A Developmentally Regulated Splice Variant from the Complex lola Locus Encoding Multiple Different Zinc Finger Domain Proteins Interacts with the Chromosomal Kinase JIL-1*

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Using a yeast two-hybrid screen we have identified a novel isoform of the lola locus, Lola zf5, that interacts with the chromosomal kinase JIL-1. We characterized the lola locus and provide evidence that it is a complex locus from which at least 17 different splice variants are likely to be generated. Fifteen of these each have a different zinc finger domain, whereas two are without. This potential for expression of multiple gene products suggests that they serve diverse functional roles in different developmental contexts. By Northern and Western blot analyses we demonstrate that the expression of Lola zf5 is developmentally regulated and that it is restricted to early embryogenesis. Immunocytochemical labeling with a Lola zf5-specific antibody of Drosophila embryos indicates that Lola zf5 is localized to nuclei. Furthermore, by creating double-mutant flies we show that a reduction of Lola protein levels resulting from mutations in the lola locus acts as a dominant modifier of a hypomorphic JIL-1 allele leading to an increase in embryonic viability. Thus, genetic interaction assays provide direct evidence that gene products from the lola locus function within the same pathway as the chromosomal kinase JIL-1.

Chromatin structure as well as the differential expression of transcription factors plays an important role in the regulation of gene expression (1–3). We have recently identified a chromosomal tandem kinase, JIL-1, that modulates chromatin structure in Drosophila (4–6). JIL-1 is an essential kinase, and in JIL-1 nulls and hypomorphs euchromatic regions of chromosomes are severely reduced and the chromosome arms are condensed (6). These changes are correlated with decreased levels of histone H3 Ser-10 phosphorylation (6). JIL-1 has been implicated in transcriptional regulation, as it localizes to the gene-active interband regions of interphase larval polytene chromosomes (4) and has been found to associate with at least one chromatin-remodeling complex, the male specific lethal (MSL)1 dosage compensation complex (5). The MSL complex is required for the necessary hypertranscription of genes on the male X chromosome for dosage compensation in flies (reviewed in Ref. 7). This enhanced transcription is thought to arise from MSL complex-induced histone H4 acetylation generating a more open chromatin structure (8). The increased histone H3 Ser-10 phosphorylation levels that JIL-1 promotes on the male X may also play a role in maintaining a more open and active chromatin structure (5, 6). However, it is not known whether histone substrates of JIL-1 may include other proteins such as transcription factors or whether there are proteins directly regulating the function of JIL-1. To identify proteins that interact with JIL-1 we carried out yeast two-hybrid screens using different JIL-1 regions as baits. Here we report that a novel splice form from the lola locus, which we have named Lola zf5, was identified in such a screen to interact with the first kinase domain (KDI) of JIL-1.

The lola locus was first characterized as a mutation affecting longitudinal axon growth within the central nervous system (9). Two isoforms from the lola locus have been previously described, Lola long and Lola short (10). Lola long contains two zinc finger motifs and is a transcription factor with DNA binding activity (10, 11). The lola locus mediates decreased copia retrotransposon mRNA expression in the central nervous system while upregulating its expression in gonads (11). In addition, lola is required for proper expression of the axonal guidance proteins Robo and slit in the central nervous system (12). Although both Lola isoforms share a BTB/POZ domain (13–15), Lola short contains no zinc finger domains (10). BTB domains are known to mediate dimerization (16, 17), which includes the ability to promote heterophilic interactions of different BTB domain-containing isoforms (15, 18, 19). BTB domain-containing zinc finger proteins have been strongly implicated in regulation of chromatin structure and gene expression (20). For example, human B cell lymphoma (BCL-6) and promyelocytic leukemia zinc finger oncoproteins have been shown to act as transcriptional repressors (21–23). Specific recruitment of repressor complexes to target promoters occurs through binding of corepressors to the hydrophobic BTB dimer pocket (24). Corepressor binding recruits a complex containing a histone deacetylase (25, 26) that represses transcription by inducing condensed chromatin architecture (reviewed in Ref. 27).

