Functional Studies of the Carboxy-Terminal Repeat Domain of Drosophila RNA Polymerase II *In Vivo*

W. June Brickey and Arno L. Greenleaf

Department of Biochemistry, Duke University Medical Center, Durham, North Carolina 27710

Manuscript received November 1, 1994
Accepted for publication March 4, 1995

ABSTRACT

To understand the *in vivo* function of the unique and conserved carboxy-terminal repeat domain (CTD) of RNA polymerase II largest subunit (RpII215), we have studied RNA polymerase II biosynthesis, activity and genetic function in Drosophila *RpII215* mutants that possessed all (*C4*), half (*W81*) or none (*IIt*) of the CTD repeats. We have discovered that steady-state mRNA levels from transgenes encoding a fully truncated, CTD-less subunit (IIt) are essentially equal to wild-type levels, whereas the levels of the CTD-less subunit itself and the amount of polymerase harboring it (Pol IIT) are significantly lower than wild type. In contrast, for the half-CTD mutant (*W81*), steady-state mRNA levels are somewhat lower than for wild type or *IIt*, while W81 subunit and polymerase amounts are much less than wild type. Finally, we have tested genetically the ability of CTD mutants to complement (rescue) partially functional *RpII215* alleles and have found that *IIt* fails to complement whereas *W81* complements partially to completely. These results suggest that removal of the entire CTD renders polymerase completely defective *in vivo*, whereas eliminating half of the CTD results in a polymerase with significant *in vivo* activity.

RNA polymerase II, a complex multi-subunit protein, is the enzyme responsible for transcription of protein-coding genes in eukaryotes. Its activity is modulated by interactions with an array of regulatory components (reviewed in ZAWEL and REINBERG 1992; CONAWAY and CONAWAY 1993; DRAPKIN *et al.* 1993; BURATOWSKI 1994). While much current research is aimed at describing the nature and mechanism of action of factors that regulate RNA polymerase II (pol II) activity, with a focus especially on the process of transcription initiation, achieving a thorough understanding of how pol II is regulated also requires detailed knowledge of pol II itself.

Highly purified RNA polymerase II is composed of 10 or more subunits in most eukaryotic species (YOUNG 1991). The largest subunit (~215 kD, product of the *RpII215* locus in Drosophila) is usually referred to as IIa, and the second-largest subunit (~140 kD, product of the *RpII140* locus in Drosophila) is usually referred to as IIc. Extensive sequence and functional homologies between the largest and second-largest subunits, respectively, of eukaryotic and prokaryotic RNA polymerases have been documented (ALLISON *et al.* 1985; AHEARN *et al.* 1987; FALKENBURG *et al.* 1987; SWEETSER *et al.* 1987; JOKERST *et al.* 1989; and see PALENIK 1992). The eukaryotic pol II largest subunit is unique, however, in containing an unusual C-terminal addition. In all eukaryotes investigated to date, with the exception of certain protozoa, this pol II-specific carboxyl-terminal domain (CTD) consists of multiple tandem repeats of the consensus heptad sequence, YSPTSPS (reviewed in CORDEN 1990; YOUNG 1991). Although the repeat number varies between species, the sequence characteristics are widely conserved. Furthermore, the CTD has been shown to be essential for viability in widely divergent eukaryotes (NONET *et al.* 1987; BARTOLOMEI *et al.* 1988; ZEHRING *et al.* 1988).

Modifications to the CTD, most notably hyperphosphorylation, have been correlated with different transcriptional states of pol II (reviewed in DAHMUS and DYNAN 1992). When the largest subunit carries an unmodified CTD, it is referred to as subunit form IIa and the corresponding polymerase is called pol IIA. This form of the enzyme has been correlated with preinitiation complex formation *in vitro* (LAYBOURN and DAHMUS 1990; LU *et al.* 1991; CHESNUT *et al.* 1992) and has been found in promoter proximal paused complexes *in vivo* (WEEKS *et al.* 1993; O'BRIEN *et al.* 1994). When the largest subunit carries a hyperphosphorylated form of the CTD, its mobility is reduced in sodium dodecyl sulfate (SDS)-polyacrylamide gels and it is known as IIo; the corresponding enzyme is called IIO (CADENA and DAHMUS 1987; LEE and GREENLEAF 1989; LAYBOURN and DAHMUS 1990; ZHANG and CORDEN 1991b; PAYNE and DAHMUS 1993). The phosphates are primarily on serine residues and to a lesser degree on threonine and tyrosine residues (DAHMUS 1981; ZHANG and CORDEN 1991a; BASKARAN *et al.* 1993). Pol IIO is thought to be the major actively transcribing form of the enzyme, with phosphorylation of the CTD having been correlated with RNA chain elongation in

Corresponding author: Arno L. Greenleaf, Department of Biochemistry, Box 3711, Duke University Medical Center, Durham, NC 27710. E-mail: arno@bchm.biochem.duke.edu

nuclei and nuclear extracts (BARTHOLOMEW *et al.* 1986; CADENA and DAHMUS 1987), in fractionated extracts (PAYNE *et al.* 1989; KANG and DAHMUS 1993), and *in vivo* (WEEKS *et al.* 1993; O'BRIEN *et al.* 1994). The identification and characterization of multiple kinases that phosphorylate the CTD have strongly supported the notion that phosphorylation of the CTD plays a major role in the modulation of polymerase activity *in vivo* (LEE and GREENLEAF 1989, 1991; FEAVER *et al.* 1991, 1993; LU *et al.* 1992; SERIZAWA *et al.* 1992; STONE and REINBERG 1992; PAYNE and DAHMUS 1993).

The CTD has been suggested to play a crucial role in the regulation of transcription of protein-coding genes by RNA polymerase II. Initially genetic approaches (ALLISON and INGLES 1989; SCAFE *et al.* 1990) and more recently biochemical experiments (LIAO *et al.* 1991; CONAWAY *et al.* 1992; KOLESKE *et al.* 1992; USHEVA *et al.* 1992; THOMPSON *et al.* 1993; KIM *et al.* 1994; KOLESKE and YOUNG 1994) have shown that the CTD can interact with certain transcriptional regulatory components, suggesting that the CTD plays an important role in modulating transcription initiation, at least for some genes. It also seems likely that the CTD is involved in more than just the initiation phase of the transcription process, and several investigators have proposed additional functions for the CTD. These include localization of polymerase within the nucleus, interaction with histones during elongation, and participation in pre-mRNA processing (CORDEN 1990; CHAO and YOUNG 1991; GREENLEAF 1993).

The issue of whether the CTD plays a role in initiation of all genes was not resolved by the above studies. In particular, genetic studies of YOUNG and coworkers indicated that diverse promoters respond differently to CTD shortening (NONET and YOUNG 1989; SCAFE *et al.* 1990). Similarly, *in vitro* transcription experiments gave mixed results. For example, a number of studies demonstrated that pol II lacking a CTD was capable of accurate and even regulated initiation from certain promoters *in vitro, e.g.,* actin (ZEHRING *et al.* 1988; ZEHRING and GREENLEAF 1990) and Ad2MLP (KIM and DAHMUS 1989; BURATOWSKI and SHARP 1990). Conversely, transcription initiation from promoters such as DHFR seems to require the CTD (THOMPSON *et al.* 1989; BUERMEYER *et al.* 1992).

We wanted to expand upon previous results by investigating the function of the CTD within the nucleus. We therefore sought to compare the *in vivo* activities and properties of Drosophila RNA polymerases II with a CTD that was either intact, partially deleted, or completely removed. Biochemical and genetic examination of defects exhibited by polymerases with a mutant CTD might reveal aspects of wild-type CTD function.

Our plan was to investigate three different strains, each containing pol II with a distinct CTD (intact, half truncated, or completely truncated). Since the truncations would be recessive lethals (NONET *et al.* 1987; BARTOLOMEI *et al.* 1988; ZEHRING *et al.* 1988), we would have to study them in heterozygotes also carrying a wild-type allele; we therefore required a means to distinguish the mutant pol II from the intact wild-type pol II. Two of the three desired alleles had actually been generated in earlier work, and each harbored a mutation allowing separation of its activity from that of background wild-type pol II. One was the previously described amanitin resistant polymerase encoded by the *C4* allele; it retains an intact CTD and remains active at α-amanitin concentrations that inhibit wild-type polymerase (GREENLEAF *et al.* 1979, GREENLEAF *et al.* 1980; GREENLEAF 1983). The *C4* allele differs from wild type at a single nucleotide position (shown as amaR in Figure 1) (G-3973 to A transition that changes Arg741 to His) (CHEN *et al.* 1993). The other allele was a previously described mutant with a partially truncated CTD, namely *W81* (VOELKER *et al.* 1985; ZEHRING *et al.* 1988). This mutant allele of *RpII215* was isolated as a recessive lethal caused by insertion of a defective *P* element into the *C4* allele (VOELKER *et al.* 1985). Sequence analysis of the *W81* allele indicated that 631 bp of a *P* element had been inserted into CTD repeat #20 of 42 (ZEHRING *et al.* 1988). These *P*-element sequences gave rise to a premature termination product due to the introduction of five novel amino acids, followed by a translation stop codon encoded by the *P* insert (see Figure 1, shown as HDQIT*); therefore the W81 subunit only contains the first 19 of the normal 42 CTD heptad repeats. Even though *W81* is a recessive lethal (VOELKER *et al.* 1985), this mutant enzyme actively transcribes DNA *in vitro* in an amanitin-resistant manner (ZEHRING *et al.* 1988; ZEHRING and GREENLEAF 1990).

Finally, to generate the third mutant for our study, we designed a complete CTD truncation mutant, which we refer to as *IIt*, that also carried the α-amanitin resistance mutation. The *IIt* gene was introduced into flies by *P*-element-mediated germline transformation. We then used the three mutant strains to carry out a comparative study of the *in vivo* properties of pol II with intact, partial, or no CTD, investigating both genetic and biochemical features of the mutant subunits. Besides its specific role(s) in transcriptional events, the data suggest that the CTD is essential for enzyme or protein integrity or stability *in vivo*. In addition, our results indicate that, in contrast to the *in vitro* situation, a full truncation mutant may be completely defective *in vivo*.

MATERIALS AND METHODS

Drosophila stocks: Fly stocks were maintained on standard cooked cornmeal/yeast/molasses/agar media. Stocks used for germline transformation were as described (ROBERTSON *et al.* 1988; also see Table 1). The *RpII215* alleles used are described in Table 1. Other genetic markers used in this study are described in LINDSLEY and ZIMM (1992).

