has not been determined, but hybridization and sequence comparisons of the pC1 cDNA clone with genomic DNA indicate that *cdi* contains at least three exons (see Fig. 2). Exon 1 contains only 5′-UTR. Exon 2 was mapped only by hybridization and lies at least 4.9 kb from exon 1. The size of exon 1 and the *Xba* fragment that hybridizes with pC1 can accommodate at most only half of the *cdi* transcript, indicating that at least half of the RNA sequences are contained in additional exons greater than 5.4 kb 3′ to fragment g. Sequencing the genomic DNA 5′ to the *cdi* cDNA clone sequences revealed the presence of the ATP synthase chain D gene, located 1.2 kb upstream.

The cdi *gene encodes a protein kinase highly related to human TESK1*

The conceptual protein sequence of *cdi* was compared with the GenBank protein database using a BLAST search. The re-

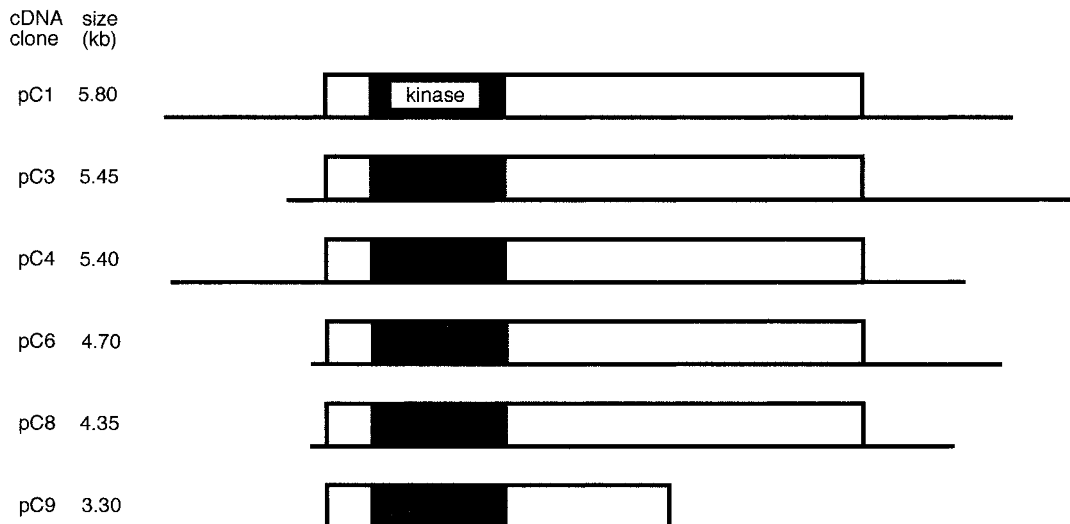

FIG. 5. Sequence organization of *cdi* cDNA clones. Sequence analysis and restriction mapping of six *cdi* cDNA clones indicated that they have the same internal sequence and differ only at the 5′ and 3′ ends. The size of each cDNA clone except pC9 includes the poly(A) region. Lines indicate UTRs; boxes represent coding sequences, and the filled box marks the location of the predicted kinase domain.