In this study we characterized the genomic organization of the lola locus and show that it is a complex locus from which at least 17 different splice variants are likely to be transcribed. All isoforms from the lola locus share a common BTB domain, whereas 15 of the splice variants each contain a different zinc finger domain. We show that one of these variants, Lola zf5, physically interacts with the JIL-1 kinase and that its expres-
guantishion is developmentally restricted to early embryogenesis. Furthermore, we show that a P element insertion in the *lola* locus enhances the viability of a hypomorphic JIL-1 allele, indicating opposing functions of the JIL-1 and *lola* loci. Although only *lola* long and *lola* short have so far been studied in detail, it has long been known that P insertion mutations in *lola* that prevent the expression of some if not all the isoforms have very complex functional roles in different cellular and developmental contexts. Gene products from the *lola* locus are likely to serve diverse functional roles in different cellular and developmental contexts.

**Materials and Methods**

*Drosophila Stocks—*Fly stocks were maintained according to standard protocols (29). Oregon-R was used for wild type preparations. JIL-1P506052 and JIL-1P506150 alleles have been previously described (6). Balanced chromosomes and mutant alleles are described in (30). The *lola*50642 mutant clone c1 P*+/+ c2 = P*lola*50642/Cyo. *ry*506 was obtained from the Bloomington *Drosophila* Stock Center. Hatch rates were determined by counting the number of eggs laid on standard apple juice/agar plates and then counting the number of unhatched eggs at 22 °C and again at 48 h after egg laying. All genetic crosses and interactions were performed at 25 °C.

**Identification and Molecular Characterization of *Lola* zf5—**JIL-1 cDNA sequence encoding a 304-amino acid fragment (Tyr251-Glu554) comprising the first kinase domain (JIL-1 KDI) of JIL-1 was subcloned in-frame into yeast two-hybrid bait vector pGBK77 (Clontech) using standard methods (31) and verified by sequencing (Iowa State University Sequencing Facility). The *JIL-1* KDI bait was used to screen the *lola*00642 mutant clone *C*+/+ yeast two-hybrid cDNA library described above and confirmed by sequencing. After stripping the *lola*506 signal, labeled *lola*506 cDNA was used to probe the same membrane to normalize mRNA loading levels. cDNA extracts were prepared from staged dechorionated embryos, larvae, pupae, or adults that were homogenized in immunoprecipitation buffer (20 mM Tris-Cl, 150 mM NaCl, 10 mM EDTA, 1 mM EGTA, 0.1% Triton X-100, 0.1% Nonidet P-40, 2 mM NaVO$_4$, pH 8.0) with added protease inhibitors 1.5 μg/ml aprotonin and 1 μg/ml phenylmethylsulfonyl fluoride (Sigma). Proteins were boiled in SDS-PAGE buffer, separated on SDS-PAGE gels, transferred to nitrocellulose, blocked in 5% Blotto, and incubated with anti-JIL-1 or anti-*lola* antibody overnight. Blots were then washed three times for 10 min in TBST (0.9% NaCl, 100 mM Tris, pH 7.5, 0.2% Tween 20), incubated with horseradish peroxidase-conjugated goat anti-rabbit or anti-mouse secondary antibody (Bio-Rad) for 1 h at room temperature, washed in TBST, and visualized using the ECL chemiluminescence kit (Amersham Biosciences). In *vitro Protein Interaction and Co-immunoprecipitation Assays—*Approximately 5 μg of GST-KDIJ1 (*lola* zf5 C terminus) fusion protein or GST protein alone was coupled to glutathione-agarose beads (Sigma) and incubated with 0.5 ml of 52 cell lysates (3 × 10$^6$ cells) overnight at 4 °C. The beads were pelleted at low speed and washed three times with 1 ml of immunoprecipitation buffer (20 mM Tris-Cl, 150 mM NaCl, 10 mM EDTA, 1 mM EGTA, 0.1% Triton X-100, 0.1% Nonidet P-40, 2 mM NaVO$_4$, 1 mM phenylmethylsulfonyl fluoride, and 1.5 μg/ml of aprotinin, pH 8.0) for 10 min at 4 °C. The proteins retained on the beads were analyzed by Western blot analysis using affinity-purified JI-anti-JIL-1 polyclonal antibody. For co-immunoprecipitation experiments, anti-JIL-1, anti-*lola* zf5, or control normal rabbit antibodies were coupled to protein G beads as follows: 30 μl of *Odin* anti-JIL-1 serum, 30 μl of normal rabbit control serum, or 1 ml of 7F1 hybridoma supernatant was coupled to 25 μl of protein G-Sepharose beads (Amersham Biosciences) for 2 h at 4 °C on a rotating wheel in 50 μl of immunoprecipitation buffer. The appropriate antibody-coupled beads were incubated overnight at 4 °C with 300 μl of 0–6 h embryonic lysate on a rotating wheel. Beads were washed four times for 10 min each of 1 ml of immunoprecipitation buffer with low speed pelleting of beads between washes. The resulting bead-bound immunocomplexes were analyzed by SDS-PAGE and Western blotting techniques as described in Jin et al. (4) using Hope antibody to detect JIL-1, mAb 7F1 to detect *lola* zf5, or *lola* polyclonal antiserum against the *lola* common core domain (10) to detect all *lola* isoforms.

