A New Enhancer of Position-Effect Variegation in *Drosophila melanogaster* Encodes a Putative RNA Helicase That Binds Chromosomes and Is Regulated by the Cell Cycle

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**ABSTRACT**

In *Drosophila melanogaster*, position-effect variegation of the *white* gene has been a useful phenomenon by which to study chromosome structure and the genes that modify it. We have identified a new enhancer of variegation locus, *Dmrnahel* (*hel*). Deletion or mutation of *hel* enhances white variegation, and this can be reversed by a transformed copy of *hel*. In the presence of two endogenous copies, the transformed *hel* behaves as a suppressor of variegation. *hel* is an essential gene and functions both maternally and zygotically. The HEL protein is similar to known RNA helicases, but contains an unusual variant (DECD) of the DEAD motif common to these proteins. Potential HEL homologues have been found in mammals, yeast and worms. HEL protein associates with salivary gland chromosomes and locates to nuclei of embryos and ovaries, but disappears in mitotic domains of embryos as chromosomes condense. We propose that the HEL protein promotes an open chromatin structure that favors transcription during development by regulating the spread of heterochromatin, and that HEL is regulated by, and may have a role in, the mitotic cell cycle during embryogenesis.

**POSITION-EFFECT** variegation (PEV) of euchromatic gene expression occurs when chromosome rearrangements place euchromatic genes next to heterochromatic regions. Variable spreading of heterochromatin into the juxtaposed euchromatic genes during development is thought to inactivate the genes, creating a clonally inherited variegated, or mottled, pattern of gene expression (Eisenberg et al. 1984; Henikoff 1990; Reuter and Speier 1992). PEV is manifested as a complete inactivation of gene expression in individual cells and is thought to occur at the level of transcription (Henikoff 1981; Rushlow et al. 1984; Kornher and Kauffman 1986).

The product of the *white* gene of *Drosophila* is required for red eye pigmentation and is sensitive to PEV. A chromosomal inversion, *In(1)wm***, places *white* next to the heterochromatic region of the X chromosome and causes variegation in *white* gene expression, resulting in a mosaic pattern of red eye pigmentation (Schultz 1936). Deletions and mutations in a number of loci dominantly suppress or enhance *In(1)wm** variegation.

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These genetic properties facilitate the identification of loci that are involved in PEV. Several genes that act as haplo-suppressors of PEV have been cloned, and most, if not all, appear to encode proteins that associate with or modify chromatin (Eisenberg et al. 1990, 1992; Reuter et al. 1990; Garzino et al. 1992; Tschiersch et al. 1994). HF-1 is a heterochromatin-associated protein encoded by *Su(var)*205 (also known as *Suvar(2)5*); *Su(var)*7 (also called *Suvar(3)7*) encodes a putative zinc-finger protein and the *modulo* gene produces a DNA-binding protein. *Su(var)*3-9 contains regions of similarity to both negative and positive transcription regulators. In addition, a protein phosphatase encoded by *Suvar(3)6* acts as a haplo-suppressor of PEV and has been postulated to modify structural components of heterochromatin, such as the zinc-finger protein encoded by *Su(var)*7 (Baksa et al. 1993). Two cloned haplo-enhancers of PEV also appear to encode chromatin-associated proteins. *E(var)*3-9D encodes a protein that contains a domain found in transcriptional regulators and that has been localized to polytene chromosomes (Dorn et al. 1993a), and *Triithorax-like* encodes another putative zinc-finger protein that most likely is the Drosophila GAGA factor (Farkas et al. 1994). The GAGA factor, initially deemed a transcription factor, has been implicated in ATP-dependent chromatin remodeling (Lu et al. 1993; Tsukiyama et al. 1994). Recently, modifier of *mdg4*, which turns out to be identical to *E(var)*3-9D, was shown to interact with suppressor of *Hairy wing* (*su(Hw)*) and to impart directionality on the chromatin-insulating ability of

Here we describe the cloning and identification of Dnmahel (hel), encoding a protein, HEL, with extensive similarity to known ATP-dependent RNA helicases. We demonstrate that the hel gene is a new holo-enhancer of PEV in Drosophila, and we further show that the HEL protein localizes to the nucleus and associates with chromosomes, intimating a role for a putative RNA helicase in chromatin structure. These results suggest that the HEL protein promotes an open chromatin structure that favors transcription by preventing the spread of heterochromatin. Furthermore, we have found that the HEL protein is present in transcriptionally competent nuclei (during interphase), but disappears as chromosomes condense and enter mitosis. The remarkable disappearance of the HEL protein in mitotic domains of embryos is of particular interest in light of the finding by WARBRICK and GLOVER (1994) that a controlling entry into mitosis.

Stock Center in Bowling Green, Ohio. The balancer chromosome in the lethal stocks in region 25E (SZIDONYA and REUTER 1988) were several of the mutant chromosomes contain additional lethal mutations positioned outside of the balancer chromosome.

MATERIALS AND METHODS

Genetic stocks: Deficiency stocks containing Df(2L)Jc120 and Df(2L)Jc49 (SZIDONYA and REUTER 1988), and the In(1)He1 mutant stock were provided by the Mid-America Drosophila Stock Center in Bowling Green, Ohio. The balancer chromosome in the deficiency stocks was replaced by the CyO, wg[wg] balancer chromosome (KASSIS et al. 1992), which expresses β-galactosidase in the wingless pattern. EMS and X-ray-induced lethal stocks in region 25E (SZIDONYA and REUTER 1988) were provided by J. SZABAD. The following alleles from complementation groups J25-J30 of the SZIDONYA and REUTER lethal mutant collection were confirmed by genetic complementation to lie within Df(2L)Jc49, b24, z15*, x59, z18*, a7, z63, b2*, b7, a26 and b23. These alleles were tested as heterozygotes over Df(2L)Jc49 for rescue of lethality by transformed hel insertions of several of the mutant chromosomes contain additional lethal mutations positioned outside of Df(2L)Jc49 also was tested over Df(2L)Jc49 for modification of PEV with the In(1)He1 chromosome. The hel[wg] mutation in hel was recombined away from a second z15* lesion in the ike gene (see below) and recombinant hel[wg] recombinants were balanced with the CyO, wg[wg] balancer chromosome.

Molecular cloning and localization of the hel gene: A cDNA clone encoding nuclear lamin (GRUENBAUM et al. 1988) was provided by P. FISHER and used to screen a dp on bw genomic DNA library in the cosmids vector pWE16 (R. PADGETT and W. GELBART, unpublished results). Two cosmids clones, cosmids 4 and 8, were isolated and used to screen a 4- to 8-hr embryonic cDNA library (BROWN and KAFATOS 1988). A 1.6-kb cDNA encoding the hel gene was recovered.

The hel cDNA was used to probe genomic Southern blots containing DNA from wild-type flies and from flies heterozygous for deficiencies Df(2L)Jc120, Df(2L)Jc49, and Df(2L)Jc49 to locate the hel gene by dosage comparison.

Sequencing and sequence analysis: Genomic and cDNA clones of D. hel were sequenced using Sequenase (United States Biochemical) as described by DEL SAL et al. (1989). Similarities to DEAD box family members, the rat homologue, and recently, the human and pig homologues were found by searching protein databases at NCBI using the BLASTP program (ALTSCHUL et al. 1990). D. MILLER discovered the homology between HEL and the yeast Saccharomyces cerevisiae protein, HER1, during a homology search of GenBank. He also pointed us toward a possible homologue in the worm, Caenorhabditis elegans. A partial sequence of the worm homologue was available as an expressed sequence tag under GenBank accession no. Z14797. The corresponding C. elegans cDNA, cm5d4 (WATERSTON et al. 1992), was provided by R. WATERSTON, and sequencing of the cm5d4 cDNA was completed for this study. Comparisons of homologues were done using the GCG Sequence Analysis Software Package (GENETICS COMPUTER GROUP 1991). The Drosophila and complete C. elegans sequences can be found under GenBank accession nos. L06018 and U08102, respectively.

The hel[wg] mutation was cloned from homozygous hel[wg] mutant pupae (identified as animals that failed to differentiate abdominal structures) by PCR. Three hel clones were sequenced as described above or according to the Sequenase manual provided by United States Biochemical.

P-element-mediated transformation: The hel gene is included in a 4.5-kb Eagl/BglII genomic DNA fragment. This fragment first was cloned from cosmid 8 into the Ncol/BamHI sites of pCaSpeR2 (THUMMEL et al. 1988) for P-element transformation of Drosophila. The transformation construct contains ~700 bp of genomic DNA 5' to the putative translation initiation site of the hel protein, and ~3000 bp 3' to the putative translation termination site. Flies were transformed by injection of y w embryos containing the endogenously expressed Δ2-3 transposase (ROBERTSON et al. 1988) and recovered as described (SPRADLING 1986). One of three transformant lines contained a second chromosome insertion of the pCaSpeR2 construct suitable for studying effects of PEV in concert with the In(1)He1 chromosome. The pCaSpeR2 vector expresses low levels of white from a minitransgene construct (w[wg]) as a transformation marker, and produces an orange background throughout the eye in this transformant line. Visual observation of PEV was possible with the transformant line containing pCaSpeR2, but because the orange background made spectrophotometric quantitation of PEV difficult, a second transformation construct was made using the Y.S.E. vector (PATTON et al. 1992), which expresses the yellow body pigmentation gene (y) as a transformation marker. For this, the 4.5-kb Eagl/BglII genomic DNA fragment was removed from pCaSpeR2 as an Eagl/Xbal fragment and cloned into the Ncol/Xbal sites of the Y.E.S. vector to obtain transformants without background eye color from the transformation vector. One transformant line containing the Y.S.E. construct was used to measure PEV effects of hel on white gene expression from In(1)He1. Similar visual results were obtained using the pCaSpeR2 transformation vector, expressing low levels of w[wg] as a transformation marker and yielding orange eyes (not shown), as were obtained with the Y.E.S. vector, which expresses the y body pigmentation gene as a marker (shown in Figure 3).

Genetic crosses and quantitation of PEV: Transformants containing the Y.E.S. construct (see above) were used to quantitate effects of hel on PEV. To assay the gene dosage effect of Df(2L)Jc49 on PEV, we used spectrophotometric quantitation of the eye pigments in females offspring from reciprocal crosses of y In(1)He1; Df(2L)Jc49/CyO with y w; P[hel, y]/+. To test the effect of hel[wg] on PEV we assayed the eye pigment levels in the female offspring of In(1)He1; z2/2;sz15/CyO males crossed with y w; P[hel, y]/+ females. Mean OD492nm readings
for four (hel[P]) or five (Df(2L)hel4) separate eye pigment extractions per genotype were determined. For each extraction, heads of 12 flies aged 5-7 days were homogenized in 200 μl chloroform and 200 μl 0.1% ammonium hydroxide and centrifuged for 2 min, and 100 μl of the aqueous phase was used for spectrophotometry without dilution (ASHBURNER 1989). The extracts were normalized relative to the two dose values in each of the four or five trials after subtracting the basal reading (from yw heads).

**Generation of mutant hel germ line clones**: Homozygous mutant hel clones were generated in the germ line using the autosomal FLP recombinase-dominant female sterile technique (CHOU and PERRIMON 1996). This technique employs the yeast FLP recombinase, which induces recombination at homozygous FRT sites, and the ovo" dominant female sterile mutation for selection of clones. Because the original hel mutant chromosome, za15, also carries a mutation in the closely linked thick sens (tku) gene, we used three independent hel el17 recombinants, which remove the tku" mutation, to put onto the FRT<sup>10</sup> chromosome. To generate clones, females of genotype y<sup>el17</sup> P<sup>(neo FRT<sup>40</sup>A)cyO</sup> were crossed to males of genotype y w FLP<sup>2/+</sup>; P<sup>rwo<sup>mc</sup> ovo<sup>1/d</sup></p>
WATERSTON similarity) from the rat was found (p47, 82% amino acid identity; NAIR et al. 1990; LIANG et al. 1994). We found that two deficiencies that delete region 25E, and may fall into the DM family of helicases (BORK et al. 1988) with deficiencies (Df) that encompass the hel gene was confirmed. Placement of nuclear lamin (nlam) was based on molecular analyses (data not shown). The relative order of complementation groups within Df(2L)ch4 is undetermined.

(DEC'D rather than DEAD). At least one other helicase-like protein, the plum pox virus C1 protein, has cysteine in this position (LAIN et al. 1989), but it also contains a histidine in place of the second aspartic acid (DECH), and may fall into the DEA family of helicases (BORK and KOONIN 1993). At least four members of the DEAD box family, eIF4A, p68, An3 and VASA, and the viral C1 protein have been shown to possess RNA helicase activity (HIRLING et al. 1989; ROYEN et al. 1990; LAIN et al. 1990; GURURAJAN et al. 1994; LIANG et al. 1994). Other members are assumed to unwind RNA on the basis of sequence comparison to the proven helicases.

**HEL homologues in other organisms:** The amino acid sequence of the HEL protein was used to search databases for homologues in other organisms. A possible functional homologue of HEL (based on sequence similarity) from the rat was found (p47, 82% amino acid identity; NAIR et al. 1992). After submitting the HEL sequence to Genbank, a second putative homologue from the budding yeast, S. cerevisiae, surfaced (HER1, 63% identity; D. MILLER, personal communication). In addition, partial sequence of a cDNA recovered from the nematode worm, C. elegans (Cernahel; WATERSTON et al. 1992), suggested the existence of another homologue of HEL. We completed the sequencing of the C. elegans CDNA (see MATERIALS AND METHODS), revealing a high degree of similarity between the encoded protein and HEL (79% identity). A comparison of the four protein sequences is shown in Figure 2B. A recent BLAST search for homologues of HEL has identified additional apparent homologues in humans and pigs (BAT1, 83% identity; PEELMAN et al. 1995).

**hel modifies PEV:** Previously, SZIDONYA and REUTER (1988) described two loci in region 25F-26B of the second chromosome that behave as haplo-enhancers and one triplo-suppressor of In(1)w* variegation in the presence of a strong suppressor of PEV, Su-var(2)F1*. We found that two deficiencies that delete region 25E, Df(2L)ch4 and Df(2L)ch4, also act as haplo-enhancers of variegation from In(1)w* in the absence of any suppressors (data not shown, see Figure 1). Southern blot analyses revealed that the hel gene is removed by the two deficiencies (data not shown), so we tested whether hel could influence PEV.

Flies were transformed with genomic DNA encoding hel by P-element-mediated transformation. Transformation constructs contained ~700 bp of genomic DNA 5' to the putative translation initiation site of the HEL protein, and ~1300 bp 3' to the putative translation termination site. Crosses were made to see whether transformed copies of hel could rescue reduced white gene expression from In(1)w* caused by Df(2L)ch4 enhancement of PEV. Transformed hel was capable of restoring white expression to unmodified In(1)w* levels in the presence of Df(2L)ch4 (Figure 3; cross A in Table 1). The eye pigment level in single dose flies was significantly lower than in flies with two hel gene doses (paired t-test; P < 0.001). The enhancement of variegation was rescued by a transformed copy of hel to a level not significantly different from that of two endogenous copies. Therefore, hel encodes a gene that, when deleted, causes enhancement of PEV. We have identified a mutation in hel (see next section) that behaves in a similar way as the deficiency. In addition, transformed hel mediates an increase in eye pigment level over that of the two endogenous copies (P < 0.05), suggesting that hel also behaves as a triplo-suppressor of PEV (Table 1).

**hel is an essential gene:** In addition to the haplo-enhancer of variegation function we describe here, at least five homozygous lethal complementation groups are uncovered by Df(2L)ch4 (SZIDONYA and REUTER 1988; see Figure 1). We have found that P-element transformation constructs containing a genomic copy of the hel gene fully rescue lethality caused by a single homozygous recessive mutation, sz15*, indicating that the sz15* mutation resides in the hel gene and that hel...
is an essential gene. We also have determined that the \( \text{he}^{\text{157}} \) mutation acts as a haplo-enhancer of \( \text{In}(1)\text{w}^{\text{m4}} \) variegation in \( \text{he}^{\text{157}}/+ \) heterozygotes (cross B in Table 1; \( P < 0.05 \)), indicating a loss-of-function phenotype similar to that seen with \( Df(2L)\text{c4}^* \). Furthermore, the enhancement of variegation caused by one copy of the \( \text{he}^{\text{157}} \) mutation is reversed by a transformed copy of \( \text{hel} \) (Table 1). In cross B, we again saw the triplo-suppressor effect of \( \text{hel} \) on PEV (Table 1; \( P < 0.01 \)). Thus, heterozygosity of \( \text{hel} \) (in \( \text{he}^{\text{157}}/+ \) animals) results in enhancement of PEV, while homozygosity of \( \text{hel} \) (in \( \text{he}^{\text{157}}/Df(2L)\text{c4}^* \) animals) is lethal. Lethality caused by a loss of \( \text{hel} \) occurs during embryonic, larval and pupal stages of development. This variability is most likely related to the strong maternal expression of \( \text{hel} \) (next section). It is also possible that the \( \text{he}^{\text{157}} \) mutation is not a complete null mutation since \( \text{he}^{\text{157}} \) may enhance PEV to a lesser extent than does \( Df(2L)\text{c4}^* \) (Table 1; we recommend caution in comparing the results between these crosses done at different times). This could alternatively be explained if more than one enhancer of PEV lies within \( Df(2L)\text{c4}^* \). A small percentage of mutant embryos show signs of morphological defects such as incomplete involution of the head and gut, \( \text{he}^{\text{157}} \) homozygotes surviving to the pupal stage develop small heads and appendages, and thoraces that are often truncated posteriorly, but most or all abdominal structures fail to differentiate. These characteristics were used to identify \( \text{he}^{\text{157}}/\text{he}^{\text{157}} \) homozygous pupae for PCR cloning of the mutant \( \text{hel} \) gene. Upon DNA sequencing, a glutamic acid to lysine substitution was identified at position 245 of the mutant \( \text{HEL} \) protein.

Effects on PEV by \( \text{hel} \) include a maternal component: The influence of \( \text{hel} \) on \( \text{In}(1)\text{w}^{\text{m4}} \) variegation includes a maternal effect. Male offspring of \( \text{In}(1)\text{w}^{\text{m4}}, Df(2L)\text{c4}^*/+ \) females (with one dose of \( \text{hel} \)) express little \textit{white}, whether or not they receive the \( Df(2L)\text{c4}^* \) chromosome (data not shown), and the female progeny express less \textit{white} than female progeny of \( \text{In}(1)\text{w}^{\text{m4}} \) females (with both doses of \( \text{hel} \)). Different overall levels of eye pigments in female offspring from the two reciprocal crosses shown in Table 1 (cross A) illustrate the maternal effect associated with \( \text{hel} \). \( \text{HEL} \) mRNA is supplied to embryos maternally and is present throughout embryogenesis (data not shown). These results suggest that maternal \( \text{HEL} \) protein, in addition to zygotically expressed HEL, promotes transcriptional competence during development.

\( \text{hel} \) is required during oogenesis: Homozygous mutant \( \text{hel} \) clones were generated in female germlines using the autosomal FLP recombinase-dominant female sterile technique (CHOU and PERRIMON 1996) and the \( \text{sz15}' \) mutation in \( \text{hel} \) (see MATERIALS AND METHODS). Females with \( \text{he}^{\text{157}}/\text{he}^{\text{157}} \) germlines lay very few eggs, and the eggs that are laid are small, often collapsed and sometimes have fused dorsal appendages. No obvious defect before late oogenesis was detected upon examination of dissected ovaries with germline clones. Egg chambers develop to late stages before showing signs of degeneration and resorption. Similar results were obtained with three independent \( \text{he}^{\text{157}} \) recombinants. These results indicate that wild-type \( \text{hel} \) activity is required during oogenesis and are consistent with the maternal effect on PEV.

The \( \text{HEL} \) protein is nuclear and associates with chromosomes: \( \text{HEL} \) protein was expressed in bacteria and used to generate a rabbit polyclonal antibody against \( \text{HEL} \). The polyclonal anti-HEL antisera was used on Western blots of total protein from 0- to 4-hr-old embryos (Figure 4). A strongly reacting, major band of the expected molecular weight of the endogenous HEL protein (~47 kD) was seen with immune serum, but not with preimmune serum. A few weakly reacting, minor bands were seen with both immune and preimmune sera.

The antibody then was used to determine the cellular location of endogenous HEL protein in embryos, larval salivary glands and adult ovaries (Figure 5). In all three cases HEL protein was found in the nucleus in cells that express it. In no case was nuclear staining detected when preimmune serum was substituted for antibody-containing serum. A relatively low cytoplasmic background color reaction occurred with both preimmune and immune sera.

HEL protein clearly is found in association with chromosomes in salivary glands, although it sometimes appears to be located around the chromosomes as well (Figure 5A). It appears to be associated with all regions of the chromosomes; there is no evidence that HEL binds only at particular loci. Strong nuclear staining is apparent in most cells on the outer, largely epidermal, surface of embryos (Figure 5B), but notable areas of much lower staining intensity, corresponding to mitotic domains described by FOE (1989) and by FOE \textit{et al.} (1993), can be seen in early-mid embryonic stages when mitotic cell cycles become asynchronous in the Drosophila embryo (Figure 6). HEL also is expressed in ovaries where heavy staining is seen in large, polyplody nurse cell nuclei and in smaller follicle cell nuclei as the follicle cells migrate between the nurse cells and across each egg chamber (Figure 5C).

Nuclear expression of \( \text{HEL} \) protein disappears during mitosis: Because we observe regionally reduced HEL staining in the mitotic domains of asynchronously dividing embryos (described by FOE 1989 and FOE \textit{et al.} 1993), we examined in more detail the relationship between HEL and the mitotic cell cycle. To visualize areas of mitosis in developing embryos more clearly, we used confocal microscopy on embryos double-stained with the HEL antibody and an antibody against \( \beta \)-tubulin to detect disintegration of cytoplasmic microtubule networks and subsequent formation of nuclear mitotic spindles (see FOE 1989 and FOE \textit{et al.} 1993). In individual and composite images generated from the
The *hel* gene encodes a protein with similarity to RNA helicases. (A) DNA sequence and deduced protein sequence of the Drosophila *hel* gene (GenBank accession number L06018). DNA sequence found in both the cDNA and genomic clones of this gene encodes a protein with similarity to RNA helicases. (B) Schematic of the exon/intron structure of the *hel* gene. (C) Triple staining for HEL, β-tubulin and DAPI (Figure 6), though a slightly scattered pattern can be seen upon careful inspection. Various states of microtubule breakdown and spindle formation are apparent (Figure 6). Triple staining for HEL, β-tubulin and DAPI (data not shown) has allowed us to interpret the mitotic stages of these structures under our experimental conditions. In preparation for mitosis, depolymerization of cytoplasmic microtubules can be identified by loss of microtubule breakdown and spindle formation are apparent. In preparation for mitosis, depolymerization of cytoplasmic microtubules can be identified by loss of microtubule breakdown and spindle formation are apparent. A closer look at each domain reveals that different cells within a domain can be at various stages of mitosis. Cells near the center of a domain are often the first to enter mitosis and then mitosis often proceeds toward the periphery of a domain (Foe et al. 1993; Figure 6), though a slightly scattered pattern can be seen upon careful inspection.
to enter mitosis, and thus are at relatively early mitotic stages. Robust HEL protein expression is seen in nuclei as these cells enter mitosis. Moving slightly more interiorly into the domain (Figure 6), HEL still is detectable as spindles begin to form, although at reduced intensity. As the spindle continues to form, condensing chromosomes move toward the metaphase plate, and HEL seems to follow this movement as it fades. By the time the chromosomes gather on the metaphase plate, HEL is absent (see FOE et al. 1993; data not shown). HEL protein persists in nondividing cells as they become mitotically quiescent and migrate into the embryo and in nuclei throughout the outer surface of embryos (data not shown) when most of these cells have stopped dividing and have settled in G1. HEL protein accumulates soon after mitosis (when cytoplasmic microtubule networks begin to reassemble) in the epidermis of embryos completing cycle 14, before acquisition of a G1 phase (see FOE et al. 1993), and gaps in HEL immunoreactivity in the epidermis of embryos other than in the mitotic domains do not arise, suggesting that HEL also is present during DNA synthesis or S phase. Polyten salivary gland cells, which alternate between G and S cycles, contain HEL protein, although we do not yet know

The HEL protein appears to be present throughout interphase in embryos. HEL must be present in G2 since it is evident in nuclei as cells prepare for mitosis at an embryonic stage that includes a G2 phase (preceding M), and because HEL protein persists in nondividing cells that later become the amnioserosa and that arrest in G2 (see FOE et al. 1993; data not shown). HEL also is found in germ cells as they become mitotically quiescent and migrate into the embryo and in nuclei throughout the outer surface of embryos (data not shown) when most of these cells have stopped dividing and have settled in G1. HEL protein accumulates soon after mitosis (when cytoplasmic microtubule networks begin to reassemble) in the epidermis of embryos completing cycle 14, before acquisition of a G1 phase (see FOE et al. 1993), and gaps in HEL immunoreactivity in the epidermis of embryos other than in the mitotic domains do not arise, suggesting that HEL also is present during DNA synthesis or S phase. Polyten salivary gland cells, which alternate between G and S cycles, contain HEL protein, although we do not yet know
whether it is during the G or the S phase (or both) that HEL associates with the chromosomes in these cells. Taken together, our results suggest that HEL protein is expressed throughout G1, S and G2 phases of the cell cycle in Drosophila.

DISCUSSION

We have identified a new gene of Drosophila, hel, that encodes a protein that is similar to members of a family of ATP-dependent RNA helicases. We have shown that hel is both an enhancer of PEV and an essential gene. The maternal contribution of hel also influences PEV, and examination of mutant hel germline clones indicates that hel is required for oogenesis. An antibody generated against bacterially expressed HEL protein recognizes a protein of expected size on Western blots, and reveals that HEL is expressed in nuclei and associates with salivary gland chromosomes. The HEL protein disappears in mitotic domains of embryos, indicating that HEL is regulated by the cell cycle and opening the intriguing question of whether HEL is involved in cell cycle control during development.

HEL shares several motifs with known RNA helicases: Several of the regions conserved between HEL and members of the DEAD box-containing RNA helicase family (see Figure 2C) have been implicated in particular molecular functions (refer to LINDER et al. 1989; PAUSE and SONENBERG 1992; PAUSE et al. 1993). The ATPase A/I domain (AXXXYGKT) is required for ATP binding, and the ATPase B/II motif (DEAD) is involved in ATP hydrolysis and the coupling of ATPase and helicase activities. Motif III (SAT) has been implicated in RNA unwinding, and region VI (HRIGRXXR) is required for RNA binding and ATP hydrolysis. A mutagenesis study of the VASA protein of Drosophila has revealed that a glycine to glutamic acid change in the ARGXD domain abrogates the ability of this DEAD box-containing helicase to unwind RNA (LIANG et al. 1994). DORN et al. (1993b) have reported the cloning of the translation initiation factor eIF4A of Drosophila. The fly eIF4A protein is 73% identical to the mouse translation factor. HEL is ~35% identical to both mouse and fly eIF4A proteins.

HEL is likely to function as an ATPase: DNA encoding the potential homologue of HEL in the rat (p47) was obtained through screening of an expression library with antibodies against an ATPase (NAIR et al. 1992). ATP binding and hydrolysis domains (see LINDER et al. 1989; PAUSE and SONENBERG 1992) of all of the putative HEL homologues (including HEL itself) are conserved with members of the DEAD box family of ATP-dependent RNA helicases. One notable exception is the DECQD variation itself, which is found in all HEL homologues. We suspect that this substitution would not preclude ATPase activity (see below).

Does HEL unwind RNA? Although variegation is thought to act through a change in chromatin structure, we consider the possibility that HEL may act on RNA, rather than DNA, because of its similarity to RNA

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**FIGURE 3.** hel is a modifier of PEV. Transformation with hel rescues the Df(2L)clP4-induced enhancement of white variegation. Shown are eyes of selected flies with identical X chromosome genotypes (y In(1)y++;y w) but different hel gene dosages. In(1)y++ induces variegation of the white gene in the presence of the two endogenous copies of hel (+/CyO) but this variegation is enhanced if one copy of hel is deleted (+/Df(2L)clP4). Addition of a transformed copy of hel (Plhel y+/Df(2L)P4) restores the variegation to the original level. Three copies of hel (Plhel y+/CyO) moderately suppress the variegation. See Table 1 for quantitation of the eye pigments in these genotypes.
the plum pox virus Pase-HEL fusion protein when incubated in vitro, indicating RNA helicase activity and which is required for RNA binding (PAUSE et al. 1992), also is conserved in HEL. In addition, a highly conserved glycine in the RGXD domain, shown to be essential for RNA unwinding activity of the VASA protein (LUNG et al. 1993). A second motif, SAT, which is critical for RNA unwinding by VASA (LUNG et al. 1993), is conserved in HEL. However, we have not been able to demonstrate helicase activity in an initial attempt with bacterially expressed glutathione S-transferase-HEL fusion protein when incubated in vitro with a double-stranded RNA template (provided by N. METHOT and N. SONENBERG). If HEL is a functional RNA helicase, it may require additional factor(s) and/or specific template(s).

How might HEL influence PEV? Strong association of HEL protein with salivary gland chromosomes and nuclear localization of HEL in embryonic and ovarian tissues intimate a direct role for HEL in chromatin structure. HEL may act at the level of the chromosomes, perhaps by unwinding or removing mRNA from the site of transcription, consequently keeping the chromosome accessible to further transcription. Another possibility is that HEL may discourage heterochromatin spreading by associating with chromosomes in a complex that may include an RNA component. Alternatively, HEL may affect PEV indirectly by regulating the expression of specific genes, such as chromosomal proteins.

Clues that implicate RNA in the regulation of transcriptional competence have been uncovered. The MALELESS (MLE) protein of Drosophila regulates dosage compensation by associating with the X chromosome in males, resulting in increased transcription from the single male X chromosome (KURODA et al. 1991). MLE is a likely homologue of human RNA helicase A (Lee and Hurwitz 1993), a demonstrated RNA helicase, and can be removed from chromosomes with RNase (BASHAW and BAKER 1996). Two recently identified Drosophila male-specific RNAs, roX1 and roX2, associate with X chromosome chromatin (Amrein and Axel 1997; MELLER et al. 1997). Perhaps HEL affects PEV by acting on chromatin-associated RNAs such as these. Mammalian dosage compensation occurs via X chromosome inactivation in females. A nuclear RNA transcribed from the XIST/Xist locus has been implicated.
FIGURE 4.—Western blot analysis of HEL. Western blot analysis of protein from 0- to 4-hr wild-type embryos. Although both the preimmune serum (left) and the anti-HEL immune serum (right) recognize a few minor proteins, only the anti-HEL immune serum identifies a prominent protein of molecular weight expected for the endogenous HEL protein (~47 kD).

in X inactivation (BROCKDORFF et al. 1992; BROWN et al. 1992). The XIST RNA complexes with the inactive X chromosome during interphase (CLEMSON et al. 1996). One might expect that an RNA helicase (or RNA-dependent ATPase) might prevent such a complex from forming on the X chromosome that remains transcriptionally active. Further experiments are required to determine if HEL affects PEV by any of these mechanisms.

HEL and the cell cycle: We have begun to characterize the possible role of HEL in cell cycle regulation by looking for cell cycle defects in hel mutant embryos and by overexpression of HEL in wild-type embryos, but both of these approaches led to inconclusive results. We have found that heF11'/Df(2L)F4 heterozygotes and heF11'/ homozygotes sometimes suffer what appear to be problems in morphogenetic movements during embryogenesis. These may be significant because FOE et al. (1993) report that the timing of mitosis is important in cells that undergo complex morphogenetic movements. However, these hel mutant defects occur at very low frequencies; stronger effects are most likely masked by the strong maternal contribution of HEL mRNA. Overexpression of HEL during cycle 14 of embryogenesis, using a transformed hel cDNA driven by a heat shock promoter, has not prompted obvious passage through mitosis. Nor is it clear that mitosis is delayed in these

overexpression experiments, because heat shock treatment of wild-type embryos can cause chromosome condensation, cell cycle delay and mitotic synchronization (MALDONADO-CODINA et al. 1993; L. J. LORENZ, personal observations), as well as microtubule breakdown (L. J. LORENZ, personal observations).
Figure 6.—HEL protein disappears during mitosis. Confocal images of mitotic domains (domains 1 and 5 as per Foe 1989) in the head region of an embryo, anterior to the cephalic furrow (CF) during early cell cycle 14 are shown. (A) A composite Z-series of optical sections through the outer lateral cell layer of an embryo double stained for HEL protein (red) and β-tubulin (green) clearly indicates that HEL disappears during mitosis. Bright HEL nuclear signals (red) are surrounded by cytoplasmic β-tubulin staining (green) in interphase cells, imparting a “pimento and green olive” appearance to these cells. (Yellow halos around nuclei are produced by the fusion into one dimension of two-dimensional cytoplasmic β-tubulin signals, slightly above and below nuclei, with nuclear HEL signals.) Mitotic domains 1 and 5 (numbered) appear as obvious dark regions (where HEL protein fades) containing β-tubulin positive mitotic spindles. Black and white photos show HEL alone (B) and β-tubulin (C). (D) Closeup view of domain 1 region boxed in A showing mitotic details. Cytoplasmic β-tubulin staining disappears before HEL is affected (▲) and subsequently, HEL begins to disappear (diamond). (E) Magnified view of region boxed in D. Dynamics of HEL and β-tubulin staining progress in the following steps: (1) cytoplasmic microtubules break down (▲), (2) HEL begins to fade (diamond), (3) spindle microtubules begin to appear when HEL is almost gone (→), (4) HEL is undetectable in anaphase (filled arrowhead) when the spindle is prominent with equatorial gapping and (5) in telophase (open arrowhead) when spindle remnants are seen between daughter nuclei.

Lorenz, personal observations), all of which complicate these studies. Nevertheless, HEL may have a regulatory role in the cell cycle that we have not yet been able to identify, because it has been shown that overexpression of a hel cDNA in the fission yeast, S. pombe, can suppress premature mitosis induced by loss of the two known inhibitory kinases of CDC2, Weel and Mik1, (Warbrick and Glover 1994), though there is no effect on wild-type yeast. Perhaps we could uncover a role for HEL in cell cycle regulation in Drosophila embryos by overexpression of a stable form of HEL that cannot be degraded at mitosis. This approach was useful in characterizing the roles of the A and B CYCLINS (Sigrist et al. 1995).
If there is a role for HEI in cell cycle regulation, it would help to consolidate the possibility of a link between PEV and cell cycle control. There is growing evidence that these two processes may be intimately associated. A cloned haplo-suppressor of PEV, Su(var)3-6, encodes a protein phosphatase involved in mitosis (AXTON et al. 1990; BASKA et al. 1993). Mutations in Su(var)3-6 can prompt over-condensed chromosomes and abnormal chromosome segregation. Furthermore, the transcriptional activator and cell cycle regulator, EZF, also influences PEV (SEUM et al. 1996). Finally, the Drosophila gene encoding PCNA is a recessive suppressor of PEV (HENDERSON et al. 1994). PCNA functions during DNA replication and repair, and exists in complexes with various cyclins and CDKs (cdk-like kinases), including CYCLINS A and B and CDC2 (ZHANG et al. 1993). HEI clearly affects PEV, and understanding the functional basis of the mitotic disappearance of HEI will further illuminate the relationship between these two processes.

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