***RpII215* mutagenesis and germline transformation:** The

CTD truncation mutant *RpII215^{IIt}* was created by oligonucleotide-directed mutagenesis of a *Kpn*I to *Sal*I 3′ subclone of *RpII215* (JOKERST *et al.* 1989). A complementary set of 32-bp primers was annealed to form an oligonucleotide fragment with 5′ cohesive ends that encoded the amino acid sequence QQFFGLM**, where * denotes a translation termination codon. This fragment was cloned into the unique *Bsp*MII restriction site (*RpII215* nucleotide position 6576) of the *Kpn*I to *Sal*I subclone. Standard methods (SAMBROOK *et al.* 1989) were then used to reconstruct the entire mutant *RpII215* gene and subclone it into pCaSpeR (PIRROTTA 1988), generating *P[w$^+$, RpII215^{IIt}]*. Subsequent sequence analysis of *P[w$^+$, RpII215^{IIt}]* revealed the unexpected presence of two 32-bp oligonucleotide sequences in a 5′:3′::5′:3′ arrangement inserted at the *Bsp*MII site. Note that the first insert encodes the desired amino acid sequence and stop codons; the second insert is irrelevant with respect to protein coding information.

Germline transformants carrying *P[w$^+$, RpII215^{IIt}]* inserts were created essentially as described by HAMILTON *et al.* (1993). *P[w$^+$, RpII215^{IIt}]* transformants were selected and stabilized with the appropriate balancer chromosomes. Subsequent genetic crosses were carried out to prepare a transgenic fly line, having transgenes in the background of an endogenous *RpII215* allele that was temperature-sensitive (*i.e., y RpII215^{ts} f/Y; IIt(48A)/CyO* and see Table 1). Transgenes were mapped by *in situ* hybridization to polytene chromosomes essentially as described (HAMILTON *et al.* 1993).

Genomic DNA isolation and Southern blot analysis: Genomic DNA was isolated from adult flies by homogenization in aqueous buffer (10 mM Tris-HCl, 60 mM NaCl, 10 mM EDTA, plus spermine and spermidine), followed by extraction with phenol:chloroform (ASHBURNER 1989, Protocol #47). The DNA was digested with restriction endonucleases and electrophoresed through a 0.8% agarose gel. The DNA was transferred to positively charged nylon membrane (Boehringer Mannheim) by upward capillary transfer in 10 or 20× SSC (SOUTHERN 1975) and hybridized at 42° or 65° with a random prime [^{32}P]- or digoxigenin-dUTP-labeled 1.3-kb *Bgl*II-*Bgl*II *RpII215* DNA fragment (see Figure 2) in formamide or aqueous buffer, respectively. After high stringency washing, autoradiography with Kodak XAR X-ray film was performed on the blots.

Purification and activity assays of RNA polymerase II: RNA polymerase II was partially purified from adult flies according to GREENLEAF *et al.* (1980). DEAE fractions containing peak enzyme activity were pooled and concentrated using Amicon Centricon-30 concentrators. RNA polymerase II activity was measured using the nonspecific transcription assay conditions (U/20) as described by COULTER and GREENLEAF (1982). Preliminary assays were carried out to determine the range of enzyme for which the amount of incorporated nucleotide was linearly dependent upon enzyme added. This range of enzyme concentrations was then used to test the amanitin sensitivity of polymerase purified from mutant flies. Triplicate assays were done at each α-amanitin concentration. To determine the amount of α-amanitin-sensitive activity present, the data were then analyzed using the program AMACURVE originally developed by A. SLUDER based upon known and theoretical amanitin sensitivity curves (see Figure 2 in GREENLEAF *et al.* 1980).

Protein extract preparation and immunoblot analysis: Crude protein extracts were quickly prepared by homogenizing adult flies in cold 95% EtOH with phenylmethyl sulfonyl fluoride (PMSF) and then resuspending the protein pellet in boiling SDS-loading buffer (WEEKS *et al.* 1993). The protein extracts were subjected to SDS-PAGE in a 5% gel. Separated protein was electroblotted onto nitrocellulose (BIO-RAD) using the transfer buffer (49.6 mM Tris, 384 mM glycine, 20% MeOH, 0.01% SDS) described by OTTER *et al.* (1987) to efficiently transfer high molecular weight proteins like RpII215.

The largest pol II subunit forms were visualized using affinity-purified goat anti-exon2 antibodies (WEEKS *et al.* 1993 and references therein), followed by rabbit anti-goat alkaline phosphatase-conjugated secondary antibodies according to manufacturer's instructions for the Western Light kit (Tropix, Inc.). The final step of the detection involved either colorimetric reaction of immunoblotted membrane with NBT/BCIP or chemiluminescent reaction using CSPD or AMPPD substrates supplied in the Western Light kit followed by exposure to Kodak XAR X-ray film. The relative amounts of pol II largest subunit forms were determined by imaging the reacted blots and/or autoradiograms using a CCD Camera (Photometrics) with Star 1.3 image capture program and analyzing the images using the National Institutes of Health Image v1.52 data analysis program.

RNA isolation and Northern blot analysis: TRIzol Reagent, a monophasic solution of guanidinium thiocyanate:phenol:chloroform (CHOMCYNSKI and SACCHI 1987) was used according to manufacturer's instructions (GIBCO BRL) to extract total RNA from adults flies. Additionally, total RNA was independently prepared by homogenizing adult flies in SDS-urea buffer containing 5 mM vanadyl-ribonucleoside complex as previously described (BIGGS *et al.* 1985), followed by phenol:chloroform extraction and ethanol precipitation. Polyadenylated RNA was isolated from the total RNA samples by oligo-(dT)-cellulose affinity chromatography (AVIV and LEDER 1972) according to the protocol of the mRNA Purification Kit (Boehringer Mannheim). Equivalent amounts (as assayed by absorbance at 260 nm) of total or poly(A$^+$) RNA samples were electrophoresed on 0.8% agarose-formaldehyde gels (AUSUBEL *et al.* 1989) and transferred to GeneScreen nylon membrane (DuPont/NEN) by upward capillary transfer in 20× SSC. The membranes were hybridized at 42° in formamide containing buffer plus the following random-prime labeled ^{32}P-DNA probes: the 1.2-kb *Bam*HI-*Hin*dIII fragment of *Ras2* (MOZER *et al.* 1985) as a control and the 1.3-kb *Bgl*II-*Bgl*II central fragment of *RpII215* (JOKERST *et al.* 1989; also see Figure 2). The blots were washed and exposed to a Storage Phosphor Screen (Molecular Dynamics). The radioactive signals were quantitated using the PhosphorImager ImageQuant program.

Genetic complementation analysis: To prepare a stock containing a *IIt* transgene on the *X* chromosome along with the *L5* allele at the normal *RpII215* locus on the *X*, the stock *y ras v L5 f/FM7/Y* (referred to as *L5/+*; also see Table 1), generously provided by M. MORTIN, and the *+ IIt(13F)* transgenic strain were recombined as described here. Crossover events on the *X* chromosome between *f* at 15F1-3 and *IIt* at 13F were generated in non-*FM7* daughters from the following cross: *y ras v L5 f/FM7* females mated to *RpII215$^+$ [IIt(13F)]f$^+$/Y* transgenic males. These non-*FM7* daughters were crossed to *FM7/y$^+$ Y v$^+$* males, and the resulting male progeny were examined for loss of the *forked* marker plus retention of the lethal *L5* allele by mating to *L5/FM7* females. The appropriate *ras v L5 [IIt(13F)]?f$^+$/FM7* daughters were backcrossed to *FM7/y$^+$ Y v$^+$* males to create the following stable stocks, *ras v L5[IIt(13F)]?/FM7/y$^+$ Y v$^+$*. Subsequent PCR amplification and Southern blot hybridization analysis of these flies confirmed the presence of *IIt(13F)* in *f$^+$* progeny in one out of 28 candidates. This stock is referred to as *L5 IIt(13F)/+*; also see Table 1. The *FM7* and *y$^+$ Y v$^+$* chromosomes, both harboring a wild-type allele of *RpII215*, are as described (VOELKER *et al.* 1984; and see Table 1).

The *in vivo* activity of *W81* and *IIt* mutants was tested by complementation assays using a series of strains carrying partially functional *RpII215* alleles. The tester fly stocks resulted

from hybrid dysgenic reversion of the *P*-element-caused *D50* lethal mutation (VOELKER *et al.* 1984) and thus are referred to as *D50* revertants or "R", or by their allele number designation. For the reversion strains, part or all of the original *D50* mutagenic *P* element present within the 5′ transcribed region of the *RpII215* locus had excised, such that precise excision revertants restored full *RpII215* activity and rescued the null *L5* allele completely (LEFEVRE 1971; MORTIN and LEFEVRE 1981), whereas imprecise excision revertants only partially restored *RpII215* function, rescuing the null *L5* allele slightly to not at all. The level of rescue is considered to be a reflection of the level of active RpII215 subunit in each of the *R* strains.

For the genetic complementation experiments, crosses between a set of test female virgins (*i.e., L5/FM7, L5 IIt(13F)/FM7,* or *W81/FM7,* respectively) and hemizygous males carrying a *D50* revertant allele (see Table 1) were established. Flies were grown at 25° in half-pint milk bottles on modified Instant Drosophila Media (GREENLEAF *et al.* 1979). The fly broods were transferred to fresh bottles every other day over a period of 2 wk. The progeny from each bottle were counted for ~5 days after the first adults emerged. The total progeny for each set of crosses numbered from 600 to 5000, with an average number of 2500 progeny counted for each cross.

RESULTS

Transgenic flies carrying a truncated, CTD-less subunit, IIt: To extend our *in vivo* functional analysis of RNA polymerases II with altered CTDs, we constructed a mutation of *RpII215*, called *IIt*, that truncates the subunit such that the CTD is completely removed; we then generated transgenic flies carrying the *IIt* allele. An oligonucleotide whose sequence encoded seven novel amino acids plus two translation termination codons (shown in Figure 1) was inserted at a unique restriction site in the coding region upstream of CTD consensus repeat 1 (see MATERIALS AND METHODS). The resulting construct caused the altered C-terminus of the prematurely truncated subunit to be immunotagged by seven novel amino acids (shown in Figure 1 as QQFFGLM) encoding antibody-reactive C-terminal amino acids of substance P (CUELLO *et al.* 1979; MUNRO and PELHAM 1984). This tag was added to permit immunodetection of the truncated subunit (IIt) and detection and purification of RNA polymerase II containing the truncated subunit (pol IIT). We constructed the *IIt* mutant gene (see Figure 1) using the amanitin-resistant *C4* allele of *RpII215* (JOKERST *et al.* 1989) for introduction into wild-type (amanitin sensitive or amaS) flies, expecting that transgenic flies would contain a mixture of intact pol IIA/II0 and truncated IIt; the amanitin resistance (amaR) property would therefore allow us to distinguish functionally between amaR enzyme from IIT and amaS (wild-type) forms IIA/II0.

