Reverse Genetics of Drosophila RNA Polymerase II: Identification and Characterization of *RpII140*, the Genomic Locus for the Second-Largest Subunit

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ABSTRACT

We have used a reverse genetics approach to isolate genes encoding two subunits of *Drosophila melanogaster* RNA polymerase II. *RpII18* encodes the 18-kDa subunit and maps cytogenetically to polytene band region 83A. *RpII140* encodes the 140-kDa subunit and maps to polytene band region 88A10:B1,2. Focusing on *RpII140*, we used *in situ* hybridization to map this gene to a small subinterval defined by the endpoints of a series of deficiencies impinging on the 88A/B region and showed that it does not represent a previously known genetic locus. Two recently defined complementation groups, *A5* and *Z6*, reside in the same subinterval and thus were candidates for the *RpII140* locus. Phenotypes of *A5* mutants suggested that they affect RNA polymerase II, in that the lethal phase and the interaction with developmental loci such as *Ubx* resemble those of mutants in the gene for the largest subunit, *RpII215*. Indeed, we have achieved complete genetic rescue of representative recessive lethal mutations of *A5* with a *P*-element construct containing a 9.1-kb genomic DNA fragment carrying *RpII140*. Interestingly, the initial construct also rescued lethal alleles in the neighboring complementation group, *Z6*, revealing that the 9.1-kb insert carries two genes. Deleting coding region sequences of *RpII140*, however, yielded a transformation vector that failed to rescue *A5* alleles but continued to rescue *Z6* alleles. These results strongly support the conclusion that the *A5* complementation group is equivalent to the genomic *RpII140* locus.

EUKARYOTIC transcription, the process by which information encoded in genomic DNA is transduced into a primary RNA transcript, requires an intricate assembly of RNA polymerase and transcription regulatory proteins. Prokaryotic transcription by comparison is less complex. A unique eubacterial RNA polymerase together with specificity factors (sigma factors) is sufficient to direct all cellular transcription. The archaebacteria also contain a unique RNA polymerase with structural features that vary between orders, but in general are intermediate between the eubacterial and eukaryotic RNA polymerases (ZILLIG, SCHNABEL and STETTER 1985). In contrast, eukaryotes contain three different classes of nuclear RNA polymerase that transcribe distinct subsets of genes. RNA polymerase I transcribes rRNA precursors, RNA polymerase II transcribes mRNA precursors, and RNA polymerase III transcribes tRNA, 5S and other small RNAs (ROEDER 1976).

Structural features of the three classes of eukaryotic RNA polymerase reflect a common evolutionary origin and similar functional properties. All three classes of eukaryotic RNA polymerases are structurally complex, consisting of 10 or more component subunits. Each contains two large subunits (M_r greater than 100 kDa) that are class-specific, three small subunits (M_r less than 30 kDa) that are shared in common among the three classes of enzymes, and several subunits that are specific to one or two classes (SENTENAC 1985; SAWADOGO and SENTENAC 1990, for reviews). Because the two largest RNA polymerase subunits together account for approximately 40–70% of the mass of these enzymes and because many of their domains have been evolutionarily conserved, it is clear that they perform fundamental roles in the process of transcription. Ideas of what some of these roles might be are emerging as a result of biochemical, genetic and molecular biological studies of the enzymes.

Cloning and sequence analysis of genes for several subunits of eukaryotic RNA polymerases have confirmed and extended ideas of their evolutionary relatedness. For example, interspecies DNA sequence comparisons revealed not only that the largest subunit of eukaryotic RNA polymerase II was a homologue of the *Escherichia coli* largest subunit, β' (ALLISON *et al.* 1985; BIGGS, SEARLES and GREENLEAF 1985), but also that the largest subunits of all three eukaryotic polym-

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erases were homologous (ALLISON *et al.* 1985; MEMET *et al.* 1988). Further studies indicated that similar relationships held for the second largest subunits of eukaryotic polymerases and the *E. coli* β subunit (FALKENBURG *et al.* 1987; SWEETSER, NONET and YOUNG 1987). Cloning and sequencing of representive archaebacterial RNA polymerase genes also confirmed earlier immunological studies which indicated that both large eukaryotic subunits share extensive homologies with their archaebacterial counterparts and that the archaebacterial RNA polymerases are more similar to the nuclear enzymes than to the eubacterial polymerase (PUHLER, LOTTSPEICH and ZILLIG 1989; LEFFERS *et al.* 1989).

While all the low molecular weight subunit genes have been isolated for RNA polymerase II from the yeast *Saccharomyces cerevisiae* (YOUNG 1991, for review) only two have been isolated from metazoans, a mammalian 23-kDa subunit gene (PATI and WEISSMANN 1989) and a Drosophila 15-kDa subunit gene (HARRISON, MORTIN and CORCES 1992). Thus, detailed molecular genetic information about most of the smaller subunits of higher eukaryotic RNA polymerase II is limited.

In addition to the approaches already mentioned, biochemical analyses of the RNA polymerases and their transcription factors (ZAWEL and REINBERG 1992, for a review) have provided much valuable information on the function and structure of the components of the transcriptional machinery. However, genetic analysis of RNA polymerases remains important, for example, to test the functional significance of apparent subunits and to establish that the biochemical functions assigned to specific subunits have physiological relevance. With these goals in mind, investigators have sought to isolate mutations that alter RNA polymerase II. The mycotoxin α-amanitin, a specific inhibitor of RNA polymerase II at low concentrations, has been very useful in this regard. For example, amanitin was used to select for mutations altering RNA polymerase II in mammalian cell lines. Regulation of amanitin resistant enzyme was demonstrated upon addition of amanitin (GUIALIS, MORRISON and INGLES 1979). Furthermore, certain amanitin-resistant myogenic cell lines display defective differentiation (CRERAR *et al.* 1983). In *Caenorhabditis elegans*, analysis of α-amanitin resistant RNA polymerase II mutants indicated that many of these mutants are defective in either oogenesis or gonadal development (ROGALSKI, BULLERJAHN and RIDDLE 1988).

Genetic analysis of RNA polymerase II in Drosophila was initiated with the isolation of an amanitin-resistant mutation (GREENLEAF *et al.* 1979) that was shown to alter the largest RNA polymerase II subunit (GREENLEAF 1983). Additional genetic analyses of this *RpII215* locus revealed that certain alleles displayed pleiotropic interactions with unlinked loci (MORTIN and LEFEVRE 1981). For example, a subset of alleles of the *RpII215* locus interact with *Ultrabithorax* mutations to give a specific homeotic transformation of the distal segment of the haltere toward wing (VOELKER *et al.* 1985; MORTIN and LEFEVRE 1981), a phenomenon referred to as the Ubx effect. In view of the later finding that homeodomain proteins such as Ubx function as specific transcription factors, it is likely that the RNA polymerase mutations alter in a fairly restricted way either the expression or functioning of such factors (MORTIN *et al.* 1992; MORTIN, KIM and HUANG 1988). Investigation of this type of RNA polymerase mutation potentially will afford new insights into transcriptional regulation during development.

The approach of selecting RNA polymerase II mutants with amanitin was limited to the isolation of mutations that map to the largest RNA polymerase II subunit (VOELKER *et al.* 1985). This approach has, however, been effectively expanded by the recovery of second site suppressors of certain *RpII215* alleles (MORTIN 1990). Selecting second site suppressors is potentially an extremely powerful way to identify components that interact with the largest subunit at the protein level, but it cannot be directed specifically to identify other subunits.