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ggatccacgataaacatccgctatcacgagcccaggaagattatcaaggtaacgttagttactataacttaacgaaaccataattaacgagtaccatctactagcttcccttggatctgagcaccaaggtcaagatcatggaggaggtgg   -228
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                                                                                         P87|   P242|    CME
TTTCGCGAGTGCAGACATAAATGAAAGCAACATATCTTTAGCTCCATTCCAGACTGGATCATAGGCTATAGCCCGTTAGAGCAGTGCCAGTGAATCGGTTACCGAATTATAATCTCGCTCTGCGTCGGCTGAGTGGTAATGTTGAATGGA   232
CAGTCGCGTCTAACAAAAAAAAAAAAAAAACAAGTACACACAACCAACGACGAATGCACATAAACAAGACGAGACAGCAAACGCAGAATCGAGTGAAAACCTATTTTAAAACACCAGAGCTATATTTATTTTGTTGACGCTCAGGATTTTC   382
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                                                                                                                      CME
TACACAAAATTCTCTATTACGAAGCAAGCAAAAAGTGATTGATAGTTAGTTCTCCACAATTTTTTTTCCTGTTTCAAAATTTTTAAAATTGTCAAATTCGGACCTATGGGTCCCATAAATGTTTCGTGCTGCTGCGTTCCATAAGCAAACACT   832
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CATCACCCCCACAACAAGCAGGCAAGGCGAGGTCGTCCAACGCCAAAAAAAAACAAACTTAAACCAACTTCACATACACATCCGTTTACGACAGCAACATACGTCGTAGCTGATAAACATGAGCCTGAATCAGCACAGTGGACCCGGCCC   1132
                                                                                                                         M  S  L  N  Q  H  S  G  P  G  P   11
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 P  S  E  I  C  G  L  S  V  D  Q  V  D  S  S  A  A  S  P  F  I  W  P  S  T  A  G  M  C  T  T  A  T  T  V  T  T  S  T  N  G  D  A  T  A  E  A  P  V  K   61
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 V  K  E  K  I  G  S  G  F  F  S  E  V  Y  K  V  T  H  R  T  T  G  Q  V  M  V  L  K  M  N  Q  L  R  A  N  R  P  N  M  L  R  E  V  Q  L  L  N  K  L  S   161
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 G  I  A  R  G  M  S  Y  V  H  D  A  G  I  F  H  R  D  L  T  S  K  N  V  L  I  R  N  L  A  N  D  Q  Y  E  A  V  V  G  D  F  G  L  A  A  K  I  P  V  K   261
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 K  L  T  L  L  L  E  K  Y  E  H  E  S  S  A  G  A  S  P  L  S  S  L  E  L  I  N  C  S  P  G  G  S  D  S  E  N  V  T  L  T  P  R  A  S  V  P  T  V  A   411
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 K  K  K  Q  Q  Q  G  G  *                                                                                                                              1219
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CTTACCGACTTGAGGTTTTAAGACTTAAAGCCCCCTTTCCCTCGAAAATTGTACAGATGATCCCGGATCGATTTGAGAAGCAAGAACACTTATCATTGGCCAATCCCATTCGGCTTAAACACGACTTATGACTTTAGTTTTAGATTCATA   5632
CGTTACAAATATGTGGGTATCTGCATTACAAATAAATACATAAGTATACATT   5684
```

FIG. 6. Complete nucleotide sequence of the pC1 *cdi* cDNA clone (5684 bp), along with 368 bp of genomic DNA that precedes the 5′ end of the clone. Genomic sequence is in small letters and cDNA clone sequence in capital letters. The 5′ end of the clone is designated nucleotide position 0, and nucleotide numbers are to the right of the sequence. The *cdi* ORF is shown below the nucleotide sequence using the one-letter amino acid code. Numbering of the protein is on the right, below the nucleotide number. The putative transcriptional start sequence (ATCGGTT) is underlined at a site 47 nt preceding the start of the cDNA clone, and an alternative transcriptional start site (ATCCGTT) is underlined beginning at residue 843. The pC1 5′-UTR is 1100 bp, the coding sequence is 3657 bp, and the 3′-UTR is 927 bp. The P[87] and P[242] insertion sites are indicated by a (|) below the sequence. They reside at +28 and +36, respectively. Two CMEs (ACGTG) present in the *cdi* gene are underlined (+40 and +628). Residues constituting the kinase domain (1449 to 2159) are underlined beneath the protein sequence. The consensus polyadenylation signal (AATAAA) is underlined 22 nt from the end of the sequence. The cDNA and genomic sequences have been deposited with GenBank under Accession Numbers AF139812 and AF139811.

sults indicated that *cdi* likely encodes a protein kinase. It possesses a kinase domain that stretches for 237 aa (Cdi residues 117–353). Protein kinase domains are typically 250 to 300 aa long with 12 subdomains containing a number of highly conserved residues (Hanks *et al.*, 1988). The Cdi protein contains all of these domains in the correct order and has all of the invariant and nearly invariant residues (Fig. 7A). This high degree of sequence similarity to other kinases suggests that Cdi is likely to be a functional kinase.