The truncation mutant construct $RpII215^{IIt}$ was subcloned into the *P*-element transformation vector pCaSpeR (PIRROTTA 1988) creating $P[w^+, RpII215^{IIt}]$. To generate transgenics, this *P* vector was microinjected into *w/w*; ry^{506} *P*[ry^+ Δ*2-3] (99B)/TM3* or *TM6B* Drosophila embryos (ROBERTSON *et al.* 1988). $P[w^+, RpII215^{IIt}]$-transformed progeny were selected and stabilized with balancer chromosomes for further analysis (see HAMILTON *et al.* 1993 for details on *P*-element transformation). Two transgenic lines were chosen for analysis in this study: +;*IIt(48A)* and *IIt(13F)* (see Table 1 and Figure 1).

Molecular characterization of *IIt* transgenes: To confirm that the complete clone of *IIt* had integrated into the genome of the transgenic flies without rearrangement and to assess the number of transgenes present, we isolated genomic DNA from wild-type and transgenic flies and carried out Southern blot hybridization (SOUTHERN 1975) as described in MATERIALS AND METHODS. *Xba*I digestion of the *IIt* transgene would produce a 9.4-kb hybridizable fragment if *IIt* integrated completely into the genome. The results in Figure 2B, lane 3, indicate the presence of a 9.4-kb *Xba*I restriction fragment (represented by an arrow) in +; *IIt(48A)* transgenic flies as expected. To determine the number of *IIt* inserts, genomic DNA was digested using *Kpn*I or *Sac*II restriction endonucleases that cut at unique sites within the *RpII215* gene and 5′ to the *RpII215* gene. As shown in Figure 2B, in addition to the wild-type-specific *Kpn*I band (indicated by the arrowhead) at about 10–11 kb, two larger DNA fragments hybridized to the *RpII215* probe, suggesting the presence of two *IIt* transgenes (compare +; *IIt(48A)/Kpn*I in lane 5 to +/*Kpn*I-digested DNA in lane 4). Similar results were obtained with *Sac*II-digested DNA as presented in lanes 8 and 7 of Figure 2B. Additionally, we prepared a stock of flies carrying the chromosome *2* balancer *CyO*, a chromosome *2* homologue having a *IIt* insert, and an *X*-linked temperature-sensitive *RpII215* allele, namely *ts; IIt(48A)/CyO* (see Table 1). Southern analyses of genomic DNA from these flies confirmed the presence of an intact *IIt* insert (see Figure 2B, lane 2). As seen previously, *Kpn*I and *Sac*II digests of *ts; IIt(48A)/CyO* genomic DNA produced two larger hybridizable bands in addition to the endogenous allele-specific band (see Figure 2B, lanes 6 and 9). Because *ts; IIt(48A)/CyO* flies share one chromosome in common with +; *IIt(48A)* and Southern analyses of these genotypes produce similar pattern of bands, we believe two *IIt* transgenes (designated as "*IIt(48A)*") are located in close proximity on chromosome 2R at 48A, because only a single site of ectopic hybridization was detected by *in situ* localization of transgenes (data not shown). Interestingly, for the *ts/ts; IIt(48A)/CyO* genomic DNA sample, the intensity of the bands for the endogenous allele is greater than for the bands derived from the *IIt* transgenes (compare the arrowhead-designated band *vs.* the arrow-indicated bands in lanes 6 and 9), while the hybridized restriction bands (+- and *IIt*-specific) for +/+; *IIt(48A)/IIt(48A)* have relatively similar intensities (see lanes 5 and 8), consistent with a 2*ts*:1*IIt-a*:1*IIt-b* gene ratio in the former genotype compared to a 2+:2*IIt-a*:2*IIt-b* ratio in the latter genotype. Thus, +; *IIt(48A)* homozygous flies appear to contain a 2:1 ratio of *IIt* transgenes to wild-type endogenous *RpII215* gene. (Refer to Table 1 and Figure 1).

Anticipating future genetic experiments (see below),

TABLE 1

***RpII215* mutants used in this study**

Mutant stock	*RpII215* allele	Description	Reference
Wild type *P2/Y*	+	*RpII215*$^{+}$; gene encoding amaS[a] largest subunit	GREENLEAF *et al.* (1979)
v C4/Y	*C4*	*RpII215*^{C4}; amaR[b] largest subunit encoding generated by EMS mutagenesis of *P2*; *X* chr.[c] marked by *v*	GREENLEAF *et al.* (1979)
W81 m fw/FM7/Y	*W81/+*	Partial CTD truncation mutant allele resulting from *P* element hybrid dysgenesis of *C4*; marked with *m* and *fw*; balanced in females with *X* chr. balancer *FM7*; amaR	SEARLES *et al.* (1982), VOELKER *et al.* (1985)
FM7^{c}	+	Balancer for *X* chr. carrying *RpII215*$^{+}$, y^{31d}, w^{a}, v^{Of}, and *B* markers	VOELKER *et al.* (1984), LINDLEY and ZIMM (1992)
+IIt(13F)/Y	+ and *IIt*	*IIt* transgene at 13F on *X* chr. encoding amaR truncated largest subunit	This study
+/Y;IIt(48A)/IIt(48A)	+ and *IIt*	*IIt* transgenes at 48A on chr. 2R encoding amaR truncated largest subunit	This study
y RpII215^{ts} *f/Y;IIt(48A)/CyO*	*ts* and *IIt*	*IIt* transgenes at 48A on chr. 2R encoding amaR truncated largest subunit, balanced by chr. 2 balancer *CyO*; in the background of endogenous temperature-sensitive *RpII215* allele on *X* chr. marked with *y* and *f*	This study, MORTON and KAUFMAN (1982)
y ras v L5 f/FM7/Y	*L5/+*	Lethal *RpII215* allele on *X* chr. marked with *y*, *ras*, *v*, *f*; covered by *X* chr. balancer *FM7*	LEFEVRE (1971), MARTIN and LEFEVRE (1981)
ras v L5 IIt(13F)/FM7/y^{+} *Y* v^{+}	*L5 IIt(13F)/+*	*IIt* transgene at 13F recombined onto *X* chr. bearing *L5* and marked with *ras* and *v*	This study
y^{+} *Y* v^{+} or *Dp(+)-Y*^{c}	+	Duplication of 9F3 to 10E3-4 onto *Y* chr. that includes *RpII215*$^{+}$, y^{+}, v^{+}, B^{S}	VOELKER *et al.* (1984), LINDLEY and ZIMM (1992)
D50 Revertants		Partially functional hybrid dysgenic revertants of *P*-element-caused lethal allele *RpII215*^{D50}; amaS	VOELKER *et al.* (1984)
R/Y	*1-1*	~40 bp *P* element remain	VOELKER *et al.* (1984)
	4-1	~50 bp *P* element remain	VOELKER *et al.* (1984)
	10-2	0 bp *P* element remain; complete reversion	VOELKER *et al.* (1984)
	16-2	~70 bp *P* element remain	VOELKER *et al.* (1984)
	16-4	35 bp *P* element remain	VOELKER *et al.* (1984), SEARLES *et al.* (1986)
	22-1	394 bp *P* element remain	VOELKER *et al.* (1984), SEARLES *et al.* (1986)
	38-1	35 bp *P* element remain	VOELKER *et al.* (1984), SEARLES *et al.* (1986)

Homozygous stock is used if not otherwise noted.
[a] amaS, amanitin sensitive (*i.e.*, wild type).
[b] amaR, amanitin resistant (*i.e.*, *C4*).
[c] Chromosome (chr.).

we recombined an *X*-linked *IIt* transgene onto an *X* chromosome carrying the *RpII215*^{L5} null allele (here represented as "L5" and previously denoted as *l (1) L5*) (LEFEVRE 1971; MORTIN and LEFEVRE 1981) at the endogenous locus at 10C. (See MATERIALS AND METHODS for details of the recombination crosses.) The transformant line carrying a *IIt* transgene at *13F* (designated as "*IIt(13F)*") had been previously identified by genetic selection and characterized by *in situ* hybridization (data not shown). Analyzing *L5 IIt(13F)* recombinant