An alternative approach is that of reverse genetics, using antibodies directed against specific subunits to screen expression libraries. This approach has led to the isolation of genes for virtually all the RNA polymerase subunits in the yeast *S. cerevisiae* (RIVA *et al.* 1986). Subsequent genetic analyses of mutant versions of those genes have greatly increased our understanding of RNA polymerases in this unicellular eukaryote (see YOUNG 1991, for review of RNA polymerse II).

Having previously prepared antibodies that react with most subunits of Drosophila RNA polymerase II (WEEKS, COULTER and GREENLEAF 1982), we were in a position to apply the antibody screening approach to a metazoan. We report here results of our initial studies. Application of the reverse genetics approach to Drosophila RNA polymerase II has led to isolating clones for two additional subunits of the enzyme. Further analyses combining standard genetics, *in situ* hybridization and germline transformation allowed us to equate the cloned gene for the 140-kDa subunit with a previously unassigned complementation group defined *in vivo* by chemically induced lethal mutations. This work has thus defined a new gene, *RpII140*, as the genomic locus for the 140-kDa subunit. These results will facilitate more detailed investigations into RNA polymerase II subunit functions using the combined tools of genetics and biochemistry.

MATERIALS AND METHODS

λgt11 expression library screen: A *Drosophila melanogaster* OregonR strain genomic λgt11 library was obtained

from Gert Pflugfelder. Preparation and specificity of antisera used in these studies were as described (WEEKS, COULTER and GREENLEAF 1982; ROBBINS *et al.* 1984). Goat anti-RNA polymerase II IgG was diluted 1:500 and used to screen the expression library (SNYDER *et al.* 1987; YOUNG and DAVIS 1983). Primary antibody binding to filters was detected immunoenzymatically with swine anti-goat IgG secondary antibody coupled to horseradish peroxidase (HRP) (Tago Immunochemicals). The HRP substrate was 4-chloro-1-napthol in the presence of 0.2% hydrogen peroxide for 2–15 min until positive colonies could be detected above background staining of plaques. Positive plaques obtained from the initial screen were rescreened three to four times until purified.

Immunoblot analysis of β-galactosidase fusion proteins: Lysogens of immunoselected λgt11 clones were prepared in either *Y1089* or *BNN103* host strains as described (YOUNG and DAVIS 1983). Parallel cultures of lysogenic strains were incubated at 32° and either induced at 42° for 15 min and then incubated at 37° or were left uninduced at 32° throughout the same incubation period. Protein lysates from these cultures were prepared (YEN and WEBSTER 1982), concentrated with 5% trichloroacetic acid at 0°, washed with acetone, resuspended in Laemmli sample buffer and electrophoresed on 6% SDS (sodium dodecyl sulfate)-polyacrylamide gels (LAEMMLI 1970). Gels were electrophoretically transferred to nitrocellulose membranes (TOWBIN, STAEHLIN and GORDON 1979) and probed with available antisera directed to RNA polymerase II holoenzyme, or to isolated 215- or 140-kDa subunits (WEEKS, COULTER and GREENLEAF 1982) or with an antiserum directed to β-galactosidase (obtained from Cappel). Detection of primary antibody binding was achieved either immunoenzymatically as described above or by incubation with ^{125}I-coupled protein A.

Preparation of affinity purified anti-fusion protein antibodies: The procedure for preparation of bacterial lysates from induced lysogenic cultures was scaled up to accomodate a culture volume of 20–40 ml. An equivalent of 20-ml culture volume of whole lysate was resuspended in 600-μl Laemmli sample buffer and run on a 6% SDS-polyacrylamide preparative gel. Proteins were transferred to nitrocellulose, stained with India ink (HANCOCK and TSANG 1983), and a side strip excised from the blot was probed with the goat anti-RNA polymerase II antibodies to locate the position of the fusion protein on the stained nitrocellulose membrane. A membrane strip containing the fusion protein was carefully excised with a razor blade and incubated with anti-RNA polymerase II antibodies. After extensive washing, antibodies that specifically bind RNA polymerase II epitopes present on the tested fusion protein were eluted with low pH buffer as described (KELLY, GREENLEAF and LEHMAN 1986).

DNA blot analysis: Genomic DNA from transformant sublines or from wild-type OregonR strain Drosophila adults was digested with restriction endonucleases, electrophoresed on 1% agarose gels, denatured, transferred to GeneScreen hybridization membrane by the capillary blot technique (SOUTHERN 1975) or by vacuum blotting at 100 kPa for 30 min and probed with nick translated probes as described in the dextran sulfate protocol in the GeneScreen NEF-972 instruction manual.

RNA blot analysis: Total RNA was isolated from 0–12 hr embryos and from third instar larvae, then polyA$^+$ RNA was obtained by oligo dT-cellulose chromatography (BIGGS, SEARLES and GREENLEAF 1985), fractionated by formaldehyde gel electrophoresis (LEHRACH *et al.* 1977), transferred to NEN GeneScreen by the capillary blot procedure and probed with either *RpII140* or *RpII18* subclones .

Plasmid subclone constructions: Standard methods (MANIATIS, FRITSCH and SAMBROOK 1989) were used to construct plasmid subclones of *Eco*RI genomic fragments that span the *RpII140* coding region and flanking regions and that were derived from λgt11 phage clones of OregonR strain origin or from two Canton S-derived phages (λL120 and λL1a, isolated by SUSAN PARKHURST from a chromosomal walk that spans the 88A/B genetic region and obtained from RICK KELLEY). *Eco*RI genomic subclones of the *RpII18* gene were prepared from the initial λgt11 phage or from a homologous phage isolated from an EMBL4 genomic library (JOHN WEEKS, unpublished data). A 9.1-kb *Sst*I restriction fragment containing the *RpII140* transcription unit, 1.6 kb of 5′ flanking and 3.0 kb of 3′ flanking sequences was first subcloned into pUC18. Since no convenient restriction site for cloning this intact restriction fragment containing the *RpII140* gene directly into the CaSpeR transformation vector (PIRROTTA 1988) was available, the *Sst*I fragment was first cloned into pHSX, a kanamycin resistant plasmid that contains a polylinker *Sst*I site flanked by *Xba*I restriction sites. The 9.1-kb restriction fragment was then excised from the pHSX subclone with *Xba*I and cloned into an *Xba*I site in the CaSpeR *P*-element transformation vector to give pCaS*RpII140*, depicted in Figure 6.

To generate pCaS*RpII140*Δ, pCaS*RpII140* was treated with *Bam*HI, which cuts at coordinate ca. +1 (Figure 2) and at additional sites between this site and one near the 5′ end of the white gene (PIRROTTA 1988). After dilution, ligation and selection, the desired plasmid was obtained, from which the DNA between the *Bam*HI site at +1 and the white gene had been deleted. This plasmid thus retains intact "upstream" adult transcript and white genes, but carries a truncated, inactive *RpII140* gene.