Comparison with other kinases revealed that Cdi is highly related to the human TESK1 protein kinase (Toshima *et al.*, 1995) (Fig. 7A). The overall sequence identity within the kinase domain is 61% (Fig. 7B), which is much greater than the sequence identity between Cdi and other kinases. The next most

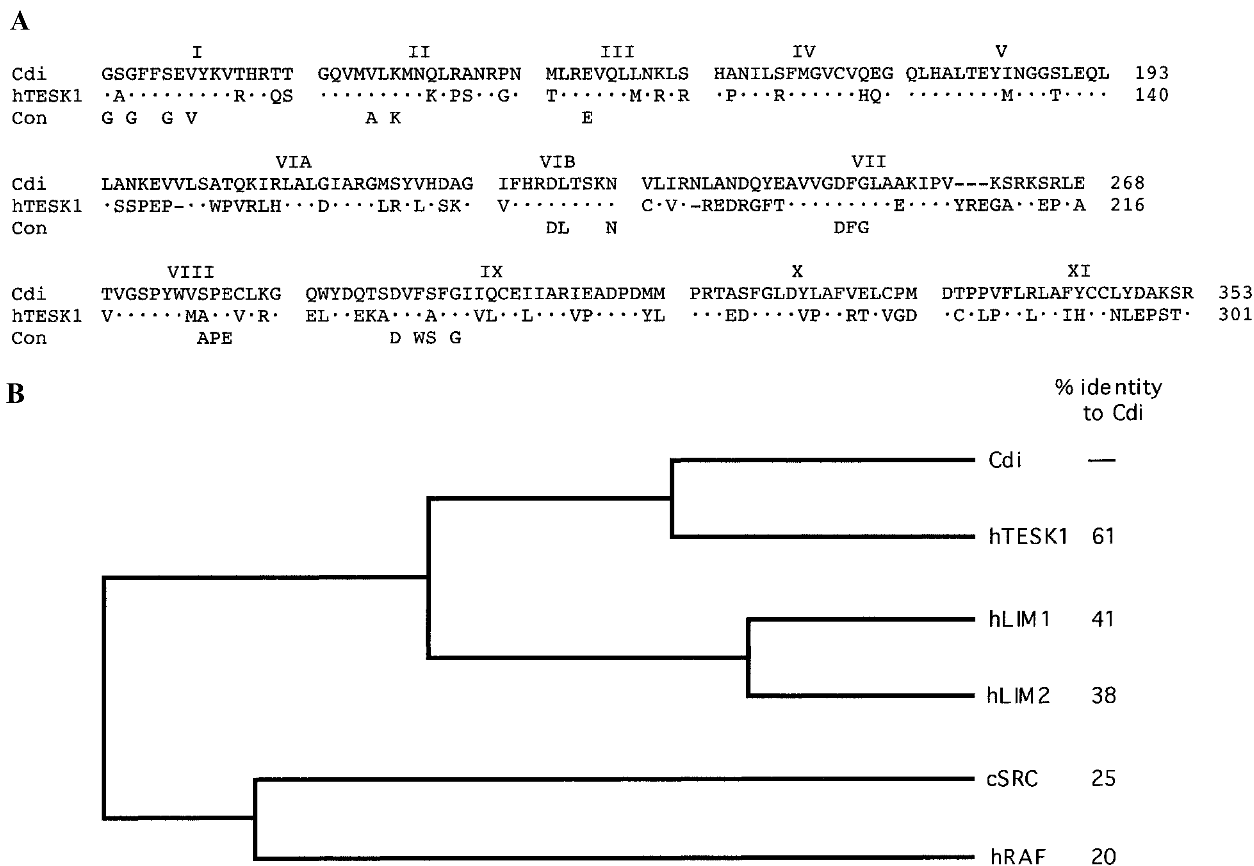
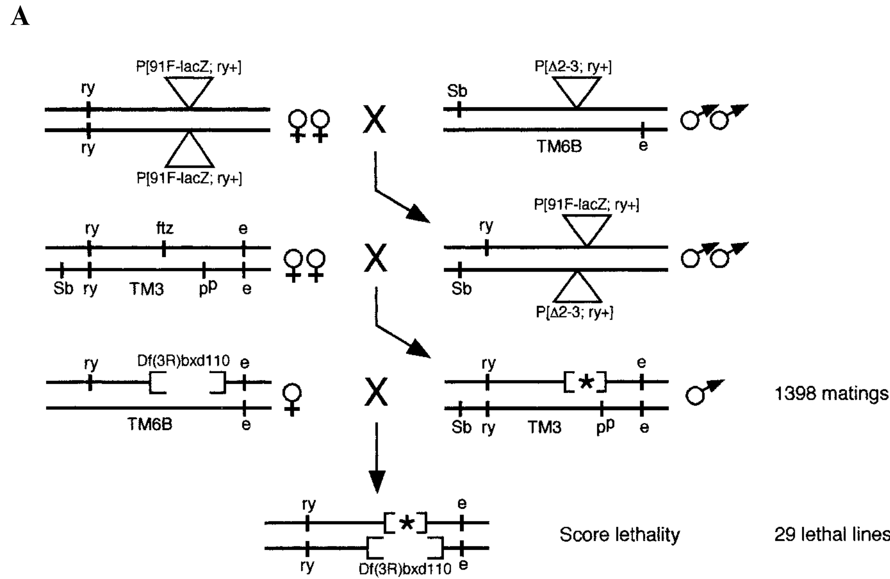


FIG. 7. Alignment of the kinase domains of Cdi and other protein kinases reveal that Cdi is highly related to TESK1. **A.** The protein kinase domains of Cdi and human TESK1 aligned for comparison. The amino acid sequence of Cdi is shown on top, and identities with hTESK1 are indicated by (●). Gaps are indicated by dashes. Each subdomain is identified by roman numeral (Hanks *et al.*, 1988). Numbering of both proteins is to the right. Consensus (Con) residues for all protein kinases are shown below the Cdi and hTESK1 sequences. **B.** Sequence identity matrix shows the % identity between Cdi and other protein kinases within the kinase domain. Sequences were aligned and the relatedness tree created using the Unweighted Pair Group Method with Arithmetic Mean Tree sequence alignment program of GeneWorks (IntelliGenetics). Proteins analyzed (with accompanying GenBank accession numbers) are: *Drosophila* Cdi, human TESK1 (Q15569), human LIM kinase 1 (D26309), human LIM kinase 2 (P53761), chicken SRC (V00402), and human bRAF (P15056). hTESK1, hLIM1, and hLIM2 have the highest sequence identity to Cdi.

closely related proteins are several LIM kinases (Mizuno *et al.*, 1994; Ohashi *et al.*, 1994), but the sequence identity within the kinase domain is no greater than 41%. For comparison, other kinases, such as the Src tyrosine kinase and Raf serine/threonine kinase, are only 25% and 20% identical, respectively, to Cdi within the kinase domain.