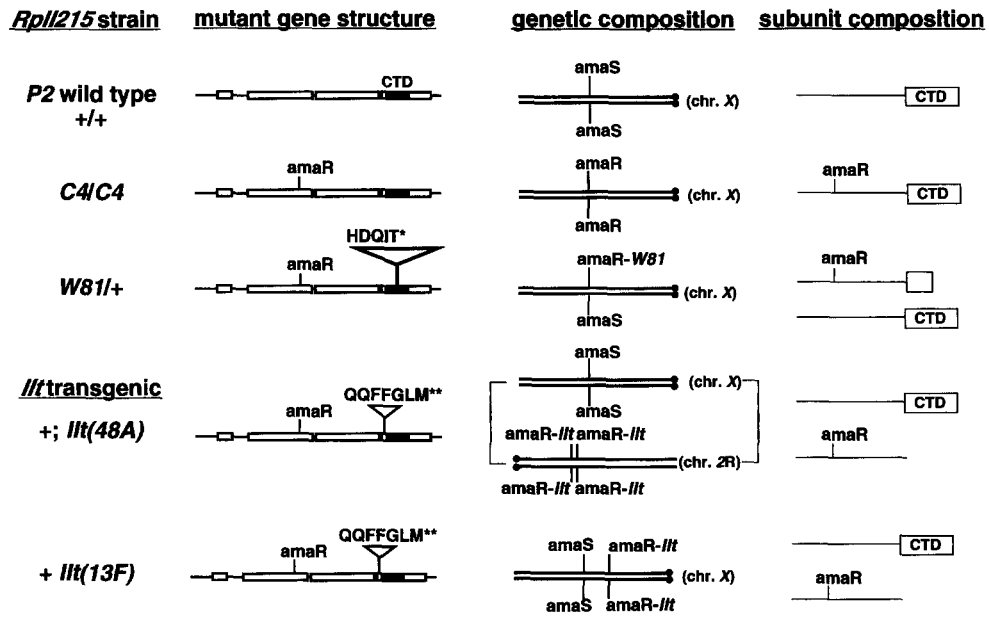

FIGURE 1.—Comparison of the structures of *RpII215* mutants analyzed in this study. The intron-exon structures (denoted as lines between boxes and □, respectively) of *RpII215* mutants are shown on the left, with the CTD heptad repeat domain coding sequences in the fourth exon indicated by ■. *C4*, *W81*, and *IIt* transgenes all carry the mutation conferring resistance to α-amanitin inhibition (amaR), whereas the wild-type gene encodes α-amanitin sensitive (amaS) subunit. *W81*, with a 631-bp *P* element inserted in the CTD coding sequence, contains sequences coding for five novel amino acids (shown by HDQIT) plus a termination codon (*) that leads to partial truncation of the CTD. The *IIt* transgenes contain an oligonucleotide inserted upstream of the CTD coding region that introduces seven novel amino acids (pictured as QQFFGLM) and two termination codons (**), producing an epitope-tagged completely truncated largest subunit construct. The +, *C4*, and *W81* alleles are all positioned at 10C, the endogenous *RpII215* locus on the *X* chromosome, whereas *IIt* transgenes have been localized to either chromosome 2R [*IIt(48A)*] or the *X* chromosome [*IIt(13F)*]. ●, the location of the centromere for each chromosome. Representations of RpII215 subunit composition for mutants are shown at the right. *W81/+* and *IIt* transgenic flies are expected to contain a mixture of amaS intact and amaR CTD mutant polymerase largest subunits.

flies by Southern blot hybridization, we confirmed the presence of the *IIt* transgene on the *L5* chromosome. In Figure 2C, the 9.4-kb *Xba*I *IIt*-specific DNA fragment as indicated by the arrow is found in *L5 IIt(13F)/+* recombinant and *+ IIt(13F)* parental transgenic flies in lanes 3 and 4, respectively. The results also indicate a length polymorphism in the *L5* allele, which has not been characterized at the molecular level (M. MORTIN, personal communication). An *L5*-specific *Xba*I band (denoted by a bar) is present in the *L5/+* control (lane 2), as well as in *L5 IIt(13F)/+* recombinant flies (lane 3), that is smaller than the wild-type *Xba*I band (shown by the arrowhead). Other hybridization experiments indicate that the *L5* mutation is due to a substantial deletion in the 5′ region upstream of the *RpII215* coding region (W. J. BRICKEY and A. L. GREENLEAF, unpublished results). Finally, by analyzing *Kpn*I-digested genomic DNA, we obtained results confirming the presence of a single *IIt* transgene on the *L5*-bearing chromosome in *L5 IIt(13F)/+* recombinant flies. (Compare lanes 5–8 of the *Kpn*I blot in Figure 2C.)

Levels of active mutant RNA polymerases: To assess the levels of active mutant pol II containing a partially or completely truncated largest subunit in *W81/+* or *+; IIt(48A)* flies, respectively, we exploited the amanitin resistance trait encoded by the *W81* and *IIt* alleles. We expected RNA polymerase II assembled with either mutant largest subunit to be resistant to the inhibitory effects of α-amanitin and therefore distinguishable from wild-type polymerase activity in nonspecific transcription assays that use denatured DNA as the template (GREENLEAF *et al.* 1979). Previous studies had demonstrated the validity of this assay for measuring levels of mutant enzyme activity and had shown that in this assay absence of the CTD does not affect catalytic activity of the polymerase (GREENLEAF *et al.* 1980; ZEHRING *et al.* 1988; A. L. GREENLEAF, unpublished results).

Pol II was partially purified from wild-type, *C4/+*, *W81/+*, and *+; IIt(48A)* adult females, and the amaS and the amaR enzyme activity in each sample was measured using the nonspecific transcription assays (GREENLEAF *et al.* 1979; JAENIKE *et al.* 1983). In the presence of α-amanitin concentrations >0.5 μg/ml, +/+ polymerase activity was nearly completely inhibited (see Figure 3A). In contrast, a substantial amount of amaR activity (~40–50% of initial activity) from C4 polymerase in the *C4/+* sample remained, and the CTD mutants (*W81* and *IIt*) displayed intermediate levels of amaR activity at α-amanitin concentrations >0.5 μg/ml.

The results presented in Figure 3B show that 100% of the total synthetic activity in +/+ flies is amaS polymerase and *C4/+* heterozygous flies contain ~50%

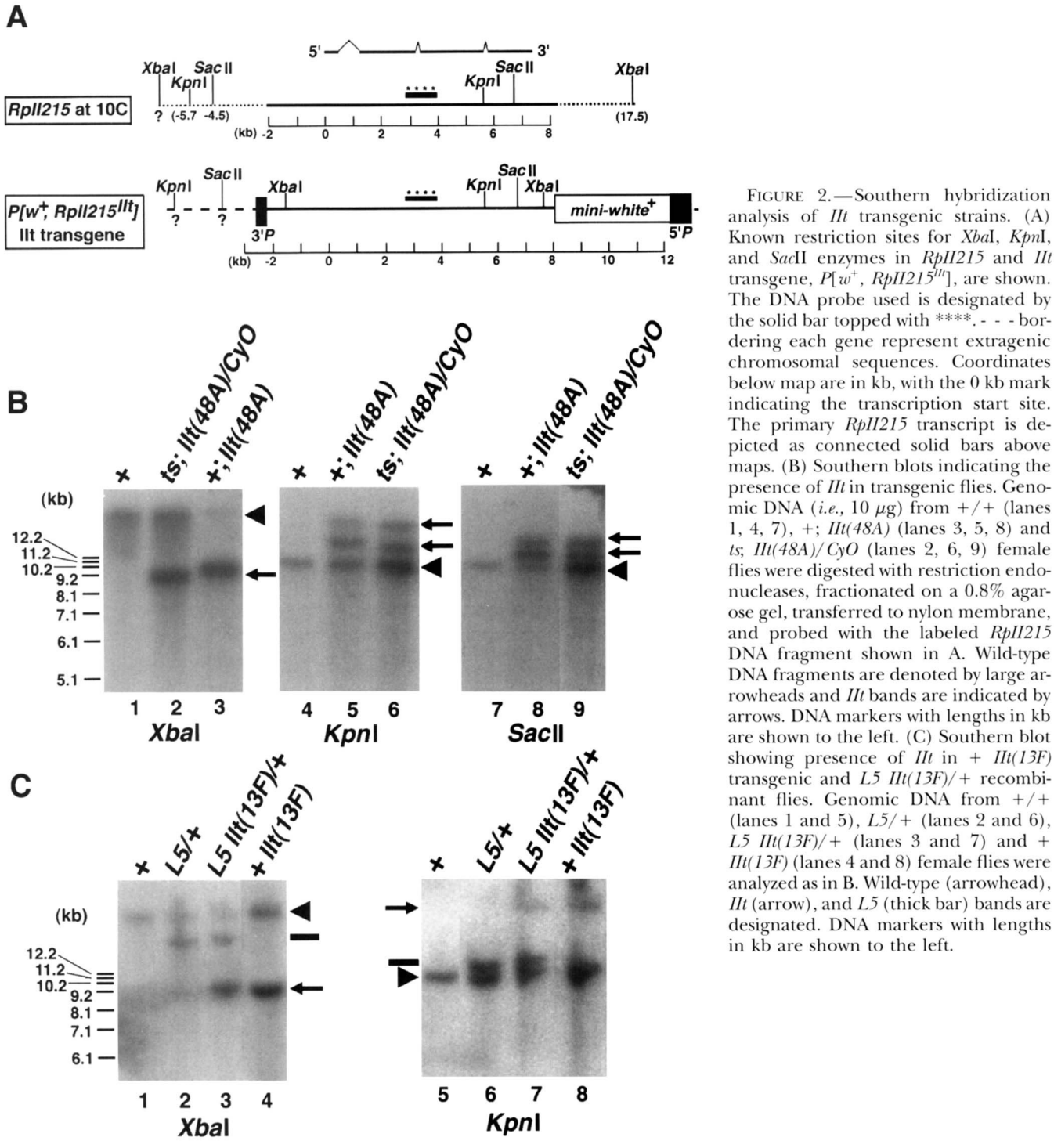

FIGURE 2.—Southern hybridization analysis of *IIt* transgenic strains. (A) Known restriction sites for *Xba*I, *Kpn*I, and *Sac*II enzymes in *RpII215* and *IIt* transgene, *P*[w^+, *RpII215*^{IIt}], are shown. The DNA probe used is designated by the solid bar topped with ****. - - - bordering each gene represent extragenic chromosomal sequences. Coordinates below map are in kb, with the 0 kb mark indicating the transcription start site. The primary *RpII215* transcript is depicted as connected solid bars above maps. (B) Southern blots indicating the presence of *IIt* in transgenic flies. Genomic DNA (*i.e.*, 10 μg) from +/+ (lanes 1, 4, 7), +; *IIt(48A)* (lanes 3, 5, 8) and *ts*; *IIt(48A)/CyO* (lanes 2, 6, 9) female flies were digested with restriction endonucleases, fractionated on a 0.8% agarose gel, transferred to nylon membrane, and probed with the labeled *RpII215* DNA fragment shown in A. Wild-type DNA fragments are denoted by large arrowheads and *IIt* bands are indicated by arrows. DNA markers with lengths in kb are shown to the left. (C) Southern blot showing presence of *IIt* in + *IIt(13F)* transgenic and *L5 IIt(13F)*/+ recombinant flies. Genomic DNA from +/+ (lanes 1 and 5), *L5*/+ (lanes 2 and 6), *L5 IIt(13F)*/+ (lanes 3 and 7) and + *IIt(13F)* (lanes 4 and 8) female flies were analyzed as in B. Wild-type (arrowhead), *IIt* (arrow), and *L5* (thick bar) bands are designated. DNA markers with lengths in kb are shown to the left.

amaS and 50% amaR enzyme, as previously documented (GREENLEAF *et al.* 1980). In contrast, *W81*/+ flies and +; *IIt(48A)* transgenics each appear to possess only ~15% amaR polymerase. This amount of amaR activity from W81 or IIT polymerase is much less than anticipated from gene dosage considerations. Recall that *W81*/+ flies possess one gene encoding the amaR largest subunit and one gene encoding the amaS largest subunit, and that in +; *IIt(48A)* transgenic flies, the ratio of amaR to amaS genes is 2:1.