**Bioinformatics—***Lola* genomic DNA sequence corresponding to nucleotides 118,125–182,622 of *Drosophila melanogaster* genomic scaffold AE003829.3 (GenBank Z97377) was used to search for zinc finger motifs. Consensus zinc finger motifs were derived from the predicted zinc finger motifs using standard PCR protocols (31). The predicted zinc finger motifs using standard PCR protocols (31). The predicted zinc finger motifs using standard PCR protocols (31).
whereas reverse PCR primers were designed to be isoform-specific, with individual reverse primers comprised of antisense sequence based on the 3' end of each separate predicted zinc finger motif. Oligonucleotides were designed using the Oligo 5.0 program. cDNA templates for PCR reactions were from the 0- to 21-h cDNA Matchmaker™ yeast two-hybrid library (Clontech) or a 3- to 12-h cDNA library (Stratagene). PCR conditions were optimized for each primer pair, respectively, and PCR products were directly sequenced.

The lola genomic sequence was also used to search for homologs in the Drosophila EST databases with FLYBLAST (available at www.fruitfly.org/blast/) and with the Geneseqer program (available at bioinformatics.iastate.edu/cgi-bin/gs.cgi) (37). Returned ESTs were compared with lola genomic DNA sequence using BLAST2 (available at www.ncbi.nlm.nih.gov/blast/bl2seq/bl2.html) at NCBI (National Center for Biotechnology Information) (38). Putative zinc finger motifs were aligned using Clustal W available at www2.ebi.ac.uk/CLUSTALW (39).

RESULTS

The JIL-1 Kinase Interacts with a Novel Isoform of the lola Locus—To identify proteins that have direct interactions with the JIL-1 kinase, we performed a yeast two-hybrid screen of a Canton-S 21-h embryonic library using the first kinase domain of JIL-1 as bait. True clones detected in the primary screen were confirmed by β-galactosidase two-hybrid interaction assays on filter paper following retransformation of the candidate clones and JIL-1 KDI bait plasmid into the yeast strain AH109 (data not shown). One of the positive clones identified in this way was sequenced, and searches of the Drosophila genome database revealed it to be the COOH-terminal domain of a novel splice variant from the lola locus, which we have named Lola zf5. Subsequently, the full-length cDNA sequence for Lola zf5 was assembled from overlapping ESTs obtained from the Drosophila genome project and is currently available as AY058586 as well as the SW59 clone (Z97377). Fig. 1A shows the amino acid sequence of the predicted open reading frame of a protein of 748 residues with a calculated molecular mass of 79.4 kDa. The BTB domain and zinc finger domains are underlined and boxed, respectively. To further characterize the protein, a Lola zf5-specific monoclonal antibody, 7F1, was generated against a GST fusion protein containing the COOH-terminal region unique to Lola zf5. On immunoblots of embryo protein extracts (0–6 h) mAb 7F1 detects Lola zf5 as a single band migrating at 105 kDa (Fig. 1B). The Lola zf5 protein is highly acidic with a pI of 5.54 accounting for its anomalous gel migration. Immunocytochemical labeling of early Drosophila embryos with mAb 7F1 revealed the Lola zf5 protein to be localized to nuclei in a pattern similar to that obtained by Hoechst labeling (Fig. 1, C and D).