TESK1 is a serine/threonine kinase that is highly expressed in testis and has not been detected at significant levels in other tissues by Northern blot analysis (Toshima *et al.*, 1995). Interestingly, it has been postulated to be a novel class of signaling proteins because it is distinct in sequence from other protein kinases (Toshima *et al.*, 1995). Within kinase subdomain VIB,



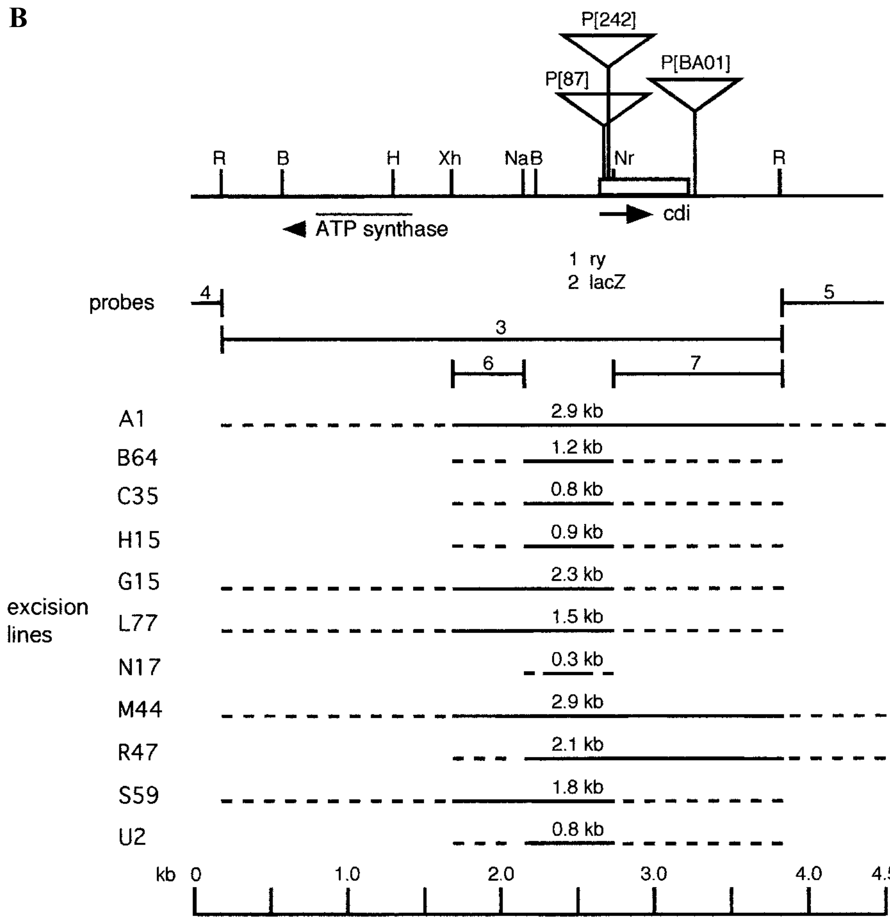


FIG. 8. Creation and molecular mapping of *cdi* deletion mutants. Transposase-mediated imprecise excision of the P[87] and P[242] P elements was used to generate deletion mutants of *cdi*. **A**. The genetic scheme used to create *cdi* mutations. P[91F-*lacZ*] signifies either P[87] or P[242]. P-element lines were crossed with Δ2-3 transposase and 1398 *ry* flies screened for lethality over *Df(3R)bxd*^{110}. **B**. The extent of each *cdi* deletion is indicated below the restriction map of the *cdi* gene, which also shows the location of the P-element insertions, *cdi* exon 1, and the upstream ATP synthase gene. Arrows indicate the transcriptional orientation of both genes. The probes used in Southern blots of restriction enzyme-cut genomic DNA to define the deletion breakpoints are numbered 1 through 7. Probes 1 and 2 were the *ry* and *lacZ* genes, respectively, and determined whether the P element was absent from the original site of insertion. Probes 3 through 5 are adjacent *Eco*RI (R) fragments, probe 6 is an *Xho*I (Xh)-*Nae*I (Na) fragment, and probe 7 is an *Nru*I (Nr)-*Eco*RI fragment. The *Bam*HI (B) and *Hin*dIII (H) sites are also shown. The size (kb) and extent of the deletion breakpoints are shown for 11 excision lines (named at left). The solid line indicates that this fragment of DNA was absent in the deletion. The dashed lines indicate that the deletion breakpoints reside in this interval of DNA. Six of the deletion lines do not lack the ATP synthase ORF; this has not been determined for the other five. The scale (kb) is shown at bottom.