Levels of mutant RpII215 subunit: Next, we examined the steady-state levels of W81 and IIt subunits in mutant flies to determine if the reduced levels of active mutant pol II represented all or just part of the total truncated subunit. Levels of CTD-truncated subunits relative to intact wild-type subunits were estimated by immunoblot analysis of quickly prepared whole fly extracts. Due to the extreme sensitivity of the CTD to proteolytic degradation (and phosphatase action), we prepared total extracts by grinding flies in cold ethanol

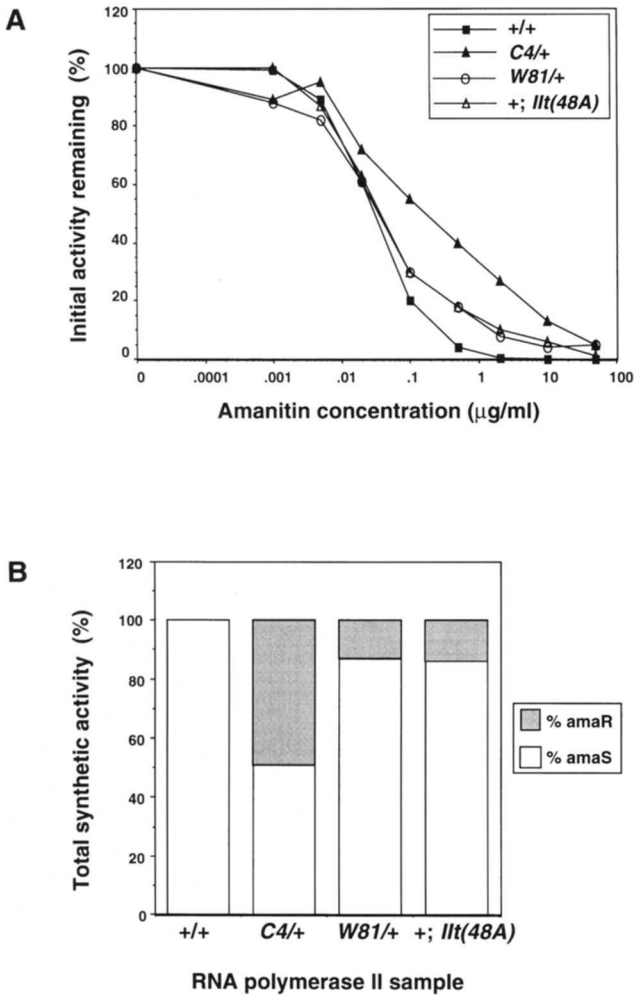

FIGURE 3.—Levels of active mutant RNA polymerase II in mutant *RpII215* flies. (A) RNA polymerase II activity in the presence of increasing amounts of α-amanitin. Crude preparations of polymerase from wild-type and mutant flies (+/+, *C4/+*, *W81/+*, and +; *IIt(48A)*) were assayed using denatured DNA template in the presence of increasing amounts of α-amanitin. The amount of nucleotide incorporation at each amanitin concentration was compared to the productivity in the absence of amanitin (defined as 100% activity) to determine percentage initial activity remaining. (B) Determination of levels of α-amanitin-resistant polymerase in *W81/+* and +; *IIt(48A)* mutants. Data in A were analyzed using the AMACURVE program developed (by A. SLUDER) to convert the amount of nucleotide incorporation at known α-amanitin concentrations to percentage initial α-amanitin-sensitive activity in polymerase sample according to the equation described by GREENLEAF *et al.* (1980) (see also JAENIKE *et al.* 1983). The difference between total synthetic activity (defined as 100%) and α-amanitin sensitive synthetic activity (□) equaled the amanitin-resistant activity (▨). The error for α-amanitin-sensitive synthetic activity determined for each sample was 2–3%.

and boiling the ground material in SDS sample buffer (see MATERIALS AND METHODS). Immunoblots were reacted with antibodies directed against determinants in the second exon of the *RpII215* gene (denoted as "anti-exon2" antibodies) (WEEKS *et al.* 1993 and references therein) to detect all forms of the largest subunit, independent of the state of the CTD [*e.g.*, form IIo (phosphorylated), form IIa (unmodified), and W81 or IIt (partially or completely truncated, respectively)].

Immunoblotting of *C4/C4* protein extracts revealed predominantly IIo (indicated by the thick arrow) and IIa (designated with a long thin arrow) subunits (and small amounts of intermediately phosphorylated species migrating between IIo and IIa) as shown in lanes 1 and 2 in Figure 4. More importantly, no bands migrating faster than IIa that would have resulted from proteolytic degradation of the CTD were evident. In contrast, for *W81/+* fly extracts, anti-exon2 antibodies revealed a protein species migrating faster than IIa at a position expected for a partially truncated subunit (Figure 4A, lanes 3 and 4, indicated by the short arrow labeled W81); in addition two bands migrating slightly ahead of the W81 band, possibly representing proteolytic products, were noticeable. Finally, for the +; *IIt(48A)* transgenic flies, a novel species with a mobility expected for a complete truncation was present in addition to IIa and IIo (Figure 4A, lanes 5 and 6, shown by arrowhead labeled IIt). (An antibody-reactive band at ~120–130 kD was sometimes seen on these immunoblots, but since we do not know its origin or structure, we have not included it in this analysis).

The amount of completely or partially truncated subunit (IIt or W81) relative to intact subunit (IIa plus IIo) in mutant fly samples was estimated by measuring and comparing the intensities of the protein bands in each sample. For *W81/+* heterozygotes, W81 protein was found to comprise ~25% of antibody-reactive species (Figure 4B, lanes 3 and 4). The bands migrating faster than W81 were considered to be breakdown products of W81, since similar bands were not present in *C4/C4* or +/+ (data not shown) extracts. It also should be noted that W81, possessing 19 of the 42 natural CTD repeats, may be phosphorylated and the modified partially truncated form(s) may migrate between IIa and IIo. We are currently unable to distinguish hyperphosphorylated W81 subunit forms from other intact *RpII215* subunits in *W81/+* protein extracts. Similar analysis indicated that IIt represents ~17% of the largest subunit forms in +; *IIt(48A)* transgenic flies (Figure 4B, lanes 5 and 6). Thus, in parallel with the activity assays described above that measured a property of assembled polymerase, this immunoblot analysis indicates lower than expected amounts of total truncated subunit present in CTD mutant flies. In view of the fairly close agreement between the two sets of values and considering the nature of the measurements, we conclude that most of the subunit we detected immunologically was probably part of assembled, catalytically active pol II.

Last, we discovered that subunit IIt, which should contain a portion of the substance P peptide at its COOH terminus, was undetectable by immunoblotting or by immunofluorescence staining of polytene chromosomes with several anti-substance P antibodies (J. R.

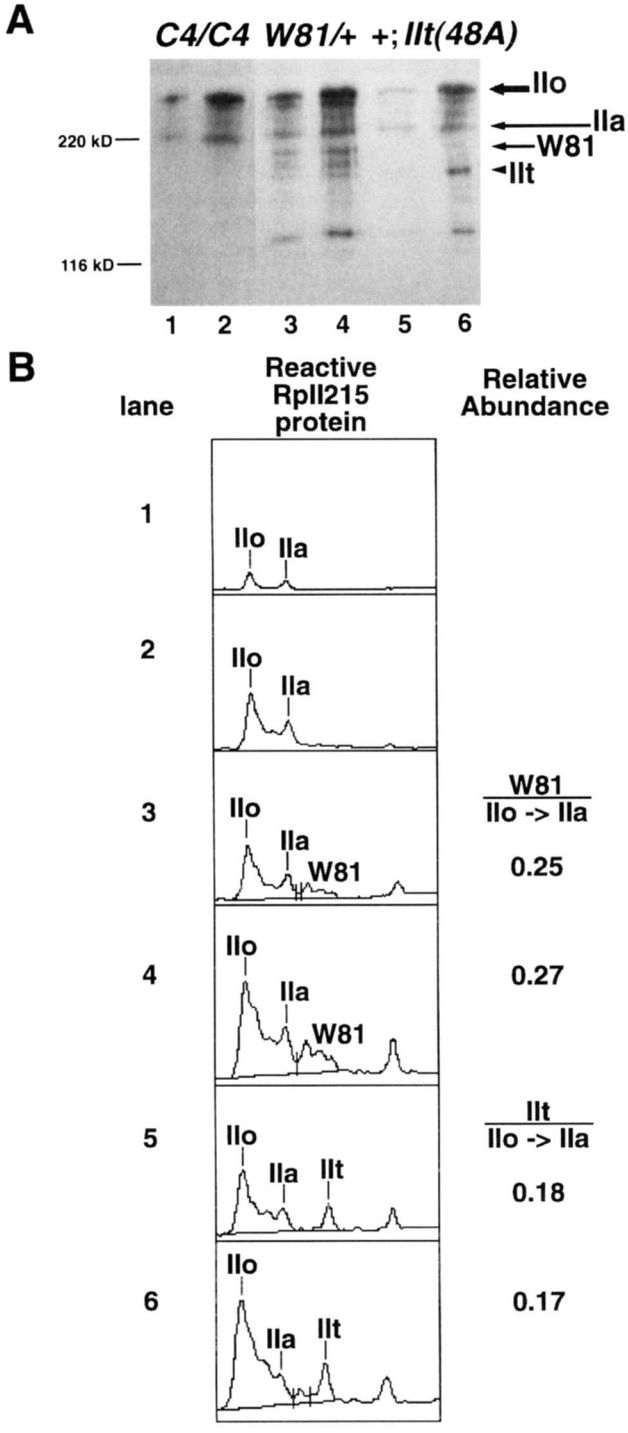

FIGURE 4.—Levels of mutant *RpII215* subunit detected by Western blot analysis. (A) Immunoblot detection of largest subunit forms in *RpII215* mutant fly extracts. Whole fly extracts were stringently prepared, electrophoresed on a 5% polyacrylamide gel, transferred to nitrocellulose, and probed with anti-*RpII215* exon2 antibodies (WEEKS *et al.* 1993). Increasing amounts of fly extracts were analyzed in paired lanes: *C4/C4* in lanes 1 (9 μg total protein) and 2 (23 μg), *W81/+* in lanes 3 (45 μg) and 4 (90 μg), and +; *IIt(48A)* extracts in lanes 5 (10 μg) and 6 (30 μg). Intact phosphorylated IIo (thick arrow), intact unmodified IIa (thin long arrow), partially truncated W81 (short arrow), and fully truncated subunit IIt (arrowhead) are indicated. The mobility of protein markers (Sigma) of apparent molecular weight 220 and 116 kD are indicated at left of blot. (B) Analysis of detected proteins reveals relative abundance of mutant subunits to intact subunits in CTD mutant flies. Intensities of antibody-reactive RpII215 subunit bands in A were quantitated by imaging the blots using a CCD camera, followed by analysis with the NIH Image v1.52 program. The peaks for IIo, IIa, W81, and IIt subunit forms for each scan are indicated. The relative abundance of mutant to intact subunits was determined by dividing the intensity of the W81 or IIt peak by the intensity of the total peak representing IIo, IIa, plus all intermediate phosphorylated forms for each respective sample.