To further explore the interaction between Lola zf5 and JIL-1 that we observed in the yeast two-hybrid assays we performed pull-down assays with the Lola zf5 COOH-terminal GST fusion protein using protein extracts from the S2 cell line. The Lola zf5-GST fusion protein or a GST-only control were coupled with glutathione-agarose beads, incubated with S2 cell lysate, washed, fractionated by SDS-PAGE, and analyzed by immunoblot analysis using JIL-specific antibody (Fig. 2A). Whereas the GST-only control showed no pull-down activity, Lola zf5-GST was able to pull down JIL-1 as detected by the JIL-1 antibody. In addition, we performed co-immunoprecipitation experiments using embryonic lysates. For these immunoprecipitation experiments, proteins were extracted from 0–6 h embryos, immunoprecipitated using either JIL-1- or Lola zf5-specific antibodies, fractionated on SDS-PAGE after the immunoprecipitation, immunoblotted, and probed with antibodies to Lola zf5 and JIL-1, respectively. Fig. 2B shows an immunoprecipitation experiment using Lola zf5 antibody where the immunoprecipitate is detected by JIL-1 antibody as a 160-kDa band that is also present in the embryo lysate. This band was not present in lanes where immunobeads only were used for the immunoprecipitation. Fig. 2C shows the converse experiment: JIL-1 antibody immunoprecipitated a 105-kDa band detected by Lola zf5 antibody that was also present in embryo lysate but not in control immunoprecipitations with immunobeads only. These results strongly indicate that Lola zf5 and the JIL-1 kinase are present in the same protein complex.

The Complex lola Locus Encodes Multiple BTB Domain-containing Proteins Each with Different Zinc finger Motifs—Two alternatively spliced isoforms of lola, Lola long and Lola short, have been previously characterized (10). Lola long is a sequence-specific DNA-binding protein with C4HC and C4H2
zinc finger motifs, whereas Lola short only has a very short COOH-terminal tail segment without zinc finger motifs (10, 11). Fig. 3A shows the domain structure of these two isoforms as compared with Lola zfs. Lola zfs has an NH2-terminal common region shared by all cloned Lola isoforms that is followed by an isoform-specific COOH-terminal region (Fig. 3A). The Lola common region is encoded by four exons and contains a 120-amino acid NH2-terminal BTB domain as well as a nuclear localization signal (Fig. 3, A and B). The COOH-terminal domain of Lola zfs contains tandem C2H2/C2HC zinc finger motifs (Fig. 3A). The position of the Lola zfs zinc fingers are very close to the common region in contrast to the Lola long isoform in which the zinc fingers are positioned at the COOH-terminal end.

Our identification of Lola zfs as a novel zinc finger-containing protein within the locus prompted us to survey the region for additional exons with zinc finger motifs. Consensus zinc finger motifs include the sequences CXXC, HXXXXH, and HXXXXXH. We searched the lola genomic region for long open reading frames (ORFs) containing any of these zinc finger motif consensus sequences. A total of fifteen putative exons containing zinc finger motifs were identified following this strategy as summarized in Fig. 4A. From this analysis we predict that at least 17 splice variants are generated from the lola locus. Fifteen of these each have a different zinc finger domain (zf1–zf15), whereas two use exons without zinc finger motifs. Fig. 4B shows an alignment of the 15 different zinc finger domains within the locus. Two isoforms, Lola zf4 and zf14, have only a single C2HC zinc finger domain, whereas the remaining splice variants have tandem C2HC/C2HC or C2HC/CHC zinc finger domains (Fig. 4B). We propose to name the various novel zinc finger domain-containing Lola isoforms according to the order of the zinc finger domain they contain, hence the name Lola zfs.