serine/threonine kinases are DLKXXN, and tyrosine kinases possess either DLRAAN or DLAARN. TESK1 and Cdi have the identical sequence DLTSKN, which matches the kinase consensus DLXXXN but is not identical to either the serine/threonine (DLKXXN) or the tyrosine kinase (DLRAAN or DLAARN) consensus sequences. Biochemical experiments have shown, however, that TESK1 is a serine/threonine kinase. These observations suggest that TESK1/Cdi represent a novel class of serine/threonine kinase signaling proteins. In addition to its kinase domain, TESK1 has homology with LIM kinases and has a LIM domain, which Cdi does not possess. Outside the kinase domain, Cdi does not have significant homology with any other protein, including TESK1. Analysis of the Cdi protein sequence using a Kyte and Doolittle hydropathy plot did not reveal any significant hydrophobic regions indicative of signal sequences or transmembrane domains. Likewise, TESK1 does not possess significant hydrophobic regions, and both Cdi and TESK1 are likely to be intracellular kinases.

Generation of cdi *mutants by P-element excision*

When transposase is introduced genetically into strains harboring P elements, the P elements excise imprecisely at low frequency and delete adjacent genomic sequences (Voelker *et al.*, 1984). The genomic DNA adjacent to the P[87] and P[242] P-element insertions was cloned by inverse PCR and sequenced. These sequences were compared with the genomic and cDNA sequences to identify the points of insertion (see Fig. 2). The 87 and 242 insertions are located 28 and 36 bp, respectively, downstream of the 5′ end of the pC1 cDNA clone. Thus, both lie within the 5′-UTR of exon 1. The insertions occurred in opposite orientations. The BA01 insertion resides further 3′ within intron 1. Despite the location of the P[87] and P[242] insertions within the 5′-UTR, both strains are homozygous viable.

In order to create mutants of *cdi*, chromosomes carrying the 87 and 242 P elements were combined with a chromosome carrying the Δ2-3 transposase (genetic scheme shown in Fig. 8A). One consequence of introducing transposase into a P-element-bearing strain is that imprecise excision events create deletions at the site of the original P element. In our scheme, flies which had lost all or part of the P element were selected on the basis of loss of the ry^+ eye color marker that resides in the 87 and 242 P elements. The resulting ry^- flies were crossed with flies having a deficiency of 91F, $Df(3R)bxd^{110}$ (91D2-92A2), and the progeny were screened for lethality. The assumption was that deletions in *cdi* would be lethal. Of 1398 *ry* males screened, 29 contained mutations that were lethal over the deficiency. Pairwise complementation tests were done between 24 of the lethal mutations, and all failed to complement, indicating that the strains all contain a lethal mutation within the same gene. There are six lethal complementation groups, $l(3)91Fa^{BE40}$, $l(3)91Fb^{BE50}$, $l(3)91Fc^{BE60}$, $l(3)91Fd^{BE70}$, $l(3)91Fe^{BE80}$, and $l(3)91Ff^{BE90}$, that map in 91F to the left of *Delta (Alton* et al., 1988) and are possible *cdi* candidates. These lethal loci were crossed with *cdi* excision mutants, and in all cases, complementation was observed, indicating that these genes do not correspond to the *cdi* excision mutations.

The extent of the DNA deleted was determined for 11 excision lines by Southern hybridization with genomic DNA of each line using seven probes including and surrounding the P-element insertions (Fig. 8B). The size of the DNA deleted in the various lines was between 0.3 and 2.9 kb. Given the location of the deletions with respect to the *cdi* transcription unit, it seemed likely that these deletions would result in a loss of *cdi* transcription. This hypothesis was tested by using a 4.8-kb probe derived from the pC1 *cdi* cDNA for *in situ* hybridization experiments on homozygous mutant embryos of cdi^{C35}, one of the smallest deletions (all but one of the other 10 deletions removed the same central stretch of DNA as cdi^{C35}). Approximately a quarter (27%; N = 84) of the embryos derived from cdi^{C35}/TM3 P[*ftz-lacZ*] adults failed to hybridize. These results are expected if homozygous mutants are transcript nulls. The fragment used contained almost all of the *cdi* cDNA coding sequence, indicating the absence of *cdi* coding transcripts in cdi^{C35}. Although the cdi^{C35} mutant, and probably the other excision mutants, are null mutations of *cdi*, the lethality associated with the mutations cannot be ascribed definitively to *cdi*. Northern analysis using probe c indicated a transcription unit just 5′ to *cdi* and an ORF in the same vicinity (see Figs. 2 and 8B). Although the upstream ORF was not deleted in a number of the excision mutations, the mutations could affect its function; and thus, the lethality of the *cdi* excision mutants could be attributable to effects on either *cdi* or adjacent genes.

cdi *mutations do not cause obvious CNS defects*

Four *cdi* mutants, three whose deletions were mapped (cdi^{A1}, cdi^{C35}, cdi^{S59}) and one that was not (cdi^{T13}), were tested for their homozygous lethal period. Heterozygous flies were crossed *inter se* or with those having the 91F deficiency, $Df(3R)bxd^{110}$, and assayed for the developmental stage when mutant individuals died. In all crosses, greater than 95% of the embryos hatched, indicating that the *cdi* mutations were not embryonic lethal. Further analysis indicated that the mutant larvae that survived fail to pupate, indicating that these mutants were larval lethals.

Because mutant embryos were able to hatch into larvae, it was unlikely that *cdi* mutations caused severe CNS defects. This idea was further examined by staining cdi^{A1}, cdi^{C35}, cdi^{S59}, and cdi^{T13} mutant embryos with a variety of reagents specific for midline cells (*sim-lacZ* enhancer trap transgene), lateral and midline nerve cells (anti-Engrailed), and axons (anti-HRP, MabBP102, MabBP104, MabBP106, and Mab22C10) (Patel, 1994). Mutant embryos were identified using a *ftz*-β-galactosidase transgene-marked balancer. In all cases, embryos appeared to be wildtype (data not shown). Embryos from other mutant lines were examined in less detail but showed similar results (data not shown). These experiments confirm that *cdi* mutants do not have a severely defective CNS.

DISCUSSION

cdi *is a* Drosophila *ortholog of TESK1*

This paper describes the isolation of a *Drosophila* protein kinase that is highly related to the human testis-specific TESK1 serine/threonine protein kinase. Given the identity between Cdi and TESK1 in the region generally associated with protein substrate specificity, it is reasonable to assume that Cdi is also a serine/threonine protein kinase. Both proteins are expressed in

a tissue-specific fashion and are likely to be intracellular proteins (Toshima *et al*., 1995, 1998). The biological and signaling function of TESK1 is unknown. However, TESK1 expression occurs during spermatogenesis, is developmentally regulated, and correlates with stages of meiosis and spermiogenesis (Toshima *et al*., 1988). Both TESK1 and Cdi would appear to be members of a novel class of signaling molecule, given their unique and conserved sequence structure.

cdi *mutants are larval lethal and do not show a severe embryonic CNS phenotype*

Mutations were generated in *cdi* using imprecise excision of the 87 and 242 P elements. These events created a set of small deletions. The cdi^{35} deletion is likely a *cdi*-null mutation, as *cdi* transcripts could not be detected, and the probe contained almost the entire coding sequence. Most likely, all of the deletions generated and analyzed, except possibly cdi^{N17}, are null, given that the extents of their deletions are equal to or greater than cdi^{C35}. Northern analysis detected transcripts adjacent to the 5′ end of the longest *cdi* cDNA clones, and the ATP synthase D chain is located 1.2 kb away. At least six of the excision mutants did not lack the ATP synthase ORF, but the mapping of the synthase transcription unit has not been carried out, so the *cdi* deletions may render that gene nonfunctional. Thus, the deletion mutants are null for *cdi*, but their lethality cannot yet be ascribed to *cdi*.

Because *cdi* is broadly expressed, absence of function could affect a number of tissues and cell types. Our analysis has focused on possible roles of *cdi* in nervous system development. Because *cdi* is expressed in the midline precursor cells and differentiated nerve cells, it could function in midline cell neurogenesis, axonogenesis, or midline-directed signaling events (Crews, 1998). The *cdi* mutations are larval, not embryonic, lethal. Consistent with the absence of embryonic lethality, the gross structure of the CNS neurons and axons resembles that in wildtype embryos. Staining *cdi* mutant embryos with Mab BP102, Mab BP104, Mab BP106, Mab 22C10, and anti-HRP that identify the axon scaffold indicated that the CNS axons were wildtype in appearance. The midline VUM cell motoneurons extend axons to the periphery via the midline fascicle (Goodman *et al*., 1984). Staining with Mab 22C10 that identifies these axons showed that the median tract was normal. Examination of the CNS midline cells using a P[*sim-lacZ*] transgenic marker coupled with anti-β-galactosidase staining and anti-Engrailed to stain midline neurons showed that the midline cells were normal. Thus, even though *cdi* is prominently expressed in the CNS midline, the midline cells were normal in *cdi* mutants.

There exist a number of explanations for the absence of severe CNS defects in *cdi* mutant embryos. First, the CNS has been examined only for severe defects in axonogenesis and midline cell appearance. There are numerous cases of genes that affect axon guidance of a subset of neurons; for example, that do not show embryonic lethality and gross axon scaffold defects (e.g., Lin *et al*., 1994; Lundgren *et al*., 1995). Thus, it is possible that defects are present but have not been detected in our assays. Second, Northern and *in situ* hybridization experiments indicate that *cdi* has a strong maternal RNA component. The maternal RNA may mask an embryonic *cdi* defect. This question can often be resolved by germline clone analysis. Third, it is possible that the developmental role of *cdi* is concealed by genetic redundancy. Other, unknown proteins, most likely protein kinases, could compensate for the absence of *cdi* activity. Finally, it is possible that *cdi* does not play a role in embryonic development.

cdi *is expressed in a dynamic cell-specific mode during embryogenesis*

One of the interesting features of *cdi* is that it is expressed in a dynamic, cell-specific fashion during embryogenesis. This pattern is in contrast to the ubiquitous expression of many protein kinases involved in cell signaling. Northern analysis indicates that *cdi* is expressed during all embryonic and postembryonic phases. There are three transcripts detected that are 5.7, 6.0, and 6.7 kb in size. All three are present at every stage of embryogenesis, except that only the 5.7-kb transcripts is present in 0- to 3-h embryonic RNA. The 5.7-kb transcript is the most abundant of the three in early embryogenesis (0–3 h), late embryogenesis (12–21 h), and postembryonic development. During most stages, the relative proportions of the three transcripts are consistent. The nature of the differences between the three transcripts has not been extensively studied, but analysis of embryonic cDNA clones has detected differences only within the UTRs.

The high concentrations of RNA detected in 0- to 3-h embryos suggest that there is a maternal contribution of *cdi* RNA, and that only the 5.7-kb transcript is maternally derived. Thus, the 6.0- and 6.7-kb transcripts are attributable only to zygotic transcription. *In situ* hybridization using a *cdi* probe on 0- to 3-h blastoderm embryos showed ubiquitous expression that is assumed to be maternal RNA. After gastrulation, *cdi* is expressed in a cell-specific fashion. This was examined in detail by *in situ* hybridization with a *cdi* cDNA clone probe and β-galactosidase staining of the BA01 and 87 enhancer trap lines. Enhancer trap line β-galactosidase expression was similar to *cdi* expression detected by *in situ* hybridization. This finding was in contrast to the 242 enhancer trap line, which revealed only CNS midline expression.

Prominent cell-specific expression was first observed in the CNS midline precursor cells at stage 9 after gastrulation. The *cdi* transcripts remained in the CNS midline cells at least until stage 15. Prominent early expression was observed in the developing foregut. By stage 13, this expression was strong in the proventriculus, which separates the foregut from the anterior midgut. This expression was observed from stage 10 through the end of embryogenesis. Additional sites of expression included the hindgut, visceral mesoderm, and epidermal cells. However, both the enhancer trap lines and *in situ* hybridization experiments revealed that *cdi* was expressed at low levels in all ectodermal cells and additional internal cell types. Thus, *cdi* has two modes of expression: one broadly distributed at low levels and the other cell specific at higher levels. Mammalian TESK1 is expressed predominantly, if not exclusively, in the testis. Expression or function of *cdi* in the *Drosophila* germ cells has not yet been studied carefully, but the expression of *cdi* in a number of other cell types suggests that the *Drosophila* kinase performs more developmental roles than the human protein.

cdi *is a target of the Sim CNS midline cell regulatory protein*

The cell-specific patterns of gene expression exhibited by *cdi* suggest control by a variety of regulatory proteins. Only the

control of *cdi* in the CNS midline cells has been investigated (Nambu *et al*., 1990). The *cdi* gene is prominently expressed in CNS midline cell precursors beginning at stage 9. The Sim::Tango (Tgo) bHLH-PAS transcription factor complex has been shown to control CNS midline precursor cell transcription and development and is localized to midline cell nuclei during gastrulation (Thomas *et al*., 1988; Nambu *et al*., 1990, 1991; Sonnenfeld *et al*., 1997; Ward *et al*., 1998). Previously, it was shown that midline expression of the P[242] lacZ transgene that resides in the *cdi* gene requires *sim* function (Nambu *et al*., 1990). In addition, ectopic *sim* neuroectodermal expression results in corresponding ectopic P[242] lacZ expression (Nambu *et al*., 1991). Because the enhancer elements that control P[242] midline expression are likely to be the same as those controlling *cdi* midline expression, these results suggest that the Sim::Tgo protein complex directly controls *cdi* midline expression, presumably through CNS midline elements (CME) (Wharton *et al*., 1994; Sonnenfeld *et al*., 1997) residing in the *cdi* regulatory DNA. Interestingly, there are two CMEs (core sequence ACGTG) that reside just downstream of the 5′ end of the *cdi* cDNA sequence at +40 and +628. The insertion points of the P[87] and P[242] P elements are close by, at +28 and +36.

Summary

The *Drosophila cdi* gene encodes a putative serine/threonine protein kinase related to TESK1 and likely participates in cellular signaling pathways related to embryonic development. The molecular targets of Cdi are unknown. Genetic analysis showed that homozygous null *cdi* mutants are lethal, and this finding suggests an important role for *cdi*, although severe embryonic phenotypes have not been observed. Additional genetic analyses of germline clones, temperature sensitive or exceptional alleles, gain-of-function transgenic flies, and sensitized mutant backgrounds will provide insight into the biologic and biochemical roles of *cdi*. The *cdi* gene is expressed in the CNS midline cells, is a target of *sim* regulation, and may play a role in CNS midline cell development. These cells must undergo differentiation into nerve cells and glia. They also play prominent roles in axonogenesis and midline signaling pathways. The *cdi* gene could play a role in any of these events.

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