WEEKS and A. L. GREENLEAF, unpublished data). The immunotag appears to be either unavailable to the antibodies or proteolytically removed from the subunit, since the presence of the substance P sequence in the transformation vector *P[w$^+$, RpII215^{IIt}]* and *IIt* transgenic flies was confirmed by PCR amplification and DNA sequence analysis (data not shown). As a result, direct and selective identification of IIt subunit by substance P antibodies unfortunately was not achievable.

Levels of mutant *RpII215* subunit mRNA: To inquire whether the low amounts of CTD-compromised enzyme in *W81/+* and in +; *IIt(48A)* flies might be due to comparably low levels of the corresponding mRNAs, we examined the steady-state levels of *RpII215* mRNA in mutant and control flies, each with a known *RpII215* gene dose. Total and poly(A$^+$) RNAs were isolated from adult females of four genotypes: +/+, *C4/+*, *W81/+*, and +; *IIt(48A)*. The RNAs were analyzed by Northern blot analysis (shown in Figure 5A and B) with the amount of hybridization to an *RpII215* probe being normalized against the signal from an internal control of a constitutively expressed RNA, namely *Ras2* (MOZER *et al.* 1985). Note that the expected length of the *IIt* transcript is only 64 bases longer than the wild-type ~7-kb message (BIGGS *et al.* 1985), and the two would not be resolved during electrophoresis. The *W81* message might consist of a heterogeneous population of RNAs, depending upon the extent of transcriptional termination or processing within the 631-bp *P*-element insert that caused the *W81* mutation. For example, 3′ end formation at a poly(A$^+$) site within the *P* insert (O'HARE and RUBIN 1983) would generate a ~6.6-kb mRNA; however, if this site is not used efficiently and if transcription continued through the insert to terminate at the normal *RpII215* site, a longer mRNA of ~7.6 kb would result. Which of these RNAs would be resolved from the wild-type transcript during electrophoresis is not known (also see DISCUSSION).

The results shown in Figure 5 indicate that, as judged by comparison with the *Ras2* internal standard, the ratio of steady-state *RpII215* transcript to *RpII215* gene dose is relatively constant for all samples examined, except for *W81/+*. That is, steady-state *RpII215* transcript per gene found in total or poly(A$^+$) RNA isolated from *W81/+* flies appears to be less than that seen for the other flies that also have two X-linked *RpII215* alleles (*i.e.*, +/

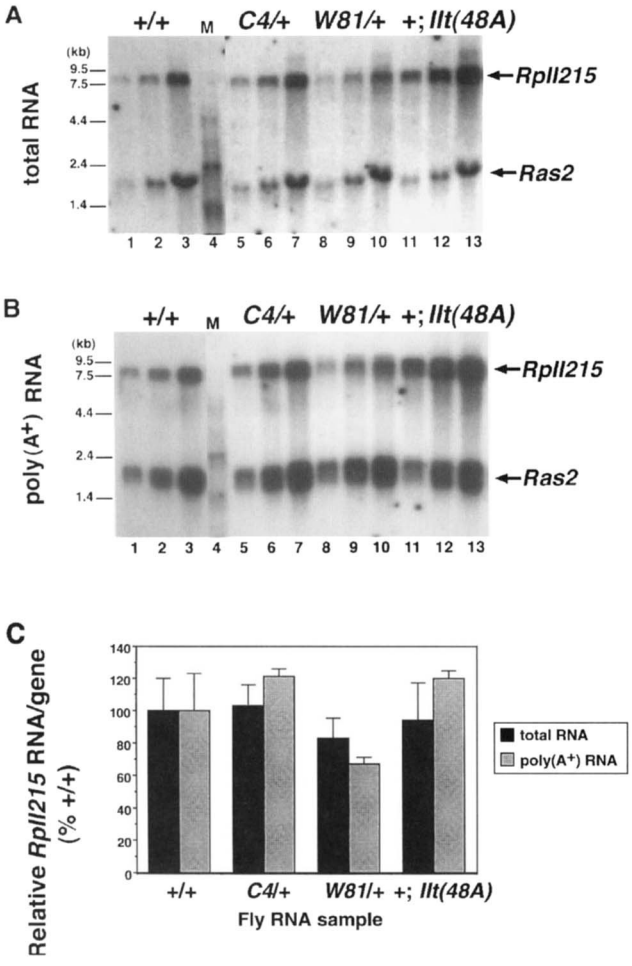

FIGURE 5.—Northern blot analysis of steady-state levels of *RpII215* subunit mRNA in *RpII215* mutants. (A) Northern blot analysis of total RNA from *RpII215* mutant flies. Total RNA was isolated from the *RpII215* strains shown, then fractionated on a 0.8% agarose-formaldehyde gel, blotted to nylon membrane, and probed with the *RpII215* DNA probe described in Figure 2. Increasing amounts (*i.e.*, 5, 10, and 25 μg) of total RNA for each sample [+/+, lanes 1–3; *C4*/+, lanes 5–7; *W81*/+, lanes 8–10; +; *IIt(48A)*, lanes 11–13] were loaded in consecutive lanes from left to right. Signals for *RpII215* and *Ras2* (internal control) transcripts (MOZER *et al.* 1985) are indicated by labeled arrows at right. M (lane 4), RNA size markers (GIBCO-BRL) with lengths in kb are shown to left. (B) Polyadenylated RNA from mutant *RpII215* flies analyzed by Northern blotting. Poly(A^+) RNA was purified by oligo-(dT)-cellulose affinity chromatography and analyzed by Northern hybridization as in A. Increasing amounts of poly(A^+) RNA (*i.e.*, 1, 3, and 5 μg RNA) were loaded on consecutive lanes from left to right for each sample (as described above). Signals for *RpII215* and *Ras2* transcripts, as well as RNA size markers (M), are indicated as in A. (C) Determination of relative levels of *RpII215* subunit mRNA in mutant flies. Relative levels of *RpII215* mRNA/gene were determined by measuring the intensity of *RpII215* and *Ras2* signals in A (■, total RNA) and B (▨, polyadenylated RNA) by PhosphorImaging, followed by using a data analysis program. The *RpII215* signal in each lane was compared to the *Ras2* signal, and a mean value of normalized *RpII215* mRNA amount in each sample was calculated from the data points in each set in which signal intensity increased with input RNA. These values were expressed as the ratio of mRNA to gene copy number relative to the wild-type value that was assigned to be 100%. The error bars represent the variability of three values used to determine the mean that were obtained through the independent analyses of two RNA preparations.

+ and *C4*/+). Interestingly, in the case of +; *IIt(48A)* flies that harbor six total *RpII215* gene copies, the ratio of the *RpII215* signal to the *Ras2* signal is higher than in the other three cases, but the ratio of *RpII215* mRNA normalized to *RpII215* gene number is equivalent to that of wild type. Thus, a lowered amount of steady-state mRNA might be a reasonable partial explanation for lowered amounts of truncated subunit and enzyme in the case of *W81* but not in the case of *IIt*.

***In vivo* complementation activity of mutant *RpII215*:** To examine the *in vivo* function of W81- and IIt-containing RNA polymerases, we chose to use a sensitive genetic complementation test that employed a series of partially functioning mutant alleles of *RpII215*, the so-called revertants of the lethal *D50* allele (VOELKER *et al.* 1984; also see Table 1). *D50* was caused by a hybrid dysgenesis-induced insertion of 1221 bp of *P* element DNA into the 5′ transcribed portion of the *RpII215* locus. In the course of subsequent genetic analysis of the *RpII215* allele, the *D50* revertant alleles (here referred to as "R," or by their allele number, *e.g.*, *22-1*) were generated as a consequence of excision of some or all of the *P*-element sequences present in *D50* (VOELKER *et al.* 1984; SEARLES *et al.* 1986). The *R* alleles vary in ability to complement the lethality of *L5*, an *RpII215* null allele (LEFEVRE 1971; MORTIN and LEFEVRE 1981). Imprecise excision of *P* sequences generated *R* alleles that complemented *L5* slightly if at all, whereas precise excision generated complete revertant alleles with wild-type complementation values (VOELKER *et al.* 1984). (Note that even the *R* alleles that do not support viability of *L5*/*R* heterozygous females, nevertheless support the viability of *R*/*Y* hemizygous males, presumably from increased expression due to dosage compensation of the X-linked *R* gene.)