To verify that these putative isoforms, the majority of which were not predicted by the genome project, were indeed expressed, we used PCR to amplify isoform-specific cDNA fragments from the 0- to 24-h yeast two-hybrid cDNA library based on the assumption that all of the Lola isoforms share the same common regions as the known isoforms. In this way the expression of thirteen out of the fifteen predicted zinc finger-containing exons was confirmed by direct sequencing of such PCR products (Fig. 4A, indicated in black). In addition, we searched the Drosophila EST database with the genomic DNA sequence of each isoform and found further EST support for expression of six of the 13 exons containing zinc finger motifs. We also identified an EST clone (GM27815) representing the second Lola isoform without a zinc finger domain and have named it Lola short-like (Fig. 4A).

The coding regions of most of the Lola isoforms are generated by splicing the four common exons together with a single exon containing the different zinc finger domains. However, by sequencing the ESTs and the PCR amplification products we identified a number of smaller exons without zinc finger motifs that are utilized by Lola zf1 and zf8 (Fig. 4A). In addition, the locus has four different 5′-UTRs (5′a through 5′d) that may
further amplify the diversity of transcripts generated from this locus (Fig. 3B). By sequencing ESTs of the various Lola splice variants, we have obtained evidence that each of the four 5′-UTRs has been utilized into at least one transcript (Fig. 3B). Interestingly, we identified two ESTs for Lola zf5 that used different 5′-UTRs (5′a and 5′c, respectively). Transcripts with different 5′-UTRs may allow for the fine regulation of their spatial and temporal expression patterns. Although Lola zf5 is the only example where this alternative utilization has been confirmed, it is likely that other isoforms are regulated by the same splicing mechanisms.

Developmental Expression of Lola zf5—To determine the expression pattern of Lola zf5, we carried out Northern blot analysis using mRNA samples from representative developmental stages (Fig. 5). As a probe we used a Lola zf5 isoform-specific cDNA fragment from the COOH-terminal coding region and 3′-UTR. Lola zf5 mRNA migrates as a single band with an approximate molecular size of 3.9 kb on 1% agarose-denaturing gels. Potential differences in size between transcripts using alternative 5′-UTRs would not be resolved on these gels. Lola zf5 mRNA was abundant in 0- to 2-h embryos, and this level of transcript was maintained in embryos 2–6 h after egg laying (Fig. 5). However, the Lola zf5 mRNA level began to decrease in 6- to 12-h embryos and could not be detected in postembryonic stages except at low levels in female adults.
The JIL-1 Kinase Interacts with a Novel Lola Isoform

The JIL-1 and Lola zf5 interact in vivo, we explored genetic interactions between mutant alleles of lola and JIL-1 by generating double-mutant individuals containing both lola00642 and JIL-1EP(3)3657. The lola00642 allele contains a recessive lethal P element insertion and fails to complement the lethality of many P element insertion alleles of lola. By PCR amplification of the flanking region of lola00642 followed by direct sequencing, we found that the insertion site is 438 bp downstream of 5’-UTRc and 54 bp upstream of 5’-UTRd (Fig. 3B). Because Lola zf5 mRNA contains either the 5’-UTRa or the 5’-UTRc at the 5’-end of the transcript, the insertion of a P element within the Lola zf5 transcription unit is likely to disrupt expression of both splicing alternatives of Lola zf5. The JIL-1EP(3)3657 allele is a hypomorphic allele that can be maintained in a homozygous stock for only a few generations due to the low hatch rate and recessive semi-lethality (6). The hatch rate of JIL-1EP(3)3657 homozygous embryos produced by homozygous parents is as low as 4–7% when compared with the hatch rate of wild type Oregon-R embryos (Ref. 6 and this study). We generated double mutants by crossing a chromosome containing the lola00642 allele into a homozygous JIL-1 hypomorphic EP(3)3657 background. Interestingly, individuals homozygous for JIL-1EP(3)3657 that also contain a lola00642 allele can be maintained indefinitely as a stock. This suggests that heterozygous lola00642 may function as a dominant suppressor of the JIL-1EP(3)3657 phenotype. To quantify the extent of rescue, we compared the numbers of JIL-1EP(3)3657 homozygous progeny with or without lola00642 from a single cross (Fig. 7A). Although equal numbers of curly- and straight-winged phenotypic classes are expected in matings of lola00642/CyO; JIL-1EP(3)3657/JIL-1EP(3)3657 males with +/+; JIL-1EP(3)3657/JIL-1EP(3)3657 females, 2.3 times more flies with straight wings were observed than curly wings (Fig. 7A). In this cross straight-winged flies carry a lola00642 allele, whereas curly-winged flies do not. The difference in numbers observed for the two classes was statistically significant (p < 0.005, χ²-test). To exclude the possibility that the apparent rescue phenotype is due to an enhancer of JIL-1EP(3)3657 phenotype on the CyO second chromosome balancer, we also set up a control cross in which the lola00642 chromosome is not present. In the control cross, we did not observe any statistically significant evidence (p > 0.1, χ²-test) that the CyO balancer chromosome affects the viability of JIL-1EP(3)3657 homozygotes (Fig. 7A). The control crosses were per-