Drosophila stocks: *Df(3R)redP93/Df(3R)293γ5e* was a generous gift of RICK KELLEY. The *red* deficiency strains were obtained from VICTOR CORCES, ALAN SHEARN, and RICK KELLEY. The relevant deletion endpoints for the *Df*s are diagrammed in Figure 3A. These *Df*s are also described in (LINDSLEY and ZIMM 1992) and (MORTIN *et al.* 1992, see especially Figure 2). Drosophila stocks were maintained on a cornmeal molasses medium that was sprinkled with dried yeast. Deficiency strains were maintained as heterozygotes over appropriate third chromosome balancer chromosomes. Stocks used for germline transformation were as described (ROBERTSON *et al.* 1988). Further description of stocks, balancers and genetic markers is given in (LINDSLEY and ZIMM 1992). Stocks used for genetic rescue experiments were constructed using the appropriate matings. Lethal mutations induced on the *red e* isochromosome were maintained as stocks balanced by *TM6B* (useful marker = Tubby, *Tb*), except for the γ-ray induced lethal mutations *A5*, *A7* and *B11* that were maintained as stocks balanced by *TM6* (useful marker = *Ubx*). Lethal mutations that map to the 88A/B genetic region were isolated in screens described by (MORTIN *et al.* 1992).

Polytene chromosome squash preparation and *in situ* hybridization: Drosophila stocks employed for preparation of polytene chromosome spreads were brooded on modified instant Drosophila medium (GREENLEAF *et al.* 1979) at a density of 15–20 flies per half pint milk bottle and incubated at 18° until larvae reached third instar stage of development. The *Df(3R)red/balancer* strains were mated to a *red/red* stock. Larvae were scored for the presence of red Malpighian tubules to select for the *Df(3R)red/red* genotype *vs.* the *balancer/red* genotype. Salivary glands were dissected from third instar larvae into 45% acetic acid and transferred to a 10-μl drop of fixative solution consisting of 1 part lactic

acid: 2 parts dH20: 3 parts glacial acetic acid on a silanized coverslip for 1–5 min, then squashed on a pretreated slide (BRAHIC and HAASE 1978) and hybridized (*e.g.*, ASHBURNER 1989). DNA probes used for hybridizations were nick translated with either Bio-11-dUTP or Bio-16-dUTP according to manufacturers instructions (Enzo-Biochem). Labeled hybrids were detected using streptavidin-peroxidase with diaminobenzidine HCl as substrate (Enzo-Biochem kit; catalog no. EBP-820–1). Chromosomes were counterstained with 5% Giemsa blood stain in PBS (10 mM $NaPO_4$ pH 7.2, 0.85% NaCl) for 30 sec, rinsed with dH_2O, air dried and mounted in a 1:1 mixture of permount:xylene. Photomicrographs of *in situ* hybridizations were taken at 320× magnification with Kodak Technical Pan film ASA 100 using a Zeiss photomicroscope III.

Germline transformants: Supercoiled plasmid DNA used for microinjections was extracted from overnight cultures of plasmid carrying *E. coli* strains by a modification of the alkaline lysis extraction protocol and then purified through two cesium chloride ethidium bromide gradients (MANIATIS, FRITSCH and SAMBROOK 1989). Purified plasmid DNA was concentrated by ethanol precipitation and resuspended in 0.1 M sodium phosphate buffer pH 6.8 at concentrations of 200–500 μg/ml. Microinjection of embryos was done essentially as described by (SPRADLING and RUBIN 1982) into *w; ry*^{506} *P[ry*$^{+}$ *Δ2–3](99B)/TM3* or *w; ry*^{506} *P[ry*$^{+}$ *Δ2–3](99B)/TM6B* genotype embryos (ROBERTSON *et al.* 1988). Surviving G_0 adults were crossed to the w^{1118} strain, w^{1118} being a null mutation of the *white*$^{+}$ marker gene carried by the CaSpeR transformation vector. G_1 progeny from this cross were scored for white^{+} eyes. White^{+} transformants were mated to either *w; Sco/CyO* or *w; TM3/TM6B* strains to generate stocks. Sites of *P*-element insertions were mapped by segregation analysis from the dominant Curly and Stubble markers present on the *CyO* and *TM3* balancer chromosomes. Homozygous transgenic lines of viable insertions were constructed. Insertions that are lethal as homozygotes were maintained as heterozygotes in combination with the appropriate balancer chromosome. *P*-element insertion sites were mapped cytologically by probing polytene chromosome spreads prepared from transformant lines with a pUC18 subclone of the *RpII140* gene restriction fragment used in the transformation experiments. DNA blot analysis of genomic DNA prepared from transformant lines confirmed the number of *P*-element insertions mapped cytologically and showed that insertions did not undergo any gross rearrangements or deletions during the integration process. The appropriate genetic matings were performed to place the second chromosome *P[w*$^{+}$*, RpII140]* insertions in the genetic background of either the *TM3* or *TM6B* balancer chromosomes or the *Df(3R)redP1* chromosome. In rescue experiments, typically $\geq 10^2$ progeny of each class were scored, if possible.

RESULTS

Cloning RNA polymerase II subunit genes

Expression library screen: A *D. melanogaster* genomic expression library was screened with a polyspecific antiserum that reacts with most component subunits of RNA polymerase II on an immunoblot. We screened 4.4×10^5 recombinant phage and obtained 37 positive clones. We expected a large number of the clones isolated in this screen would represent the largest RNA polymerase II subunit of 215 kDa, which constitutes approximately 40% of the molecular mass of the enzyme. The Drosophila *RpII215* gene encoding the largest subunit was cloned and analyzed in previous studies (SEARLES *et al.* 1982; BIGGS, SEARLES and GREENLEAF 1985; JOKERST *et al.* 1989), and we used *RpII215* gene probes in DNA hybridization experiments to determine which positive λgt11 recombinant clones contained sequences homologous to the *RpII215* gene; 12, or 33%, of our positive clones hybridized to the *RpII215* gene at high stringency and were excluded from further analysis.

Immunoblot analysis of β-galactosidase fusion proteins: The β-galactosidase fusion proteins expressed by the remaining positive λgt11 clones were analyzed on immunoblots probed with available RNA polymerase II antisera as an additional method of selecting clones that express genuine RNA polymerase II antigenic determinants. Those clones expressing β-galactosidase fusion proteins that reacted with an affinity purified fraction of the initial goat anti-RNA polymerase II serum used to screen the expression library and with a rabbit antiserum that reacts with subunits of 215, 180, 140, 34, 18, 16.5 and 16 kDa (WEEKS, COULTER and GREENLEAF 1982) were subjected to further examination.

Identification of RNA polymerase II subunits expressed by positive λgt11 clones: We affinity purified antibodies directed against fusion proteins expressed by individual λgt11 clones and used these antibodies to identify the subunit encoded by each positive clone. Antibodies directed specifically against RNA polymerase II epitopes presented by the fusion protein were fractionated from the goat anti-holoenzyme antiserum and were used to probe a blotted sample of RNA polymerase II. Two classes of clones representing two RNA polymerase II subunit genes were identified in this manner. Figure 1 depicts an immunoblot in which RNA polymerase II was probed with antisera directed to fusion proteins expressed by recombinant phage clones (phage 107 and 801, respectively) representing these two classes of clones. Comparison with a lane of RNA polymerase II probed with goat anti-RNA polymerase II shows that the anti-107 and anti-801 fusion protein antisera react uniquely with subunits of 140 and 18 kDa, respectively. These results indicate that the inserts of these phage represent the *RpII140* and *RpII18* genes, respectively. Additionally, fusion protein expressed by phage 107 clone reacts with a monospecific antiserum directed against the 140-kDa subunit of Drosophila RNA polymerase II and another monospecific antiserum directed against the corresponding subunit of wheat germ RNA polymerase II (data not shown) further confirming the identification of this clone.

The phage 107 insert represents the 140-kDa subunit gene, *RpII140*: The gene encoding the 140-kDa

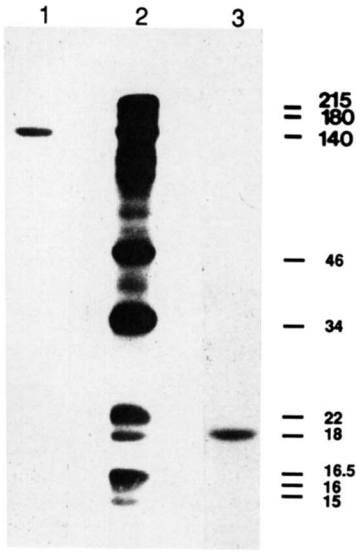

FIGURE 1.—Immunoblot of RNA polymerase II probed with affinity-purified anti-fusion protein antibodies. Approximately 1 μg per lane of partially purified embryonic RNA polymerase II was electrophoresed on an 8–15% gradient SDS gel, transferred to nitrocellulose and probed with goat anti-fusion protein antibodies or with goat anti-RNA polymerase II IgG (MATERIALS AND METHODS). Second antibody was rabbit anti-goat IgG, followed by ^{125}I-labeled protein A. Lane 1: affinity-purified anti-phage 107 fusion protein. Lane 2: anti-RNA polymerase II IgG. Lane 3: affinity-purified anti-phage 801 fusion protein.

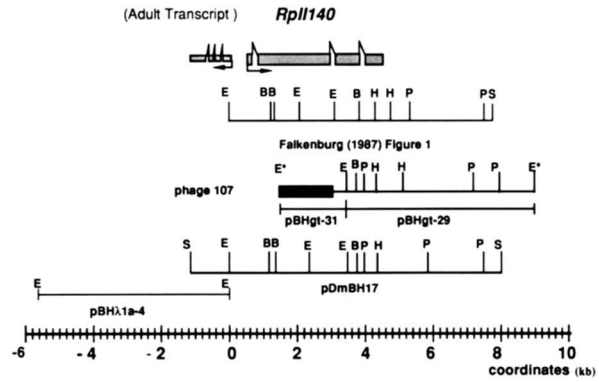

FIGURE 2.—Restriction and transcript maps of the *RpII140* region. The *RpII140* 4.0-kb transcript and a small adult RNA that is transcribed divergently (SITZLER *et al.* 1991) are aligned above the genomic map as previously presented (FALKENBURG *et al.* 1987). Each gene contains three introns as indicated. An *Eco*RI site at the beginning of the *RpII140* gene sequence data (FALKENBURG *et al.* 1987) is used as the zero point on this coordinate system. The λgt11 phage 107 insert is schematically depicted below the published map; the crosshatched box represents the translated region that is fused to β-galactosidase. pBHgt-31 and pBHgt-29 are *Eco*RI subclones of the 107 phage. pDmBH17 is a 9.1-kb *Sst*I genomic subclone derived from the CantonS strain. pBHλ1a-4 is a subclone from the λL1a phage (MATERIALS AND METHODS). Abbreviations: E, *Eco*RI (E*, phage *Eco*RI cloning site); B, *Bam*HI; H, *Hin*dIII; P, *Pst*I; S, *Sst*I.

subunit from Drosophila was cloned previously by cross-homology to the yeast RNA polymerase II 150-kDa subunit clone (FAUST *et al.* 1986; FALKENBURG *et al.* 1987). Extensive restriction endonuclease analysis (data not shown) confirmed that the genomic insert of phage 107 (and those of other related phage) corresponded to the *RpII140* gene described previously; and the phage 107 insert is schematically depicted, relative to the FALKENBURG *et al.* map in Figure 2. Note that the cloning of the same gene by a completely independent method confirms the identity of this gene. The size of the fusion protein encoded by phage 107 (165 kDa) is explained as the sum of 114 kDa of β-galactosidase and 51 kDa translation product from the genomic insert (indicated on the phage 107 insert map), with termination of translation occurring in an intron near coordinate 3.0. We also subcloned a CantonS genomic fragment representing this region and showed by the high degree of concordance of restriction endonuclease sites and by Southern blotting that this clone spanned the entire *RpII140* gene; the 9.1-kb subclone, pDmBH17, is used in later experiments and is depicted on the Figure 2 map.

***In situ* hybridization to polytene chromosomes:** *In situ* hybridization of an *RpII18* clone to OregonR strain polytene chromosomes mapped this gene to a centromere proximal location on chromosome arm 3R at 83A. Unfortunately, the 83A region is devoid of any genetic deficiencies due to its proximity to a dosage sensitive locus, Triplo-lethal at 83D (KEPPY and DENNELL 1979), making further cytological refinement of this clone impossible. Because of the lack of other well characterized markers in this region, we focused the rest of our analysis on *RpII140*. Some additional characterizations of the *RpII18* clones can be found in (HAMILTON 1990).

The *RpII140* gene mapped to a central region of chromosome arm 3R at polytene band 88A10:B1,2 (not shown), reconfirming the identity of this gene with that cloned by FAUST *et al.* (1986). This region has been well characterized genetically. Candidates for the *RpII140* gene locus, illustrated on the cytological map of the 88A/B region (Figure 3A), initially included *su(Hw)*, a suppressor of spontaneous mutations associated with insertions of the gypsy transposable element (MODOLELL, BENDER and MESELSON 1983), *l(3)k43*, a transacting factor that regulates chorion gene amplification (SHEARN *et al.* 1971; SZABAD and BRYANT 1982), and *trx*, a gene required for maintenance of the expression of the Bithorax complex genes (INGHAM 1983; GARCIA-BELLIDO and CAPDEVILA 1978). Additionally, *red*, a viable recessive mutation manifest in the adult stage as a reddish-brown eye color and in the larval stage as rusty Malpighian tubules, maps here and serves as a useful marker (LINDSLEY and ZIMM 1992).

To refine further the position of the *RpII140* gene locus, we obtained a set of overlapping deficiency strains that have breakpoints within the 88A/B region [shown in Figure 3A; described by LINDSLEY and ZIMM (1992). Note that available genetic and molec-

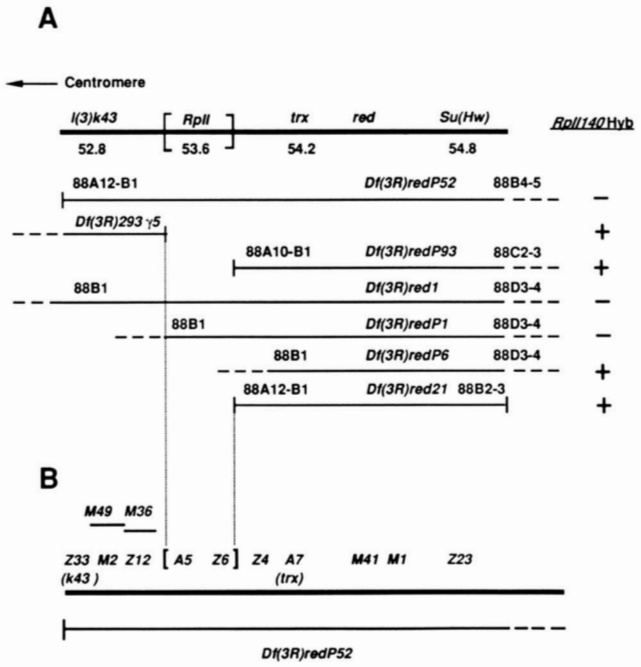

FIGURE 3.—The 88A/B region of polytene chromosome arm 3R. (A) *In situ* hybridization to deficiency chromosomes. Previously characterized genes are shown above the top thick line. Published recombinational map positions are shown directly below each locus (LINDSLEY and ZIMM 1992). The extent of the black lines below each listed deficiency chromosome indicates the deleted portion of the chromosome relative to the position of the genetic loci at the top of the figure. Published cytological breakpoints are indicated. Dashed lines indicate that the breakpoint is off the map or has not been mapped molecularly. Results of *in situ* hybridizations are summarized at right under the column "*RpII140* Hyb." (See Figure 4 for illustration of data and MATERIALS AND METHODS for experimental details). (B) Lethal complementation groups within *Df(3R)redP52*. The 11 lethal complementation groups recovered from saturation mutagenesis of the third chromosomal region deleted by *Df(3R)redP52* are depicted above the thick line [modified after MORTIN *et al.* (1992); *cf.* this reference for details]. Note that the relative order of *A5 vs. Z6* has not been determined, nor has the relative order of *A7 vs. M41 vs. M1*. Also, *M2*, *M49*, *Z12* and *M36* may represent a complex locus; the *Z33* group is identical to *l(3)k43*; *A7* is identical to *trx*; and *Z23* represents a small subunit of RNA polymerase, *RpII15* (HARRISON, MORTIN and CORCES 1992). No lethal mutant alleles of either *red* or *su(Hw)* were recovered from this screen. The *red* locus was mapped distal to *trx* and proximal to *su(Hw)* (BREEN and HARTE 1991). Thirty-one recessive-lethal mutations were recovered for the *A5* locus; four recessive-lethal mutations were recovered for *Z6*.

ular data (R. KELLEY, unpublished data; BREEN and HARTE 1991) indicate that the proximal breakpoints of these deficiencies are distinct despite the fact that the published breakpoints are identical]. Polytene chromosome preparations of the 88A/B region genetic deficiencies were hybridized to 140-kDa subunit clones in an attempt to correlate the map position of the *RpII140* locus with one of these previously characterized genes. *In situ* hybridization to a trans-heterozygous deficiency strain, *Df(3R)293γ5/Df(3R)redP93*, is shown in Figure 4, where asynapsis of chromosome homologs in the region of these deficiencies facilitated the analysis. The *RpII140* clone hybridized to both the *Df(3R)293γ5* and the *Df(3R)redP93* homologs, a result that positions the *RpII140* locus between the distal deficiency breakpoint of *Df(3R)293γ5* and the proximal deficiency breakpoint of *Df(3R)redP93* (see Figure 3A) and precludes the possibility that *RpII140* corresponds to either the *l(3)k43* function deleted by *Df(3R)293γ5* or the *trx* and the *su(Hw)* functions deleted by *Df(3R)redP93*. The position of the *RpII140* gene within the 88A/B region was further refined by *in situ* hybridization to the remaining *red* deficiencies (results summarized in Figure 3A). *Df(3R)red21* hybridized to the *RpII140* probes, whereas *Df(3R)redP52*, *Df(3R)red1* and *Df(3R)redP1* all deleted *RpII140*. Since *Df(3R)redP52* and *Df(3R)red1* overlap the *Df(3R)293γ5* breakpoint, these results define the position of the *RpII140* gene locus to an interval delimited by the proximal deficiency breakpoints of *Df(3R)redP93* and *Df(3R)redP1*.

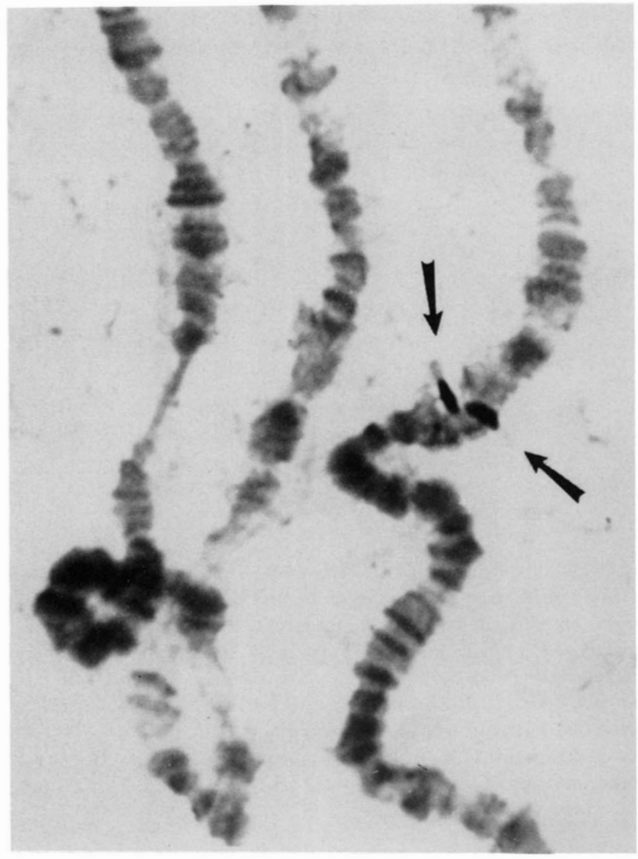

FIGURE 4.—*In situ* hybridization of the *RpII140* gene to polytene chromosomes from a deficiency heterozygote, *Df(3R)293γ5/Df(3R)redP93*. DNA from phage 107 was nick translated using biotinylated dUTP and hybridized as in MATERIALS AND METHODS. Arrows indicate hybridization signals on both asynapsed homologues.

Genetics of *RpII140*

Because the above analysis had eliminated all known loci as corresponding to *RpII140*, we sought to isolate

new mutations that might represent alleles of the *RpII140* locus. An extensive mutagenesis of the 88A/B genetic region yielded 11 complementation groups that were mapped within *Df(3R)redP52* (MORTIN *et al.* 1992); see Figure 3B. Two of these lethal complementation groups, *A5* and *Z6*, mapped within *Df(3R)redP1* and outside of *Df(3R)redP93*, namely into the same interval defined by our *RpII140* gene *in situ* hybridization results. We thus concentrated our attention on these two groups for further analysis.

Since two of the *A5* mutations, namely *Z36* and *Z43*, were temperature-sensitive conditional lethal mutations, they were useful for isolating homogeneously mutant RNA polymerase II from homozygotes for *in vitro* thermolability testing (COULTER and GREENLEAF 1982). Enzymes isolated from both temperature-sensitive *A5* mutants showed thermal stability identical to that of the host *red e* strain in which the mutations were recovered (Hamilton 1990). Thus, this biochemical approach was not useful for demonstrating the congruence between *A5* and *RpII140*. It should be noted, however, that this result does not argue strongly against this identity, because several known temperature-sensitive mutants of eukaryotic and bacterial RNA polymerases produce enzymes that are thermostable once assembled (COULTER and GREENLEAF 1982; GROSS, FIELDS and BAUTZ 1976).

To address further the question of which lethal complementation group within the *Df(3R)redP93* to *Df(3R)redP1* deficiency interval represents the *RpII140* gene locus, we wanted to use a genetic approach, that of introducing a copy of the wild-type *RpII140* gene into flies by *P*-element mediated germline transformation and testing for genetic rescue by this transgene of recessive lethal *A5* or *Z6* mutations. Although we did not initially know the limits of the cis-regulatory region required for expression of the *RpII140* gene, we used as a guide previous studies that had shown that for the *RpII215* gene, 0.7 kb upstream of the transcription initiation site was sufficient for expression (JOKERST *et al.* 1989). Before finally deciding on which subunit clone to use in the case of *RpII140*, we were also interested in whether there might be other embryonically expressed genes mapping close to the *RpII140* locus that we could detect by Northern blotting.

Transcription of the *RpII140* genomic region: RNA blots were probed with subclones from the *RpII140* region to map transcripts within this region (see Figure 5). The 4-kb *RpII140* message was the only species that showed significant reaction with pDmBH17 (lane 1), a subclone containing a 9.1-kb *Sst*I restriction fragment extending from 1.6 kb upstream of the transcription start site to 3.0 kb downstream of the polyadenylation site (see Figure 2). A partially overlapping subclone extending farther upstream (pBHλ1a-4) detected three different RNAs of 2.5, 1.4 and 1.1 kb (lanes 2 and 3). However, the virtually exclusive reaction of pDmBH17 with the 4-kb *RpII140* transcript suggests that this is the unique gene transcript expressed from the 9.1-kb genomic region *Sst*I fragment in embryos.

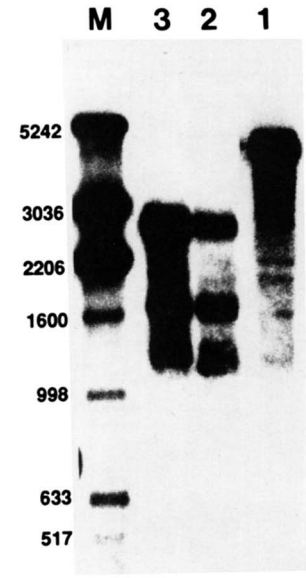

FIGURE 5.—Gel blot analysis of transcripts from the *RpII140* genomic region. Poly A$^+$ mRNA was formaldehyde gel fractionated, blotted and detected as described in MATERIALS AND METHODS. Lane 1, 0–12 hr embryo RNA hybridized with pDmBH17 (see Figure 2 for probes). Lane 2, third instar larval RNA hybridized with pBHλ1a-4. Lane 3, 0–12 hr embryo RNA hybridized with pBHλ1a-4. Lane M, [γ-^{32}P] end-labeled SV40 size markers; sizes at left in bp.

Germline transformation and genetic rescue of *A5* and *Z6* mutations: Given the immediately previous results, we cloned the 9.1-kb genomic *Sst*I restriction fragment from pDmBH17 into the CaSpeR *P*-element vector (PIRROTTA 1988) as depicted in Figure 6. Using this vector we succeeded in establishing a series of independent transformant lines that carry one or more wild-type *RpII140* transgenes as part of a P[w^+, *RpII140*] insert (see Table 1 and MATERIALS AND METHODS).

We next sought to test whether the (wild-type) *RpII140* transgene could rescue lethal alleles of *A5* or *Z6*, the candidate *RpII140* loci. Crosses were established that generated transgene-carrying zygotes, one fourth of which were homozygous for a lethal *A5* or *Z6* allele. We tested three transgenic lines representing distinct insertion sites of P[w^+, *RpII140*] on chromosome 2, namely lines G30, G60 and G70 (see Table 1), and found that all three transgenes allowed some survival of *A5* homozygotes but no survival of *Z6* homozygotes.

Because these results could have been complicated by unlinked lethal mutations on the homozygous *A5*-carrying or *Z6*-carrying chromosomes, we decided to use a more stringent test of genetic rescue, namely to

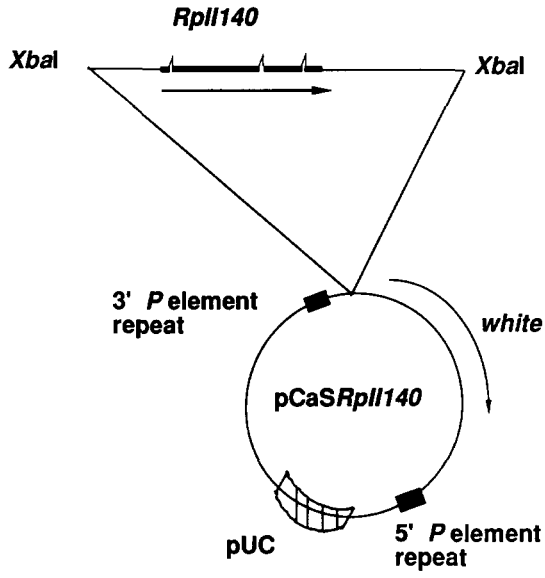

FIGURE 6.—*RpII140* P-element transformation vector construct. A 9.1-kb restriction fragment containing the *RpII140* transcribed region, 1.6 kb of 5′ flanking, and 3.0 kb of 3′ flanking sequence was subcloned into a polylinker *Xba*I site in the CaSpeR *P*-element transformation vector (details in MATERIALS AND METHODS). Direction of transcription of the *RpII140* and *white* genes are indicated with arrows. *P*-element repeats and pUC sequences in the vector are indicated.

determine whether a P[w^+, *RpII140*] insertion can rescue lethality of *A5* or *Z6* mutations as hemizygotes in trans-heterozygous combination with the *Df(3R)redP1* chromosome, which deletes both of these loci (see Figure 3A). This experiment has two advantages over testing for rescue of *A5* and *Z6* homozygotes. First, partially functional (leaky) mutations may show a more severe defect in hemizygotes. Second, the effect of second site recessive lethal or semilethal mutations that might be present on the tested chromosome will be eliminated as a result of heterozygosity with the *Df(3R)redP1* chromosome. Both the G30 and G70 insertions were able to rescue very efficiently the lethality of the *A5 red e/Df(3R)redP1* hemizygotes. Typical results are presented in Table 2. A control cross in which *A5 red e/TM6* was crossed to *w; Df(3R)redP1/TM6B* yielded no *A5 red e/Df(3R)redP1* progeny. As an additional control, the G70 homozygous subline in the *Df(3R)redP1* background was crossed to *A7* (an allele of *trx*); the G70 insertion was unable to rescue the lethality of the trx^{A7}/*Df(3R)redP1* heterozygotes.

The G30 and G70 insertion stocks were next tested for their ability to rescue additional alleles of the *A5* locus as hemizygotes. Results presented in Table 3 demonstrate that both G30 and G70 sublines afforded rescue of hemizygous *M39* and *Z43* mutations. Like G70, the G30 subline also failed to rescue the trx^{A7} mutation.

The P[w^+, *RpII140*] construct carries both the *A5* and the *Z6* complementation groups, but *A5* = *RpII140*: Unexpectedly, both the G30 and the G70 sublines rescued the lethality of the *Z6 red e/Df(3R)redP1* hemizygotes (see Table 3). Two additional *Z6* alleles, *Z30* and *Z29*, were also rescued as hemizygotes in crosses to the G70 subline. These same sublines (G30 and G70) had failed to rescue *Z6 red e* homozygotes. The most plausible explanation for this result is that a second lethal mutation induced on the *Z6 red e* chromosome during the initial mutagenesis caused the lethality of the *Z6 red e* homozygotes in the genetic background of a P[w^+, *RpII140*] insertion. When the *Z6 red e* chromosome is made hemizygous with the *Df(3R)redP1* chromosome, which does not carry the second site lethal, the lethality of the *Z6* mutation is complemented by a copy of the P[w^+, *RpII140*] transposon.

To test further whether the observed rescue of both loci could be attributed directly to the presence of the P[w^+, *RpII140*] transposon, we designed crosses in which the transposon and a *Df* that removes both *A5* and *Z6* were segregating, and we asked whether survival of *A5* or *Z6* lethal alleles in combination with the Df required the presence of the transgene. To do this, representative *A5* or *Z6* males with rescued phenotypes were backcrossed consecutively to *w; Df(3R)redP93/TM6B* and to *w; Df(3R)redP1/TM6B*. Segregation of the P[w^+, *RpII140*] transposon could be scored in the male progeny of these crosses. The results showed that survival of *A5* or *Z6* in combination with the *Df(3R)redP1* chromosome depends on inheritance of a copy of the P[w^+, *RpII140*] transgene. The same crosses generated control genotypes showing that survival of *A5* or *Z6* in combination with the *Df(3R)redP93* chromosome, which does not delete either of these gene loci, does not depend on the presence of the P[w^+, *RpII140*] insert. These findings indicate that the transposon carries information to complement lethal mutations in both the *A5* and *Z6* complementation groups.

To generate a transformation construct carrying presumably only one functional gene, we deleted most of the *RpII140* coding sequences (and all the DNA between *RpII140* and the white gene) but left the upstream region that encodes the divergently transcribed adult RNA intact (see Figure 2 and MATERIALS AND METHODS). The new construct, pCaS*RpII140*Δ, was used to generate germline transformants as before. Results with one allele from each complementation group indicate that this construct continues to rescue *Z6* but fails to rescue *A5* (data not shown). These results suggest that the upstream adult transcription unit represents *Z6*, but more importantly in the present context strongly support the conclusion that the *A5* complementation group represents the *RpII140* locus.

Map distance between and order of the *A5* and *Z6*

TABLE 1

P[w+, RpII140] transgenic lines

Transgenic line	*P[w+, RpII140]* eye color	No. of insertions	Chromosomal linkage	Cytological map position
P[w+, RpII140]$^{D10-60}$	Pale orange		X	
P[w+, RpII140]^{G10}	Pale yellow		2	
P[w+, RpII140]^{G20}	Pale yellow		2	
P[w+, RpII140]^{G30}	Yellow	1	2	27B
P[w+, RpII140]^{G40}	Orange	2	2	23A, ?
P[w+, RpII140]^{G50}	Orange		2	
P[w+, RpII140]^{G60}	Orange	1	2	26A
P[w+, RpII140]^{G70}	Pale orange	1	2	55C
P[w+, RpII140]^{G80}	Brick-red			
P[w+, RpII140]^{H10}	Yellow	1	2	
P[w+, RpII140]^{H30}	Pale orange		2	
P[w+, RpII140]^{H50}	Bright orange	1	2	
P[w+, RpII140]^{H60}	Pale orange		2	

Eye colors of flies carrying *P[w+, RpII140]* transposon insertions were scored within 24 hr post-eclosion. Chromosomal linkage, determined by segregation analysis of the *p[w+]* marker (see MATERIALS AND METHODS for details), was confirmed for some lines by *in situ* hybridization to the polytene chromosomes.

TABLE 2

Genetic crosses to test for rescue of *A5 red e* hemizygotes

$$\frac{+}{+};\frac{A5\ red\ e}{TM6}\ \text{females} \times \frac{w}{Y};\frac{P[w^+, RpII140]^{G30}}{P[w^+, RpII140]^{G30}};\frac{Df(3R)redP1}{TM6B}\ \text{males}$$

Genotype	Markers	% of expected
$\frac{w}{+};\frac{P[w^+, RpII140]^{G30}}{+};\frac{A5\ red\ e}{Df(3R)redP1}$	*red, Tb+*	74
$\frac{w}{+};\frac{P[w^+, RpII140]^{G30}}{+};\frac{TM6}{Df(3R)redP1}$	*Ubx, Tb+*	100
$\frac{+}{Y};\frac{P[w^+, RpII140]^{G30}}{+};\frac{A5\ red\ e}{Df(3R)redP1}$	*red, Tb+*	70
$\frac{+}{Y};\frac{P[w^+, RpII140]^{G30}}{+};\frac{TM6}{Df(3R)redP1}$	*Ubx, Tb+*	100

A representative cross of *A5 red e/TM6* to the G30 homozygous transformant line in the genetic background of the *Df(3R)redP1* chromosome is depicted. Genotypes of relevant progeny classes and the markers used for scoring these genotypes are indicated. The number of progeny is reported as a percentage of the expected number, as defined by the control classes (100%).

loci: Recessive visible markers from the *rucuca* third chromosome (LINDSLEY and ZIMM 1992) were recombined with the *A5* allele, *Z24*, and with *Z6* to construct four lines: (1) *ru h th st cu Z24 red e/TM6B*, (2) *Z24 red sr e ca/TM6B*, (3) *ru h th st cu Z6 red e/TM6B*, (4) *Z6 red sr e ca/TM6B*. Flies from line 1 were mated to line 4 (used in cross A of Table 4) and line 2 to 3 (used in cross B). Virgin females carrying both recessive lethal mutations were mated to *Df(3R)redP52/TM8* males. *TM8* carries a dominant temperature-sensitive lethal mutation that blocks development of flies reared at 29° (LINDSLEY and ZIMM 1992). The number of eggs laid in the A and B crosses was estimated. The eggs were then placed at 29° and allowed to develop. Only recombinants between *Z24* and Z6 that retain neither lethal mutation will survive as heterozygotes with *Df(3R)redP52*. The distance between *Z24* (*i.e.*, *A5* locus) and *Z6* was calculated to be 0.02 cM as described in Table 4.

To determine the order of *Z24* and *Z6*, we crossed the three progeny recovered from cross A (Table 4) to *rucuca* flies. Progeny from the two fertile matings displayed all the recessive visible markers from the *rucuca* chromosome, strongly suggesting that the *Z6* locus is closer to *cu* and the centromere than is the *A5* locus. Since no adult progeny were recovered from cross B, we were unable to confirm this result. However, this result is consistent with the hypothesis resulting from the molecular analyses that the adult transcript shown in Figure 2 might represent the *Z6* locus.

DISCUSSION

We have molecularly cloned and cytologically mapped two RNA polymerase II subunit genes from *D. melanogaster* to facilitate further genetic and biochemical investigations of this critical transcriptase. Our analysis of the *RpII140* and *RpII18* gene loci

TABLE 3

Genetic rescue of hemizygous *A5* and *Z6* alleles with *G30* and *G70* transposon insertions

	Index of genetic rescue			
	G30		G70	
Allele	P	M	P	M
A5 alleles				
A5	ND	1.40	ND	1.60
Z43	1.30	0.66	0.92	0.89
M39	1.40	0.75	1.08	0.87
Z6 alleles				
Z6	0.85	0.95	1.56	0.72
Z29	ND	ND	ND	0.35
Z30	ND	ND	ND	0.42
trx alleles				
A7	0.00	0.00	0.00	0.00

Crosses to determine genetic rescue were of the type *lethal/Bal(3) × G30* or *G70 w; P[w+, RpII140]; Df(3R)redP1/TM6B*. Reciprocal crosses were performed as indicated.

Index of genetic rescue is defined as the ratio of the number of progeny of the genotype *P[w+, RpII140]G/+; lethal/Df(3R)redP1* to the sibling genotype *P[w+, RpII140]G/+; lethal/TM6B*.

ND = not determined.

M designates a cross in which the *A5* or *Z6* allelic mutation is contributed by the female parent, and P, by the male parent.

TABLE 4

Meiotic mapping of the *A5* and *Z6* loci

	No. of eggs	No. of progeny	Calculated distance[a]
Cross A	36,500	3	0.03
Cross B	23,500	0	0.00
Total	60,000	3	0.02

[a] No. of progeny/no. of eggs × 4 × 100 = calculated distance in cM.

demonstrates that like the RNA polymerase II subunit genes mapped to date from *S. cerevisiae*, these genes are organized in the genome as single copies and are not part of an operon as are the large subunit genes of eubacterial and archaebacterial RNA polymerases. The refinement of the *RpII140* gene localization within the 88A/B polytene band region of chromosome 3 by *in situ* hybridization to a series of deficiency chromosomes showed that this gene was not represented by mutations in any previously known loci and consequently that it represents a new genetic locus in Drosophila.

Extensive mutagenesis of the 88A/B region produced 11 lethal complementation groups (MORTIN *et al.* 1992), two of which (*A5* and *Z6*) mapped to the cytological interval defined by *in situ* hybridization of the cloned gene. The large number of alleles recovered for *A5 vs. Z6* as well as the phenotypes of the mutants suggested to us initially that *A5* and not *Z6* represented the *RpII140* gene locus. For example, lethality in *A5* homozygotes occurred during late embryogenesis-early first larval instar, in parallel with the lethal phase for mutations in the gene for the largest RNA polymerase II subunit, *RpII215*, and as expected for a gene whose product is required throughout development (survival through embryogenesis is due to maternal RNA polymerase). In contrast, *Z6* mutations caused lethality later during development, a result difficult to reconcile with a continuous requirement during development for the *Z6* gene product. In addition, like some *RpII215* alleles, certain *A5* mutations exhibited genetic interactions with developmentally important loci, such as *Ubx* (see INTRODUCTION and below), whereas *Z6* alleles exhibited no such interactions.

In an attempt to test the hypothesis that the *A5* complementation group represented the *RpII140* locus, we used a genetic rescue approach. We generated germline transformants carrying a 9.1-kb genomic insert containing the *RpII140* coding region and including 1.6 kb upstream of the mRNA cap site and 3.0 kb downstream of the mRNA polyadenylation site. Surprisingly, this insert rescued both *A5* and *Z6* mutant alleles, indicating that both *A5* and *Z6* complementation groups were carried by the 9.1-kb genomic region introduced by germline transformation. In view of the results of the subsequent mapping experiment, this is not unreasonable as it turns out that *A5* and *Z6* map only 0.02 recombinational map units apart. In addition, whereas we found no transcripts originating from the genomic regions flanking the *RpII140* locus in embryonic (or third instar larval) RNA, SITZLER *et al.* (1991) recently sequenced an upstream region of *RpII140* that they find is transcribed in adult flies and which contains a 783 nucleotide open reading frame (see Figure 2). This transcript maps within the genomic region introduced by the *P*-element construct. It is possible that this adult RNA is the transcript of the *Z6* locus for three reasons. First, *Z6* mutant homozygotes can survive to the pupal stage when reared on certain media (B. J. Hamilton, unpublished data), suggesting that *Z6* functions after the pupal stage of development. Second, since the orientation of the molecular map in Figure 2 parallels that of the genetic map in Figure 3 (R. KELLEY and S. PARKHURST, unpublished data, and our work), the recombination experiment that places *Z6* toward the centromere from *A5* (RESULTS) is consistent with equating the small adult transcript with the *Z6* locus and the *RpII140* transcript with the *A5* locus. Third, a transformation construct carrying a truncated *RpII140* gene but an intact adult transcript gene rescues *Z6* but not *A5* alleles. This last result also provides strong support for the conclusion that the *A5* complementation group indeed represents the genomic *RpII140* locus. An approach that ultimately would rigorously prove our proposition that *A5* = *RpII140* is to identify at the molecular level the lesions causing *A5*

mutations; recently this approach has been successful (CHEN *et al.* 1993).

One of the component subunits of the prokaryotic RNA polymerase holoenzyme (β) is homologous to the Drosophila and yeast 140-kDa subunits. Studies in bacteria suggest that this subunit functions in elongation of RNA chains and in transcription initiation. Functional studies of the eukaryotic RNA polymerases have demonstrated a role for the second largest subunit in substrate binding (nucleoside triphosphates), phosphodiester bond formation, binding to the DNA template and binding to the nascent RNA chain (SAWADOGO and SENTENAC 1990). Mutational alterations in any of these functions might lead to lethality. Consequently some of the Drosophila *RpII140* mutants might affect domains of the 140-kDa subunit involved in these fundamental processes. Our initial studies of RNA polymerase II enzymes isolated from the two heat-sensitive *RpII140* alleles (*Z36* and *Z43*) showed that in an *in vitro* thermal inactivation assay these enzymes were as stable as that isolated from wild-type Drosophila. Although our studies did not demonstrate any defect in these enzymes that might be responsible for the temperature-sensitive phenotype of these mutants, they do suggest that phosphodiester bond formation and nucleoside triphosphate binding are not obviously affected. It is possible that the temperature-sensitive *RpII140* mutants are defective in assembly of functional RNA polymerase II at nonpermissive temperatures, but that once assembled at lower temperatures these enzymes are catalytically normal (COULTER and GREENLEAF 1982; GROSS, FIELDS and BAUTZ 1976). Another possibility is that the temperature-sensitive mutations are defective in some aspect of transcription *in vivo* that was not monitored in the assay used for our thermal inactivation studies, such as promoter binding or interaction with general or gene-specific transcription factors.

The existence of mutations that affect RNA polymerase II by altering the second largest subunit provides opportunities to determine what these functions are and, once the mutations are mapped at the DNA sequence level, to assign them to particular domains. For conditional lethal mutants whose enzymes can be purified in homogeneously mutant form, *in vitro* transcriptional studies (PRICE, SLUDER and GREENLEAF 1987; ZEHRING *et al.* 1988; COULTER and GREENLEAF 1985) should provide insights into defects associated with temperature sensitivity.

For mutant alleles that are not viable in homozygous form, several *in vivo* approaches are available. For example, mutant polymerases that retain some enzyme activity can be examined for abnormal utilization of different promoters fused to reporter genes; promoter-specific defects may be uncovered that would possibly be diagnostic of subtle alterations in promoter recognition or transcription factor interactions. The intriguing "Ubx effect" also provides a point of departure for *in vivo* investigations of interactions between RNA polymerase II and regulatory transcription factors. Five *RpII215* and three *RpII140* alleles enhance *Ubx* mutations to give a more severe morphological transformation of haltere to wing (MORTIN, KIM and HUANG 1988; MORTIN *et al.* 1992, for description and discussion). Because the *RpII215* alleles cause the Ubx effect in a Ubx^+ background whereas the *RpII140* alleles require a mutant *Ubx* allele, the alterations in the two large subunits may induce this effect by different mechanisms. In addition, an as yet unexplained feature is that for both large subunit genes the Ubx effect is observed only when the respective polymerase locus is heterozygous for the mutant and a wild-type allele. It seems clear that future investigations into these phenomena will uncover novel aspects of direct or indirect interactions between the core transcription apparatus and developmentally important transcriptional regulators. Finally, continued application of second-site suppressor analysis (MORTIN 1990) should provide increasingly detailed information about nuclear and chromosomal components with which RNA polymerase II interacts.

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