To determine whether the CTD-altered *RpII215* mutant alleles *W81* and *IIt* displayed *in vivo* activity, we used selected *R* alleles to ask whether some might be complemented by *W81* or *IIt*, thereby providing evidence of *in vivo* function of partially or completely truncated enzyme (recall that the null allele *L5* is not complemented by either of the recessive lethals *W81* or *IIt*). We first created a recombinant mutant stock that possessed the *IIt* transgene at 13F in an *X* chromosome that also carried the lethal *L5* allele at 10C to create a strain producing only IIT polymerase for crossing to other X-linked *RpII215* alleles (see MATERIALS AND METHODS for details). Then, *L5*/*FM7*, *L5 IIt(13F)*/*FM7*, or *W81*/*FM7* virgin females were mated to males carrying various *R* alleles (*i.e.*, *R*/*Y* males), and female

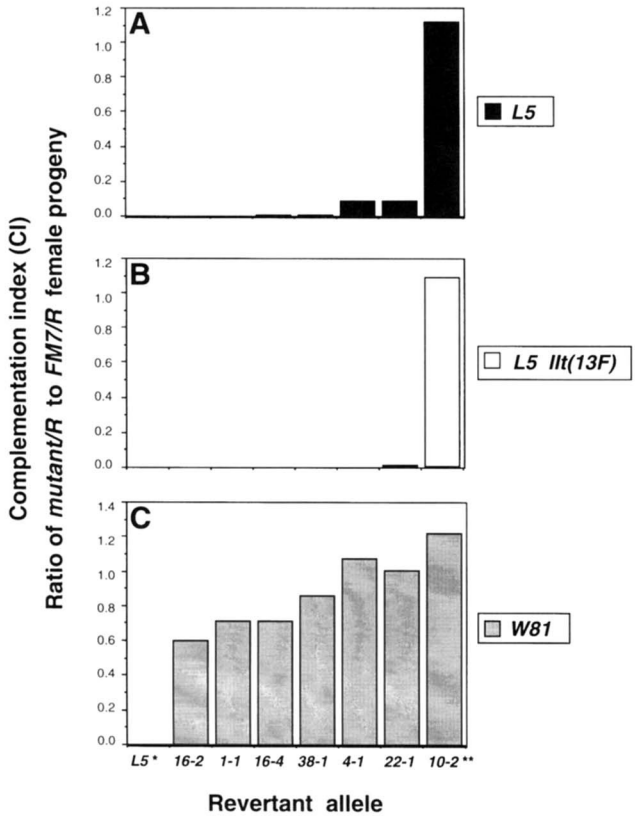

FIGURE 6.—Genetic complementation index for *L5*, *L5 IIt(13F)*, and *W81* with *RpII215 D50* revertant (*R*) alleles. *L5/FM7* (A), *L5 IIt(13F)/FM7* (B) or *W81/FM7* (C) females were mated to *R/Y* males, with revertant alleles shown below the bottom axis (with *L5*/Y* males as the null *RpII215* control and *10-2**/Y* males as the complete reversion control; note that *10-2* is a precise excision of the *D50 P* element [see Table 1]). The female progeny were counted, and a complementation index (CI) was defined as the ratio of *mutant/R* females to *FM7/R* females, which are considered 100% viable since *FM7* carries the *RpII215*$^{+}$ allele. CIs are indicated by solid (*L5*), open (*L5 IIt(13F)*) or stippled (*W81*) vertical bars above the test alleles arrayed along the bottom axis. The error for each CI value was 0.002 or less.

progeny were examined. A complementation index (CI) was defined as the ratio of *mutant/R* daughters to *FM7/R* daughters (note that *FM7/R* females represent 100% viability since *FM7* carries *RpII215*$^{+}$). If *W81* or *IIt* displayed activity *in vivo*, then the observed complementation index would be greater than that for the null *L5*. On the other hand, if either CTD mutant possessed no activity, the complementation ratio would be no greater than that seen for *L5*.

Testing of *W81* revealed that *W81/R* females (stippled columns in Figure 6C) survived with a remarkably high viability, from 60 to 100% of their *FM7/R* female siblings depending upon the respective *R* allele. In contrast to *W81*, the level of complementation between the null *L5* and the *R* alleles (solid columns in Figure 6A) was from none to slight, with CIs from 0.00 for *16-2* to 0.09 for *22-1*. [Note that as expected *W81* did not complement *L5* (CI = 0). Also, in an earlier study by VOELKER *et al.* (1984), the complementation ratios for *L5* with the *R* alleles in general were somewhat higher than we found; this may reflect slight variations in conditions or methodology.] Thus the *W81* complementation indices were 10- to 700-fold higher than those for the *L5* null allele. These results are consistent with the proposal that *W81* does exhibit partial but significant genetic function *in vivo* and that this function is revealed when *W81* is in combination with another partially functioning *RpII215* allele. (Recall that *W81* homo- and hemizygotes normally die late in embryogenesis.) Indeed, *W81* function was sufficient to support wild-type levels of viability in combination with *4-1* and *22-1* alleles.

The results for *IIt* were in marked contrast to those for *W81*. The complete truncation mutant allele (as *L5 IIt(13F)*) failed to complement significantly any of the partial revertants. The survival rate for *L5 IIt(13F)/R* females (open columns in Figure 6B) was actually lower than that for *L5/R* females (note especially the cases of *4-1* and *22-1*). Thus, *W81* displays significant function *in vivo* when heterozygous with a partially functional allele, whereas *IIt* seems to be completely defective.

DISCUSSION

To examine CTD function *in vivo*, we characterized and compared the activity, biosynthesis and genetic function of Drosophila RNA polymerase II largest subunit mutants that possessed none (*IIt*), half (*W81*), or all (*C4*) of the CTD heptad repeats. In the case of the strain +; *IIt(48A)*, we found dramatically less IIt subunit and IIT polymerase than expected for a transgenic fly strain harboring two no-CTD transgenes to each copy of the *RpII215* endogenous gene. While investigating a potential correlative down-regulation of RNA synthesis of mutant subunit due to position effect on the expression of the transgenes, we discovered that steady-state *RpII215* subunit mRNA levels in transgenic flies in fact reflected the increased gene dose, such that the amount of mRNA per gene copy was the same as in wild type. These results suggest that absence of the CTD causes a severe reduction in steady-state protein levels. For the half-CTD mutant allele (*W81*) we discovered that heterozygous flies (*W81/+*) displayed W81 subunit levels and mutant polymerase activity much lower than the corresponding wild-type levels even though each gene (+ or *W81*) is present in one dose. Subsequently and in contrast to the case for *IIt*, we discovered reduced mRNA per gene in *W81/+* heterozygotes, implying that the transcriptional regulation of the *W81* allele and/or its RNA processing and/or stability differed from wild type; these effects might be a consequence of the nature of the *W81* mutation, namely a *P*-element insertion. Finally, we showed that whereas *IIt* does not complement a set of partially functional *RpII215* alleles, *W81* complements them very well. Although these data sug-

gest that a CTD-less pol II may be completely defective *in vivo*, they indicate that an enzyme with half the CTD intact displays significant *in vivo* function.

The experiments reported here afford several useful insights into the biology of RNA polymerase II subunit expression and function. For example, we learned that the *IIt* transgene produces mRNA as efficiently as does the endogenous wild-type gene, verifying that all the necessary *cis*-acting sequences required for proper expression of the *RpII215* transgene are present in the *P*-element construct. This information is vital for designing future *RpII215* mutagenesis and transformation experiments.

The much lower than expected steady-state levels of the IIt subunit and IIT polymerase suggest the importance of the CTD in sustaining biological integrity of pol II in an organism. The observed effects could manifest themselves through several alternative mechanisms. For example, a CTD-less subunit might exhibit a shorter than normal half life, consequently being incorporated less efficiently into core enzyme. Alternatively, assembled enzyme lacking the CTD may itself be less stable than enzyme with an intact CTD. Although previous work (KOLODZIEJ and YOUNG 1991) demonstrated that removal of half of the CTD repeats in the yeast pol II largest subunit did not lead to reduction in subunit levels or in assembly of mutant subunit with the other two largest subunits, absence of part of the CTD, as for *W81*, may lead to intermediate effects on assembly or stability, and in conjunction with the somewhat reduced levels of *W81* mRNA could explain the low steady-state levels of W81 enzyme. In the future it might be possible to design experiments that would distinguish among these possibilities and pinpoint the actual mechanism by which CTD loss affects enzyme abundance.

Perhaps the most striking result was the vast difference in function, as revealed by a genetic test, between a half-CTD and a no-CTD polymerase. From the perspective of *in vitro* results available when these experiments were begun (CORDEN and INGLES 1992 and references therein), it might have been imagined that even a CTD-less polymerase would show substantial function *in vivo*. On the other hand, the recent discovery that CTD-associated components are integral parts of a pol II "holoenzyme" that may represent the initiation-competent form of RNA polymerase II *in vivo* (THOMPSON *et al.* 1993; KIM *et al.* 1994; KOLESKE and YOUNG 1994) would make the lack of activity of pol IIT much less surprising. The holoenzyme is composed of RNA polymerase II plus associated proteins that either bind to the CTD (*e.g.*, the SRB complex) (THOMPSON *et al.* 1993; KOLESKE and YOUNG 1994) or otherwise participate in transcription initiation complex formation (*e.g.*, TFIIF) (KIM *et al.* 1994; KOLESKE and YOUNG 1994). If the CTD in fact is required to mediate preinitiaton complex formation at most promoters, then a CTD-less polymerase would be completely defective in this step and would be essentially without physiological function, whereas a half-CTD polymerase might be only partially defective and would display detectable *in vivo* activity.

A distinguishing feature uncovered for the *W81* allele was that its mRNA is apparently under-represented in the steady-state poly(A^+) pool, relative to wild-type *RpII215* subunit mRNA. Examination of the *P*-element sequences present in the *W81* allele provided some clues that might explain this difference from wild type. First the *W81* allele contains a nonsense mutation, resulting in premature termination of translation ~500 nucleotides upstream of the normal termination site. The resulting *W81* RNA may thus be subject to nonsense mutation-mediated degradation, although the normal levels of *IIt* RNA would indicate a strong influence of sequence context on this effect (CHENG and MAQUAT 1993; CHENG *et al.* 1994). Second, a polyadenylation signal, AAUAAA, was found 123 nucleotides downstream from the *P*-element-encoded termination codon, followed by poorly matched consensus poly(A^+) addition sites (BERGET 1984) 16 and 28 nucleotides 3′ of this signal. If inefficient poly(A^+) addition occurs at either of these sites, the resulting message might display a stability different from wild type and contribute to the observed phenotype. Third, sequences in the *P* element downstream of this putative poly(A^+) signal were found to be relatively AU rich, and examination of 3′ untranslated *RpII215* sequences revealed the presence of multiple AUUUA motifs (AU-rich element or ARE) (SHYU *et al.* 1991); these elements are often found in unstable proto-oncogene RNAs (*i.e.*, c-myc) and are involved in determining their decay rates (SACHS 1993). Inclusion of these sequences in the mRNA (*e.g.*, if the poly(A^+) site in the *P* element is not used) would generate a message 600 nucleotides longer than the normal transcript that might be expected to differ from wild type in decay rate. Clearly additional experimentation, which for example might use RT-PCR based analyses, will be required in the future to test these possibilities by analyzing separately the allele-specific transcripts.

Our genetic experiments demonstrate that despite the low abundance of *W81* subunit and polymerase, the partial CTD mutant does in fact possess significant activity *in vivo*. Even though W81 homozygotes normally die at a late embryonic stage, our studies showed that various *W81/R* heterozygotes survived to a relatively high degree, with the rate of survival ranging from 60 to 100% of the *FM7/R* controls. In contrast, the *IIt/R* survival rate was at or near zero (comparable to that for flies carrying a null allele, namely *L5/R* females). The detailed nature of the complementation of the partially functional *R* alleles by *W81* was not determined by our experiments and revealing it will require additional molecular characterization of transcript processing and translation for both the *W81* allele and each respective *R* allele. Recall that the partially functional *R* alleles carry remnants of *P*-element sequences in the

5′ leader sequence that probably affect translational efficiency (SEARLES *et al.* 1986). A reasonable proposal is that the complementation by *W81* simply reflects the level of functional subunit generated by each *R* allele. This would be supported by the observation that the *R* alleles displaying the highest complementation with *W81* also had the highest values with *L5* (refer to A and C in Figure 6).

That W81 polymerase possesses substantial but not wild-type activity *in vivo* raises questions concerning which functional properties of a half-CTD enzyme are normal and which are defective. Is this enzyme present in some or all preinitiation complexes? Is it generally competent to initiate and elongate transcripts *in vivo*, or is it defective for certain subsets of loci? Does the mutant enzyme elongate growing transcripts at a normal or abnormal rate and with normal or abnormal efficiency? How does the phosphorylation of the partial CTD compare to that of a complete CTD? We had hoped to address some of these questions by using the epitope tag on a CTD-truncated subunit for specific immunolocalization of mutant polymerase on polytene chromosomes (WEEKS *et al.* 1993). However, since we could not detect the tag, these experiments were not possible; differently tagged constructs may allow such an approach in the future. Meanwhile, it should be possible to answer certain questions about W81 polymerase function by applying the immunolocalization approach to polytene chromosomes from amanitin-treated salivary glands from *W81/+* larvae. In fact, preliminary experiments suggest that detectable W81 polymerase function in supporting heat shock puff formation in the presence of amanitin (W. J. BRICKEY and D. LEE, unpublished data). In addition, nuclear run-on experiments with nuclei isolated from *+/W81* flies indicate W81 polymerase function in transcribing histone *H4* genes (T. O'BRIEN and J. LIS, personal communication).

Additional future experiments to obtain more specific and informative data about *in vivo* function(s) of the CTD might profitably utilize polymerase largest subunit mutants for which the CTD is less drastically altered (*e.g.*, BARTOLOMEI *et al.* 1988). In contrast to the case of mammals (CORDEN *et al.* 1985; AHEARN *et al.* 1987; BARRON-CASELLA and CORDEN 1992) and yeast (ALLISON *et al.* 1985), where the majority of heptad repeats align nearly perfectly with the consensus sequence, in *D. melanogaster* only two repeats match exactly the consensus heptamer and many repeats are distinct in sequence. If these variations are conserved among other Drosophila species (D. LEE, personal communication), that fact might implicate particular repeats for discrete and/or specialized functions and establish a foundation for future mutagenesis studies to examine structure/function correlates of the CTD. Future experiments, building on the results reported in this paper, should allow us to examine this and other possibilities, thereby increasing our understanding of how the CTD functions *in vivo*.

We are grateful to MARK MORTIN for the *y ras v L5 f/FM7/Y* and *y RpII215^{ts} f/Y* stocks and for his helpful comments on the genetic selection of *L5 IIt(13F)* recombinants. We thank TOM O'BRIEN and JOHN LIS for generously sharing unpublished data. We also appreciate WILL ZEHRING for his advice and relevant information on the genetic background and experimentation concerning the *D50* revertants. We thank JOHN WEEKS for his assistance with microinjections, continued support during this project, and assistance with computer applications. We appreciate the expert technical assistance of ELLA BOST in the preparation of fly media and care of fly stocks. We acknowledge DAVID KIRSCH for his enthusiastic assistance with polytene squashes and *in situ* hybridization experiments, ANN SLUDER for the initial development of the AMACURVE program, PAUL MODRICH for use of his CCD camera, and MICHAEL BEEN for use of his PhosphorImager. We appreciate the support and helpful discussions from other members of the GREENLEAF lab. W.J.B. was initially supported by a National Research Service Award 5 T32 GM07148. This work was funded by National Institutes of Health grant GM-28078 to A.L.G.

LITERATURE CITED

AHEARN, J. M., JR., M. S. BARTOLOMEI, M. L. WEST, L. J. CISEK and J. L. CORDEN, 1987 Cloning and sequence analysis of the mouse genomic locus encoding the largest subunit of RNA polymerase II. J. Biol. Chem. **262:** 10695–10705.

ALLISON, L. A., and C. J. INGLES, 1989 Mutations in RNA polymerase II enhance or suppress mutations in *GAL4*. Proc. Natl. Acad. Sci. USA **86:** 2794–2798.

ALLISON, L. A., M. MOYLE, M. SHALES and C. J. INGLES, 1985 Extensive homology among the largest subunits of eukaryotic and prokaryotic RNA polymerases. Cell **42:** 599–610.

ASHBURNER, M., 1989 *Drosophila, A Laboratory Manual.* Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

AUSUBEL, F. M., R. BRENT, R. E. KINGSTON, D. D. MOORE, J. G. SEIDMAN *et al.* 1989 *Current Protocols in Molecular Biology.* John Wiley & Sons, New York.

AVIV, H., and P. LEDER, 1972 Purification of biologically active globin messenger RNA by chromatography on oligothymidylic acid-cellulose. Proc. Natl. Acad. Sci. USA **69:** 1408–1412.

BARRON-CASELLA, E., and J. L. CORDEN, 1992 Conservation of the mammalian RNA polymerase II largest-subunit C-terminal domain. J. Mol. Evol. **35:** 405–410.

BARTHOLOMEW, B., M. E. DAHMUS and C. F. MEARES, 1986 RNA contacts subunits IIo and IIc in HeLa RNA polymerase II transcription complexes. J. Biol. Chem. **261:** 14226–14231.

BARTOLOMEI, M. S., N. F. HALDEN, C. R. CULLEN and J. L. CORDEN, 1988 Genetic analysis of the repetitive carboxyl-terminal domain of the largest subunit of mouse RNA polymerase II. Mol. Cell. Biol. **8:** 330–339.

BASKARAN, R., M. E. DAHMUS and J. Y. WANG, 1993 Tyrosine phosphorylation of mammalian RNA polymerase II carboxyl-terminal domain. Proc. Natl. Acad. Sci. USA **90:** 11167–11171.

BERGET, S. M., 1984 Are U4 small nuclear ribonucleoproteins involved in polyadenylation? Nature **309:** 179–182.

BIGGS, J., L. L. SEARLES and A. L. GREENLEAF, 1985 Structure of the eukaryotic transcription apparatus: features of the gene for the largest subunit of Drosophila RNA polymerase II. Cell **42:** 611–621.

BUERMEYER, A. B., N. E. THOMPSON, L. A. STRASHEIM, R. R. BURGESS and P. J. FARNHAM, 1992 The HIP1 initiator element plays a role in determining the *in vitro* requirement of the dihydrofolate reductase gene promoter for the C-terminal domain of RNA polymerase II. Mol. Cell. Biol. **12:** 2250–2259.

BURATOWSKI, S., 1994 The basics of basal transcription by RNA polymerase II. Cell **77:** 1–3.

CADENA, D. L., and M. E. DAHMUS, 1987 Messenger RNA synthesis in mammalian cells is catalyzed by the phosphorylated form of RNA polymerase II. J. Biol. Chem. **262:** 12468–12474.

CHAO, D. M., and R. A. YOUNG, 1991 Tailored tails and transcription

initiation: the carboxyl terminal domain of RNA polymerase II. Gene Expr. **1:** 1–4.

CHEN, Y., J. WEEKS, M. A. MORTIN and A. L. GREENLEAF, 1993 Mapping mutations in genes encoding the two large subunits of *Drosophila* RNA polymerase II defines domains essential for basic transcription functions and for proper expression of developmental genes. Mol. Cell. Biol. **13:** 4214–4222.

CHENG, J., and L. E. MAQUAT, 1993 Nonsense codons can reduce the abundance of nuclear mRNA without affecting the abundance of pre-mRNA or the half-life of cytoplasmic mRNA. Mol. Cell. Biol. **13:** 1892–1902.

CHENG, J., P. BELGRADER, X. ZHOU and L. E. MAQUAT, 1994 Introns are cis effectors of the nonsense-codon-mediated reduction in nuclear mRNA abundance. Mol. Cell. Biol. **14:** 6317–6325.

CHESNUT, J. D., J. H. STEPHENS and M. E. DAHMUS, 1992 The interaction of RNA polymerase II with the adenovirus-2 major late promoter is precluded by phosphorylation of the C-terminal domain of subunit IIa. J. Biol. Chem. **267:** 10500–10506.

CHOMCYNSKI, P., and N. SACCHI, 1987 Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. Anal. Biochem. **162:** 156–159.

CONAWAY, R. C., J. N. BRADSHER and J. W. CONAWAY, 1992 Mechanism of assembly of the RNA polymerase II preinitiation complex. Evidence for a functional interaction between the carboxyl-terminal domain of the largest subunit of RNA polymerase II and a high molecular mass form of the TATA factor. J. Biol. Chem. **267:** 8464–8467.

CONAWAY, R. C., and J. W. CONAWAY, 1993 General initiation factors for RNA polymerase II. Annu. Rev. Biochem. **62:** 161–190.

CORDEN, J. L., 1990 Tails of RNA polymerase II. Trends Biochem. Sci. **15:** 383–387.

CORDEN, J. L., and C. J. INGLES, 1992 Carboxy-terminal domain of the largest subunit of eukaryotic RNA polymerase II, pp. 81–107 in *Transcriptional Regulation,* edited by S. E. MCKNIGHT and K. R. YAMAMOTO. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

CORDEN, J. L., D. L. CADENA, J. M. AHEARN JR. and M. E. DAHMUS, 1985 A unique structure at the carboxyl terminus of the largest subunit of eukaryotic RNA polymerase II. Proc. Natl. Acad. Sci. USA **82:** 7934–7938.

CUELLO, A. C., G. GALFRE and C. MILSTEIN, 1979 Detection of substance P in the central nervous system by a monoclonal antibody. