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The RNA Polymerase II 15-Kilodalton Subunit Is Essential for Viability in *Drosophila melanogaster*

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A small, divergently transcribed gene is located 500 bp upstream of the suppressor of Hairy-wing locus of *Drosophila melanogaster*. Sequencing of a full-length cDNA clone of the predominant 850-nucleotide transcript reveals that this gene encodes a 15,100-Da protein with high homology to a subunit of RNA polymerase II. The RpII15 protein is 46% identical to the RPB9 protein of *Saccharomyces cerevisiae*, one of the smallest subunits of RNA polymerase II from that species. Among those identical residues are four pairs of cysteines whose spacing is suggestive of two metal-binding "finger" domains. The gene is expressed at all developmental stages and in all tissues. Two deletions within the *RpII15* gene are multiphasic lethal deletions, with accumulation of dead animals commencing at the second larval instar. Ovary transplantation experiments indicate that survival of mutant animals to this stage is due to the persistence of maternal gene product throughout embryogenesis and early larval development. The *RpII15* gene product is thus necessary for viability of *D. melanogaster*.

The complex multimeric nature of RNA polymerases has been recognized for many years. RNA polymerases in eukaryotes consist of 9 to 14 subunits (for reviews, see references 43, 45, and 53). Some of these subunits are similar or identical in all three forms of polymerase in all species, while others are form and species specific. The largest subunits of RNA polymerase forms I, II, and III of the yeast *Saccharomyces cerevisiae* are sufficiently similar to allow detection of all three proteins by using antibodies generated to any one of the peptides (18). Furthermore, the fifth-, sixth-, and eighth-largest subunits (RPB5, RPB6, and RPB8) of RNA polymerase II are not just similar to those of the other polymerases but are shared between all three forms in yeast cells (51). This sequence conservation is not restricted to *S. cerevisiae*. The largest subunit of RNA polymerase II in yeast cells has regions with a high degree of interspecies homology, including similarity to *Drosophila*, mammalian, and even prokaryotic RNA polymerases (2, 3, 7, 11, 15). While there are many similarities between RNA polymerase complexes, each polymerase has unique features as well. In fact, the number and immunological relatedness of subunits varies in different enzyme forms and in different species (45). To understand the functional and phylogenetic relationships of different polymerases, it will be necessary to molecularly analyze the components of each. With the recent cloning of *RPB9* (50), the identification of the genes for all the subunits of RNA polymerase II, the enzyme responsible for transcription of the protein coding genes, has nearly been completed in *S. cerevisiae* (reviewed in references 43 and 53). Analysis of deletions constructed for each of these polypeptides indicates that 7 of these 10 loci are required for viability.

While molecular analysis of the RNA polymerase subunits in *S. cerevisiae* is almost complete, identification of component genes in other eukaryotes is just beginning. RNA polymerase II from *Drosophila melanogaster* comprises at least 12 electrophoretically separable subunits (22). The sequences of the two largest subunits have been reported (13, 20). Both of these loci have been shown to be essential for viability. In addition, a third subunit, for which there are no known mutations, has recently been cloned and sequenced (16a). This gene encodes the protein identified as the 18-kDa subunit on denaturing protein gels. Despite the limited number of cloned subunits, *Drosophila* genetics has already proven to be a powerful tool in the study of subunit interactions and interactions with other proteins, such as transcription factors (14, 30, 31, 48). Identification of other subunit genes will facilitate this analysis.

Here we report the cloning, sequencing, and phenotypic analysis of *RpII15*, a gene encoding an essential small subunit of RNA polymerase II from *D. melanogaster*. This subunit is the homolog of the recently characterized *RPB9* gene of *S. cerevisiae* (50).

MATERIALS AND METHODS

Isolation and maintenance of *Drosophila* strains. Fly stocks were maintained at 22.5°C and 65% relative humidity. The $RpII15^{Z23}$ allele was generated as a lethal mutation in combination with *Df(3R)* red^{P52}. Males homozygous for *red*, *ebony* were fed 0.024 M ethyl methanesulfonate (25) and mated to doubly balanced *TM6B/TM3* females. Progeny were mated to *Df(3R)* red^{P52}/*TM6B* flies. Mutations which resulted in no *Df(3R)* red^{P52}/*red, ebony* flies thus delineated lethal complementation groups within the red^{P52} deficiency.

Isolation and enzymology of nucleic acids. Isolation of plasmid DNA, screening of lambda libraries, and DNA labeling and enzymology were carried out by standard procedures (27). Genomic DNA from *Drosophila* adults was prepared as described by Parkhurst et al. (35). Total RNA was isolated by homogenization in 10 mM Tris hydrochloride (pH 7.4)–0.1 M NaCl–1 mM EDTA–0.5% sodium dodecyl sulfate followed by phenol extraction and ethanol precipitation. Poly(A)$^+$ RNA was selected by chromatography on oligo(dT)-cellulose (4). Southern and Northern (RNA) analyses were done as described by Parkhurst et al. (35).

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DNA sequence analysis was performed by the dideoxy chain termination method (42).