\[ \text{Fig. 6. Western blot analysis of Lola proteins.} \]

A. protein extracts from selected stages of Drosophila development were fractionated by SDS-PAGE, immunoblotted, and labeled with the Lola zf5-specific mAb 7F1 (upper lanes) or with tubulin antibody as a control (lower lanes). The Lola zf5 protein was only detectable during early embryogenesis and was absent at postembryonic stages. B. developmental Western blot as in A but probed with a Lola polyclonal antiserum likely to recognize all the different Lola isoforms. Multiple Lola isoforms were labeled by the antiserum at the various developmental stages. C. immunoprecipitation (ip) of lysates from 0- to 6-h embryos were performed using Lola zf5 antibody (mAb 7F1) coupled to immunobeads (lane 1 and 2) or with immunobeads only as a control (lane 3) and analyzed by SDS-PAGE and Western blotting using mAb 7F1 (lane 1) and Lola antiserum (lane 2 and 3) for detection. Lola zf5 was detected as a 105-kDa protein by mAb 7F1 in lane 1. The lower bands (arrow) are comprised of 7F1 IgG antibody, which was pulled down in the immunoprecipitation. Rabbit polyclonal Lola antiserum also recognized the 105-kDa Lola zf5 band but additionally labeled two protein bands co-immunoprecipitating with Lola zf5. None of the three bands recognized by the Lola antiserum were detected in the beads only control lane.

~12 h after egg laying and could not be detected at postembryonic stages, including adult females (Fig. 6A). The lack of Lola zf5 protein in female ovaries suggests that Lola zf5 is not translated from maternally stored transcripts until after fertilization. These results indicate that the functional expression of Lola zf5 is restricted to early embryogenesis.

In previous studies a polyclonal Lola antibody was made to the Lola common region that would be expected to recognize all the Lola isoforms (10). In developmental immunoblots using this Lola polyclonal antibody we detected multiple bands throughout all developmental stages (Fig. 6B). In early embryos (0–6 h) three major bands, including one migrating at 105 kDa, can be detected on the immunoblots. In 12- to 22-h embryos at least 15 bands can be recognized by the polyclonal Lola antibody suggesting that most of the Lola isoforms are expressed at this stage. Some isoforms also appear to be present in later developmental stages such as third instar larvae as well as adults (Fig. 6B). To test whether the 105-kDa protein detected by the Lola antiserum corresponded to Lola zf5 as we would predict, we performed immunoprecipitation experiments with the mAb 7F1 of protein extracts from 0- to 6-h embryos. The immunoprecipitations were fractionated by SDS-PAGE, immunoblotted, and detected with mAb 7F1 and Lola antiserum, respectively (Fig. 6C). As shown in Fig. 6C Lola antiserum recognizes three major bands, including one of 105 kDa that is also labeled by mAb 7F1 and thus is likely to represent the Lola zf5 protein. Interestingly, the presence of the two additional bands labeled by the Lola antiserum and not present in the control lane suggests that Lola zf5 may be involved in heterodimer formation with other Lola isoforms (Fig. 6C, lane 2).
The JIL-1 Kinase Interacts with a Novel Lola Isoform

A, presence of a heterozygous lola06642 allele increases the viability of JIL-1EP33657 (JIL-1) homozygous animals (histograms to the right). lola06642/CyO; JIL-1EP33657/JIL-1EP33657 males were mated with +/+; JIL-1EP33657/JIL-1EP33657 females. JIL-1EP33657 homozygotes with a lola06642 allele (histogram in black) enclosed at a rate 2.3 times greater than JIL-1EP33657 homozygotes with a wild type lola allele (histogram in white; normalized to 100%). The difference in numbers observed for the two classes was statistically significant (p < 0.005, \( \chi^2 \) test). Control crosses were performed by mating +/CyO; JIL-1EP33657/JIL-1EP33657 males with +/+; JIL-1EP33657/JIL-1EP33657 females. Presence of the CyO balancer alone did not affect viability of homozygous JIL-1EP33657 animals, as statistically equivalent (p > 0.1, \( \chi^2 \) test) numbers of curly (normalized to 100%) and straight-winged flies were observed in the F1 progeny (left white and black histograms). B, rescue of JIL-1EP33657/JIL-1EP33657 lethality by lola06642 occurs during embryogenesis. Only 7.2% of embryos from control matings of JIL-1EP33657/JIL-1EP33657 flies hatched into larvae (white histogram). In contrast, when lola06642/CyO; JIL-1EP33657/JIL-1EP33657 flies were mated, the hatching rate of those embryos not homozygous for embryonic lethal lola06642/CyO chromosomes increased to 20% (black histogram). Thus, presence of the heterozygous lola06642 allele increased the hatch rate of JIL-1EP33657/JIL-1EP33657 flies 2.8-fold. This difference was statistically significant (p < 0.005, \( \chi^2 \) test). The numbers of animals counted in all classes are indicated at the bottom of each histogram. In B the number of embryos hatching from the total number of lola06642/CyO; JIL-1EP33657/JIL-1EP33657 individuals are shown.

In this study we provide evidence that the JIL-1 tandem kinase molecularly interacts with a novel isoform of the lola locus, Lolasf. This interaction was first detected in a yeast two-hybrid screen and subsequently confirmed by pull-down and cross immunoprecipitation assays. Furthermore, immunocytochemical labeling of Drosophila embryos shows that Lolasf is localized to nuclei. This localization is compatible with a direct interaction with JIL-1, because JIL-1 has been shown to be a nuclear kinase expressed throughout embryogenesis. An interesting feature of the lola locus is its complex splicing pattern, and we demonstrate that it contains at least 27 exons. Four of these code for a BTB domain and sequences with a nuclear localization signal common to all Lolasf splice forms. In addition, fifteen of the exons code for sequences with different zinc finger domains. From this analysis, we predict that a minimum of 17 different protein products are generated by the lola locus, fifteen of which contain zinc finger domains and two that do not. By PCR amplification of cDNAs from 0- to 21-h embryos and sequencing of ESTs, we have obtained confirming evidence that at least 15 different Lolasf polypeptides are likely to be encoded. We were not able to verify the existence of the two remaining isoforms; however, this could be due to their being only expressed at developmental stages or in tissues that we did not examine. We further provide evidence that the number of transcripts from the locus is enhanced by alternative splicing of four different 5'-UTRs. The utilization of different 5'-UTRs and exon shuffling may provide a way to finely regulate stage- and tissue-specific expression of multiple gene products from the locus that serve different functional roles. This kind of complex gene organization has previously been observed at other loci. The most extreme example may be the Dseam locus that codes for cell adhesion receptors in the Drosophila nervous system and that potentially can generate more than 38,000 Dseam receptor isoforms (41). Another example related to control of gene expression is the locus of the trithorax group protein med(mdg4). This locus encodes at least 21 different BTB domain-containing protein isoforms (42). At least one of these isoforms has been shown to associate with
Su(Hw) to exert gypsy insulator function preventing enhancer-promoter communication (43). Thus, differential splicing may be a general mechanism for BTB domain proteins to generate functional diversity. The BTB domain has been shown to promote dimerization and the residues necessary for this function have been identified for the Bab protein (16). Comparison between the Lola and Bab BTB domains show that all these residues are conserved in the Lola BTB domain indicating that it has the capacity for homodimer formation. Furthermore, our immunoprecipitation experiments strongly suggest that Lola zf5 forms heterodimers with other Lola isoforms. Formation of homo- or heterodimers between Lola isoforms, including Lola zf5 is likely to lead to different developmental consequences by modifying DNA binding specificities and/or affinities of the Lola zinc finger isoforms. The majority of BTB-containing zinc finger proteins described thus far have been observed to function as transcriptional repressors, and several of these have been shown to directly bind co-repressor components of histone deacetylase complexes to their dimerized BTB domains (reviewed in Ref. 44). Consistent with such a repressive activity it has been suggested that Lola long may reduce expression of copia transposable elements in the Drosophila nervous system (11). However, Cavarec et al. (11) also observed Lola-mediated positive regulation of copia transcription in the gonads indicating that Lola isoforms also can act as transcriptional activators. In support of this notion, they identified a D. hydei isofom of Lola that binds directly to the copia enhancer and positively regulates transcription in transfected S2 cells. However, this effect was abrogated in a dose-dependent manner when the D. melanogaster Lola long coding sequence was co-transfected. Our analysis shows that the zinc finger domains of the D. hydei Lola isoform are nearly identical to those of D. melanogaster Lola zf8, with only a single conservative substitution of serine to threonine in the second zinc finger domain. Thus, the expression of alternative Lola isoforms may determine whether Lola acts as an activator or a repressor within different tissues and at specific stages during development.

C2H2 zinc fingers are one of the most common DNA binding motifs found in eukaryotic transcription factors and are characterized by a small number of conserved residues that generate the folded ββα domain structure necessary to align the residues that coordinate binding activity (reviewed in Ref. 45). The identification of alternatively spliced isoforms of the lola locus containing different zinc finger arrangements in conjunction with the ability of Lola isoforms to generate heterodimers suggests that Lola complexes functioning as transcription factors may recognize a wide range of potential DNA regulatory sequences. However, it should be noted that many of the Lola isoforms have a very unusual arrangement of zinc finger domains that suggest they have the potential to be involved in protein-protein interactions as well. Although most zinc fingers are of the C2H2 class, in each of the Lola isoforms where the zinc finger is present as a tandem array, the first finger is of the unusual C2HC class. This atypical zinc finger domain is also found in MYST family histone acetyltransferases such as MOF, where it has been shown to be essential for nucleosome interactions (46). Additionally, this domain has been implicated in blocked non-histone proteins (47), RNA (48–50), and DNA (51, 52).

Our genetic experiments suggest that a reduction of protein levels resulting from mutation in the lola locus can act as a dominant modifier of a hypomorphic JIL-1 allele leading to an increase in embryonic viability. However, in these experiments the P element insertion into the lola locus is likely to perturb transcription of many of the Lola isoforms. Thus, we do not know which of the Lola isoforms are responsible for the genetic interaction or whether JIL-1 acts upstream or downstream. Thus, several scenarios for a functional interaction between Lola proteins and JIL-1 that enhances viability can be envisioned. On one hand, JIL-1 may act as a derepressor to counteract a potential repressive function of Lola zf5 or other Lola isoforms on gene expression. Derepression of the gene products from these loci may lead to enhanced viability. On the other hand, Lola zf5 or other Lola isoforms may normally act to down-regulate JIL-1 kinase activity by physically interacting with JIL-1. Consequently, the decrease in JIL-1 kinase activity observed in a hypomorphic mutant background may be alleviated by the reduction of its negative regulator in the lola locus. In a third scenario, the interaction of JIL-1 with Lola zf5 may indeed enhance transcription at some genes, but the loss in the lola mutant of other Lola isoforms that normally function to down-regulate gene expression in other contexts may counterbalance the reduced JIL-1 activity in the JIL-1 hypomorph. Thus, the interaction between JIL-1 and the lola locus may be highly complex and promises to provide new experimental avenues into exploring the mechanisms of modification of chromatin and/or the regulation of gene expression during early embryogenesis.

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