P-element-mediated germ line transformation. P-element-mediated transformation was carried out as described by Rubin and Spradling (40), using the *white* gene as a selectable marker in the CaSpeR vector (37). The CaS X/K 5.3 plasmid was generated by the insertion of a 5.3-kb *Xba*I-*Kpn*I fragment from the bacteriophage 13R3 (35) into the *Xba*I-*Bam*HI sites of the CaSpeR vector. The plasmid was introduced into animals which are genotypically w^{67c23} y^2 sc^1 ct^6 f^1; bx^{34e} $su(Hw)^V/TM6$, Ubx^{P15} $su(Hw)^f$.

Polymerase chain reaction and cloning of amplified DNA. Genomic DNAs were subjected to the polymerase chain reaction to amplify sequences from the *su(Hw)* region (34, 41). A pair of primers containing restriction sites was used to amplify a fragment covering the entire transcribed region of *RpII15* by use of the Perkin Elmer/Cetus Amplitaq polymerase chain reaction kit and the Perkin Elmer/Cetus thermal cycler. The primers used were GGTGTCGTAACTT TCGCTGCAGCA and CCGGGCCGTGAACTGTGGAAT TCGCA, containing *Pst*I and *Eco*RI sites, respectively. This pair amplifies from base −1989 to base +1072 (as numbered in Fig. 2). Conditions were as recommended in the kit. Annealing was performed at 56°C, and polymerization was performed for 4 min per cycle for 30 cycles. Amplified DNA was treated with 1 U of Klenow enzyme at 37°C for 30 min to ensure complete fill in of the ends of the amplified fragments. DNA was phenol extracted, ethanol precipitated, and digested with the restriction enzymes present in the oligonucleotides used to generate the amplified fragments. Digested DNA was cloned by standard ligation and transformation techniques (27). Several independent clones from each *RpII15* allele were sequenced in order to avoid possible artifacts introduced by the *Taq* polymerase.

In situ hybridization. In situ hybridization to whole-mount embryos was performed as described by Tautz and Pfeifle (47). Hybridization to frozen sections was performed in the same manner, except that proteinase K treatment of sections was omitted. Frozen sections were prepared by embedding tissues in OCT compound and cutting 6-μm sections on a Slee cryostat. The wild-type animals used were w^{67c23} y^1. *RpII15* mutant animals were generated by mating $su(Hw)^V/$ *TM6B, Tb Hu* and $RpII15^{Z23}$/*TM6B, Tb Hu* flies. The $su(Hw)^V$ allele is a deficiency that deletes the *RpII15* gene (see Results). Mutant larvae were selected as Tb^+, and mutant adults were selected as Hu^+.

Ovary transplantation. Ovaries were transplanted as described by Clancy and Beadle (12), using third-instar larval donors of the genotype $su(Hw)^V/RpII15^{Z23}$. Mutant donors were generated by mating $su(Hw)^V$/*TM6B, Tb Hu* and $RpII15^{Z23}$/*TM6B, Tb Hu* flies and screening for Tb^+ female larvae. Donated ovaries were injected into female third-instar larvae which were wild type for *RpII15* but heterozygous for the dominant female sterile mutation ovo^{D1} (10). The resulting adult female hosts were individually mated to wild-type males and allowed to lay eggs. The viability of eggs from each host animal was then scored. As a control for the transplantation technique, ovaries were transplanted from heterozygous donors of the genotype $su(Hw)^V$/*TM6B, Tb Hu* or $RpII15^{Z23}$/*TM6B, Tb Hu*.

RESULTS

Gene distal to *su(Hw)* is essential for viability. Examination of the null phenotype of the suppressor of Hairy-wing gene has been problematic for many years. All known mutations of the *su(Hw)* locus which are not deletions are viable as homozygotes or in combination with a deficiency of the region, such as *Df(3R)* red^{P52}. In contrast, all combinations of deficiencies which include *su(Hw)* are lethal. This indicated that other genes in close proximity to *su(Hw)* are likely to be essential for viability.

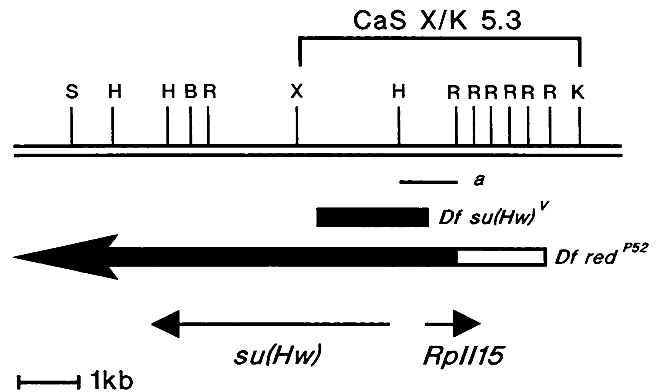

FIG. 1. DNA map of the immediate *su(Hw)* region. A restriction map of the *su(Hw)* region including the adjacent essential locus, *RpII15*, is presented. Thin lines with arrows represent the transcription units of the two genes. The upper bold line indicates the region which is deleted in $su(Hw)^V$. The lower bold line indicates the region which is deleted in *Df* red^{P52}, with the open box showing the uncertainty in the endpoint of the deficiency. Fragment *a* was used as a probe for Southern analyses and for library screening. The bracket above the map indicates the DNA fragment which was cloned into a P-element-containing vector for germ line transformation experiments. Restriction enzyme recognition sites are abbreviated as follows: S, *Sal*I; H, *Hin*dIII; B, *Bam*HI; R, *Eco*RI; X, *Xba*I; K, *Kpn*I.

One small lethal deletion is the $su(Hw)^V$ allele (described below). A map of the deletion can be found in Fig. 1. This deletion has one endpoint within the coding region of *su(Hw)* and extends 1.7 kb distally into adjacent sequences. A transcript from this region had previously been identified (35), but it was not determined whether the lethality of $su(Hw)^V$ results from loss of *su(Hw)* or of an adjacent gene. Woychik et al. have recently described the characterization of the yeast gene encoding the RPB9 subunit of RNA polymerase II and have found close homology between this gene and *Drosophila* sequences present upstream of the *su(Hw)* coding region (50). This suggests that the lethality of the $su(Hw)^V$ deletion might be due to lack of the *Drosophila* homolog of RPB9.

To address this question, a construct containing a 5.3-kb *Xba*I-*Kpn*I genomic DNA fragment lacking the complete *su(Hw)* coding region but including 4 kb of sequence distal to *su(Hw)* was made (Fig. 1). This fragment was inserted in the CaSpeR vector and reintroduced into flies of the genotype $su(Hw)^V$/*TM6* by P-element-mediated transformation. Flies homozygous for $su(Hw)^V$ were recovered from the transformed progeny, indicating that lethality of the allele had been rescued. Although the CaS X/K 5.3 plasmid contains part of the *su(Hw)* coding region, it lacks the Zn finger region that is essential for *su(Hw)* function, and it is therefore unable to rescue the *su(Hw)* phenotype. In addition, flies transformed with this plasmid do not accumulate detectable levels of *su(Hw)* protein as judged by protein electrophoresis (25) and Western (immunoblot) analysis (data not shown). It can thus be concluded that it is the gene immediately distal

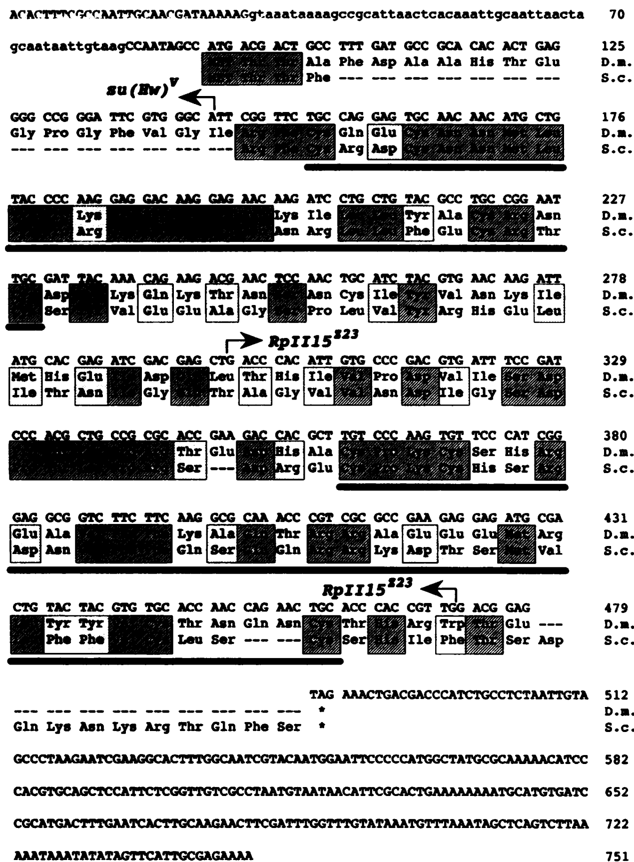

FIG. 2. Sequence analysis of the *RpII15* locus. The first nucleotide represents the start of transcription. The intron is denoted in lowercase letters. The encoded protein is shown under the nucleotide sequence, and the amino acid sequence of the *RPB9* protein from yeast cells (S.c.) is shown under that of the *Drosophila* (D.m.) protein. Identities between the proteins of the two species are indicated by cross-hatched boxes, and chemically similar amino acid substitutions are indicated by lightly stippled boxes. Chemically similar amino acids are grouped as by Schwartz and Dayhoff (44): Ala, Ser, Thr, Pro, and Gly; Asn, Asp, Gln, and Glu; His, Arg, and Lys; Met, Leu, Ile, and Val; Phe, Trp, and Tyr. Dashes are used where gaps were needed to align the protein sequences. The potential metal-binding domains are underlined. Arrows indicate the breakpoints of the $su(Hw)^V$ and $RpII15^{Z23}$ deletions.

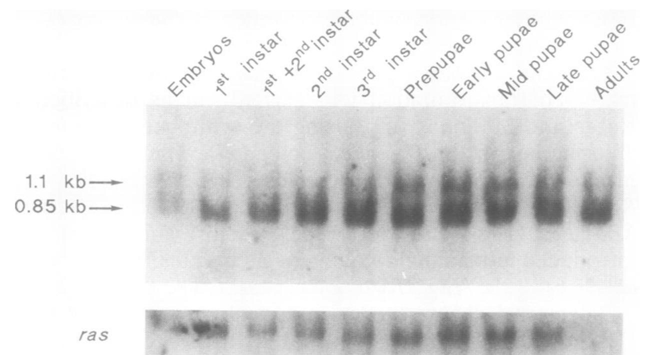

FIG. 3. Developmental transcription of the *RpII15* gene. Poly(A)$^+$ RNAs (3 μg) from successive 24-h collections of wild-type animals were electrophoresed on a 1% agarose–formaldehyde gel, blotted to a nylon membrane, and probed with a ^{32}P-labeled 0.83-kb *Hin*dIII-*Eco*RI fragment (probe *a* from Fig. 1). The lower autoradiogram shows the same blot probed with the *Drosophila ras2* gene to control for RNA loading on the blot.

to *su(Hw)*, and not *su(Hw)* itself, which is required for viability.

Essential gene distal to *su(Hw)* encodes an RNA polymerase II subunit. A probe of 830 bp spanning the *Hin*dIII-*Eco*RI sites (fragment *a* in Fig. 1) immediately distal to *su(Hw)* was used to recover recombinant lambda phages from a cDNA library constructed from the RNAs of adult female flies (38). One phage bearing a 767-bp insertion was subcloned and sequenced. An isolated genomic DNA fragment from that region was also sequenced. The results are presented in Fig. 2. Comparison of the genomic and cDNA sequences indicates that the gene consists of two exons and one intron. The exact 5′ end of the gene was not determined experimentally, but the good agreement between the size of the cDNA and the predicted size of the mRNA suggests that the cDNA is full length; in addition, the first seven nucleotides of the cDNA are a good match for the consensus sequence AT-CAG/TTC/T of *Drosophila* cap sites (19). The first nucleotide of this cDNA is a guanosine, which is not present in the genomic sequence. This likely indicates that the clone includes the true 5′ terminus of the transcript, as reflected by the presence of a noncoded G which is apparently diagnostic of reverse transcriptase attempting to copy the mRNA cap (9). The first exon is 29 nucleotides (nt) and contains no initiation codon. The 55-nt intron is followed by the 664-nt second exon, with 19 terminal adenosine residues in the cDNA clone. This exon contains a 129-amino-acid open reading frame encoding a protein with a predicted molecular mass of 15,100 Da.

Analysis of the open reading frame confirms the results of Woychik et al. (50), revealing a high homology with the RPB9 gene, which encodes a small subunit of the RNA polymerase II from the budding yeast *S. cerevisiae* (50). To be consistent with the existing nomenclature for *Drosophila* RNA polymerase II subunits, the gene will be referred to as *RpII15*, indicating that it is a subunit of RNA polymerase II and that its mass is 15 kDa. It is not clear at the moment which of the several biochemically defined subunits in this size range (15) is encoded by *RpII15*. The yeast and *Drosophila* proteins are 46% identical and more than 63% homologous, if conservative amino acid changes are considered (Fig. 2). Among the identical amino acids are eight cysteines. These are grouped in pairs with the sequence CXXC (except for one pair with CXXXXC), where X represents any amino acid. Two pairs are closely spaced in a manner suggestive of the zinc finger motif (6, 21). Potentially, the protein could then have two tetrahedral coordination sites for a metal cofactor.

***RpII15* transcripts are present throughout development.** Flies wild type for the *RpII15* locus were used as a source of RNA for Northern blot analysis. Samples were collected, and poly(A)$^+$ RNA was prepared, electrophoresed on an agarose-formaldehyde gel, and blotted to a nylon membrane. Each lane in Fig. 3 represents animals collected from successive 24-h intervals. Blots were probed first with a DNA fragment which recognizes the 5′ end of the *RpII15* transcript (*Hin*dIII-*Eco*RI 0.83-kb fragment; indicated in Fig. 1 as probe *a*) and then with a fragment from the *Drosophila ras2* gene (33) to control for the amount of RNA loaded in each lane. *RpII15* accumulates a major transcript of approximately 850 nt that is present throughout development (Fig.

3). The levels of *ras2* RNA in the adult lane cannot be seen in the autoradiogram shown in Fig. 3, but other Northern blots carried out with the same RNA preparation indicate that the adult lane contains approximately the same amount of RNA as the other lanes in the gel (data not shown). The size of the RNA correlates well with that of the cloned cDNA.

A minor transcriptional product of approximately 1,200 nt is also present in constant amounts over time. This transcript may well share the same 5′ end as the 850-nt transcript but includes a longer 3′ end. Sequencing of the genomic region 3′ to *RpII15* reveals that there are two adjacent and overlapping consensus polyadenylation signals 400 bp downstream of the signal used in the major transcript. Incomplete termination of transcription at the upstream site could therefore account for the presence of a minor product. It is unlikely that this RNA is derived from another locus with homology to *RpII15*, because no other genomic DNAs were detected by low-stringency Southern blot analysis when the same probe as described above was used (data not shown).

***RpII15* is transcribed in all tissues.** To determine whether there is spatial restriction to the expression of *RpII15*, in situ hybridization to RNA in animals of different developmental stages was performed. Figure 4 presents hybridization in wild-type embryos, larvae, and adults with the 830-bp *Hin*dIII-*Eco*RI fragment (probe *a* of Fig. 1) as a probe. It is clear that transcripts of *RpII15* are present in all tissues of the stages tested. Figure 4A and B show that embryos as early as germ band extension stage and as late as prehatching express *RpII15* in all cells. The ubiquitous nature of RpII15 RNA is also seen in all later stages of development (data not shown). Figure 4C is representative of expression in larval stages. In this panel, it can be seen that the anterior structures of the larva (brain, eye-antenna imaginal discs, etc.) express *RpII15*. The same is true for the representative adult structures shown in panels 4D and E, where expression is found in developing egg chambers of the ovaries and in thoracic flight muscle, respectively. This ubiquitous expression is not surprising, as *RpII15* shares its upstream control region with *su(Hw)*, another gene which is expressed constitutively in all tissues (16b). As can be seen in panel F, there is little or no detectable *RpII15* transcript in a mutant [$RpII15^{Z23}$/$su(Hw)^{V}$] adult escaper. These rare flies are apparently able to survive to adulthood by using only the maternally provided RpII15 product (see below). While there is no detectable transcript remaining in the animal after eclosion, this, of course, does not eliminate the possibility that RpII15 protein may be present. The lack of RNA does, however, imply that the two mutations used are incapable of producing stable *RpII15* transcripts.

Two mutations of *RpII15* are small deletions. There are two known mutations of the *RpII15* locus. The first is the mutation $su(Hw)^{V}$, already briefly described in this paper. It was generated by gamma irradiation of bx^{34e} males which were then mated to bx^{34e}, $su(Hw)^{2}$/*TM6* females (see reference 35 for a description of the mutagenesis scheme). The $su(Hw)^{V}$ allele was recovered because of strong suppression of the bx^{34e} phenotype in *trans* with the bx^{34e}, $su(Hw)^{2}$ chromosome. As mentioned above, the $su(Hw)^{V}$ allele is also homozygous lethal. $su(Hw)^{V}$ is a deletion, as evidenced by Southern blotting analysis shown in Fig. 5A. Compared with the wild-type bx^{34e} parental chromosome, $su(Hw)^{V}$ exhibits an extra fragment 1.8 kb smaller when digested with *Kpn*I-*Pst*I and with *Kpn*I-*Xba*I and probed with the 0.8-kb *Eco*RI-*Hin*dIII fragment (probe a in Fig. 5C). This lesion was confirmed by sequencing of the mutant region. Genomic DNA from heterozygous flies carrying the $su(Hw)^{V}$ allele was subjected to polymerase chain reaction to amplify the DNA region containing the *RpII15* gene. Polymerase chain reaction products were cloned, and several independent isolates were sequenced. In agreement with the Southern analysis estimate, it was found that $su(Hw)^{V}$ is a deletion of 1,747 bp from the 5′ side of the acidic domain in *su(Hw)* to the first cysteine in *RpII15* and including the 5′ control region for both genes (Fig. 1, 2, and 5C). In the parental chromosome, the sequence ACCGAA [reading the coding strand for *su(Hw)*] is present on both sides of the deleted region. In $su(Hw)^{V}$, only one copy of this sequence exists and the deletion lies directly at that site, implying that the deletion resulted from a homologous recombination event.

The second mutation known for *RpII15* was generated by ethyl methanesulfonate. Mutagenized males were mated to females carrying a third chromosome balancer. Resulting progeny were mated to flies bearing the deletion *Df(3R)* red^{P52}, and mutations lethal in combination with the deficiency were recovered (30a). One such mutation, $RpII15^{Z23}$, also exhibits a restriction fragment anomaly. DNA was prepared from flies heterozygous for $RpII15^{Z23}$ and $su(Hw)^{f3}$ and from $su(Hw)^{f3}$ homozygous flies. This DNA was digested with *Hin*dIII-*Xmn*I and *Dra*I and then subjected to Southern analysis. Fragment a in Fig. 5C was used to probe the blot. It can be seen in Fig. 5B that the $RpII15^{Z23}$ allele contains restriction fragments which are approximately 200 bp smaller than those present in $su(Hw)^{f3}$ (which is wild type for *RpII15*). DNA from the *RpII15* locus of the $RpII15^{Z23}$ allele was amplified and cloned as described above. Sequencing of $RpII15^{Z23}$ indicates that it is a deletion of 174 bp of the 3′ half of the *RpII15* gene (Fig. 2 and 5C). The deletion includes the last four cysteines, and therefore, the entire second putative finger domain is missing in this mutant.

Mutation of *RpII15* causes late larval lethality. Both *RpII15* alleles, $su(Hw)^{V}$ and $RpII15^{Z23}$, are fully penetrant lethals as homozygotes. Flies of the genotype *RpII15*/*TM8* were mated, and the progeny were reared at various temperatures under optimal growth conditions. None of the viable adult progeny was homozygous for a mutant *RpII15* allele. On the other hand, when heteroallelic [$su(Hw)^{V}$/$RpII15^{Z23}$] combinations of these mutations were made, some of the *RpII15* flies survived to adulthood. The viability was low (less than 5% of the expected). The survival rate was highly dependent on the growth temperature. Flies reared at 18°C never produced viable mutant adults, while flies reared at 22.5 or 25°C grew more slowly than their heterozygous siblings but did occasionally survive to adulthood. This finding is consistent with results obtained from the analysis of a yeast *RPB9* mutant; namely, that growth outside the optimal temperature range for the animal dramatically decreases viability of the mutants. However, in the case of the yeast mutant, viability is high in the optimal temperature range. In flies, the *RpII15* survivors are the exception rather than the rule.

To examine the onset of *RpII15* lethality, heterozygous *RpII15*/ry^{506} flies were crossed, eggs were collected, and development was observed. In all crosses, the rate of hatching of embryos to larvae was greater than 95%. The accumulation of dead animals initiated in the second larval instar. No obvious structural abnormalities were visible in these dead animals. Lethality was multiphasic, with differential rates of death at each developmental stage, depending on the genotype and growth conditions of the mutant animals.

***RpII15* is required maternally for embryogenesis.** The fact

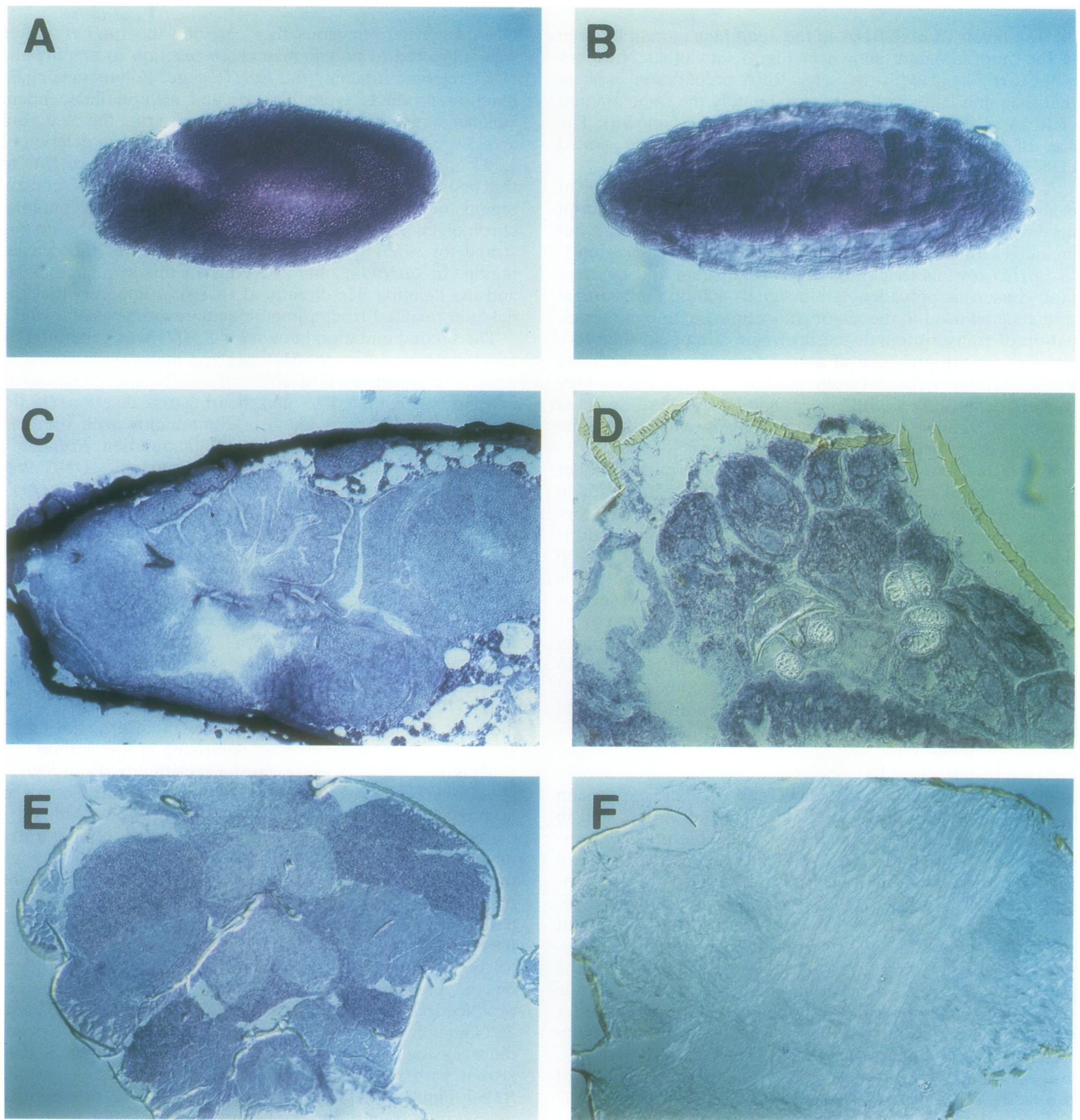

FIG. 4. In situ localization of *RpII15* transcripts. Fixed tissues were hybridized to a probe prepared by random priming a 830-bp *Hin*dIII-*Eco*RI fragment (probe *a* in Fig. 1) with digoxigenin-labeled dUTP. Wild-type animals were used as the source of tissue for the following: (A) whole mount of a germ band extension stage embryo; (B) whole mount of a late embryo near hatching; (C) frozen section of a larval head; (D) frozen section of a female adult abdomen, including developing egg chambers; (E) frozen section of adult thoracic muscle; (F) frozen section of thoracic muscle from a transheterozygous mutant adult escaper [$su(Hw)^V/RpII15^{Z23}$].

that death of homozygous mutant *RpII15* animals does not commence until mid-larval development seems to indicate that *RpII15* product is not required until that stage of development, that maternally supplied *RpII15* product is sufficient to sustain the animals through embryogenesis and early larval development, or that there is another gene which is capable of substituting for *RpII15* function during early development. It is unlikely that there is another gene with overlapping function, because low-stringency Southern analysis using the coding region of *RpII15* was unable to detect homologous DNA in the *Drosophila* genome. To distinguish between the two former possibilities, ovaries were transplanted from $RpII15^{Z23}/su(Hw)^V$ larvae into wild-type larval female hosts. Adult host animals which developed ovaries

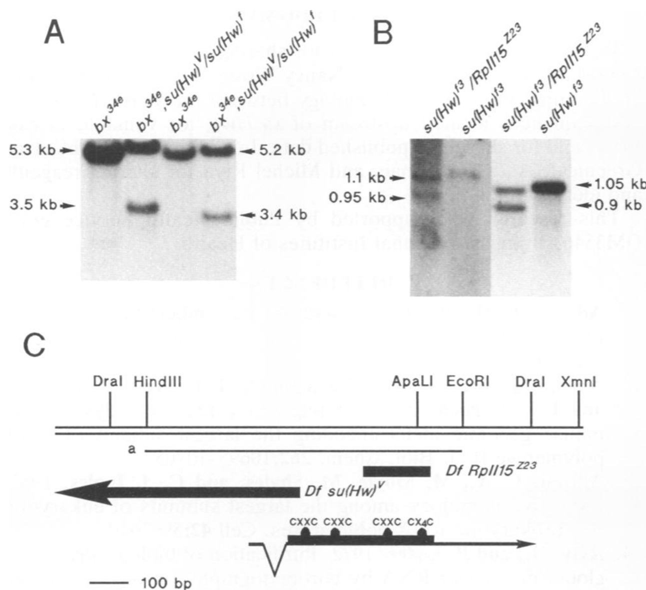

FIG. 5. Southern analysis of *RpII15* mutant alleles. Genomic DNAs from wild-type and mutant stocks were digested with various restriction enzymes, electrophoresed on a 0.7% agarose gel, transferred to nitrocellulose membrane, and hybridized with ^{32}P-labeled DNA from the 0.83-kb *Hin*dIII-*Eco*RI fragment designated probe *a* in Fig. 1. (A) DNA from bx^{34e} [the parental chromosome of $su(Hw)^V$] and from bx^{34e}, $su(Hw)^V/su(Hw)^f$ flies was digested with *Kpn*I and *Pst*I and loaded in the first two lanes. The same DNAs were treated with *Kpn*I-*Xba*I and loaded in the third and fourth lanes. (B) DNAs from $su(Hw)^{f3}/RpII15^{Z23}$ and from $su(Hw)^{f3}$ flies were digested with *Hin*dIII-*Xmn*I and loaded in the first two lanes, while digestions of these DNAs with *Dra*I were loaded in the third and fourth lanes. (C) DNA map of the *RpII15* locus. The transcription unit for *RpII15*, including the intron-exon structure, is represented at the bottom of the figure. The bold line on the transcript shows the location of the coding region, including the cysteine pairs. The bold lines above the transcript delineate the limits of the two *RpII15* deficiencies. Fragment a was used for Southern analyses and library screening.

were then mated to wild-type males and allowed to lay eggs. Five such females were capable of laying eggs. None of these eggs was competent to complete embryogenesis. Most of the eggs examined failed to undergo any recognizable embryonic morphogenesis, while others underwent defective or abortive gastrulation, and a few were capable of developing to a recognizably segmented stage. Even the relatively normal embryos arrested in embryogenesis and failed to hatch as larvae. In contrast, four control females which were injected with ovaries from a wild-type host were all capable of producing viable and fertile offspring. This suggests that there is a requirement for *RpII15* during embryogenesis but that the maternal product from heterozygous mothers is capable of promoting development of homozygous progeny until the mid-larval stage.

DISCUSSION

Sequencing of genomic and cDNA clones has revealed that the gene adjacent to *su(Hw)* is homologous to a small subunit of RNA polymerase II identified in the yeast *S. cerevisiae*. The *RPB9* locus in yeast cells was cloned by virtue of the association of its gene product with the other known subunits of RNA polymerase II (50). The high degree of homology between the two species (46% identical and more than 63% conservative at the amino acid level) is an indication that the proteins are functionally similar. The level of similarity is not surprising considering that polyclonal antibodies to the RPB9 protein in yeast cells cross-react with *Drosophila* protein extracts (18). The function of *RPB9* protein, or the *Drosophila* homolog, in the enzyme complex is presently unknown.

Eight cysteine residues are clustered in four pairs within the 129-amino-acid open reading frame of *RpII15*. The unusually high use of cysteine in this protein together with the fact that these eight residues are identical in yeast cells and *D. melanogaster* implies that these cysteines are important to the function of the protein. The first two pairs are separated by 18 amino acids, and the last two pairs are separated by 24 amino acids. This regular arrangement of cysteine pairs is often seen in metal-binding domains (5). RNA polymerase II is known to bind zinc (24, 26), so it is reasonable to speculate that RpII15 may sequester at least some of that zinc. The largest subunit of all three forms of eukaryotic RNA polymerase also contains a potential Zn-binding domain (2, 20, 28). This sequence is consistent with the Cys_2-His_2 Zn finger structure found in many DNA-binding proteins (6, 21). The first protein recognized to have this structure, transcription factor IIIA from *Xenopus laevis*, has been shown to bind both 5S DNA and 5S rRNA (17, 29, 36). In addition, the second largest subunits of the *S. cerevisiae* and *Drosophila* RNA polymerase II (B) contain potential metal-binding domains which may form Cys_4-type zinc fingers (13, 46). This is the same type of structure one would expect from the RpII15 subunit. A structure of this sort may also imply that RpII15 is involved in the interaction of RNA polymerase II with DNA. Alternatively, the RpII15 domain containing zinc fingers could be involved in protein-protein interactions with other RNA polymerase subunits, as has been shown to be the case for the largest and second-largest subunits of yeast RNA polymerase I (52).

When a genomic fragment containing the coding region of *RpII15* is used as a probe for a Northern blot, a major 850-nt transcript is detected. This RNA is present in samples derived from all developmental stages. Also, in situ hybridization has shown that the RNA accumulates in all tissues at all developmental stages. The presence of *RpII15* in all cells is not surprising in light of the fact that *su(Hw)* transcription is also constitutive. These two genes are transcribed divergently and therefore share a 500 bp upstream control region. It is reasonable to expect that an enhancer present in this region could act on both transcription units; thus their expression patterns would at least be overlapping, if not identical. Also, one could argue that expression of an essential RNA polymerase II subunit would probably be constitutive.

The two identified mutations of *RpII15*, $su(Hw)^V$ and $RpII15^{Z23}$, are small deficiencies. Both of these mutations are homozygous lethal and lethal in combination with the large deficiency $Df(3R)\ red^{P52}$. When heterozygous *RpII15* mutants were mated, the observed progeny hatched into larvae at the same high rate as the wild-type controls, indicating that lethality occurs after embryogenesis. Significant accumulation of dead animals does not begin until second larval instar. Death occurs over several developmental stages, ranging from first larval instar through pupal development. Most of the animals die in the mid- to late larval stages. This is perhaps due to the fact that a great deal of growth is occurring in the larval stages, and there is a concomitant requirement for a high rate of transcription.

Because $su(Hw)^V$ and $RpII15^{Z23}$ are deficiencies, the homozygous mutants are presumably incapable of producing any RpII15 protein. This is supported by the fact that mutant escaper animals accumulate no detectable transcripts. Despite this fact, the mutant animals are viable throughout early developmental stages. This suggests either that the RpII15 protein is not required early in development or that there is sufficient maternal product supplied to the embryo to maintain it to larval stages. The lack of temporal specificity in *RpII15* transcription indicates that it is always present. In addition, in situ hybridization indicates that this expression includes the developing egg chamber and all stages of embryogenesis. The presence of *RpII15* suggests a constant requirement for RpII15 protein; thus it is more likely that viability up to larval stages arises from the maternal contribution to the egg. This is further supported by the fact that mutant *RpII15* ovaries transplanted into hosts which are wild-type for *RpII15* cannot produce eggs which are competent to complete embryogenesis. A similar result was obtained by generation of germ line clones bearing a mutation in the large subunit of RNA polymerase II (32). If early viability of the mutants is indeed due to the maternal contribution of RpII15, then this indicates that either the RpII15 RNA or the protein or both are quite stable. This is also consistent with the finding that death does not occur until a stage of rapid cell proliferation and growth of the animal. During the second and third larval instars, the animal dramatically increases its size and cell numbers. This would dilute the remaining maternally derived RpII15 product and leave the larva incapable of maintaining a transcription rate sufficient for survival.

In contrast to *D. melanogaster*, the *S. cerevisiae* RPB9 product is apparently not essential for viability at standard growth temperatures (50). One could argue, however, that the partial deletion of the *RPB9* gene created to test viability may produce some small amount of functional gene product. While this is a formal possibility, it seems unlikely. Even if protein were produced, it would be lacking the entire first putative finger motif. Deletions and point mutations in a zinc finger are generally reported to abolish function of the protein (1, 8, 16, 39, 49). Alternatively, perhaps another protein can partially compensate for the loss of RPB9 in yeast cells. The same protein may not exist in flies, or it may have diverged functionally. Contrary to this hypothesis, low- or moderate-stringency screens for homologs in both species have failed to identify other genes (16b, 50). Perhaps the most likely explanation for the difference in requirement for this subunit from yeast cells and flies is that *RpII15* and *RPB9* serve similar or overlapping but not identical functions in the two species. More-detailed biochemical analysis of the proteins will be required to investigate this hypothesis.

The *Drosophila* and yeast mutants do have phenotypic similarity at temperature extremes. It was noted that *RPB9* mutants in yeast cells will not grow at temperatures outside the optimal range. It has also been noted that *RpII15* mutants are adversely affected by rearing at low temperature. There were no escaping *RpII15* homozygous mutant adults among thousands of heterozygous siblings when reared at 18°C. This increase in severity of the mutations at temperature extremes may indicate that in both species this RNA polymerase subunit has a role in sustaining the organism under adverse conditions. Perhaps in yeast cells this is the only essential role of *RPB9*, while in flies the *RpII15* has an additional essential function under standard growth conditions.

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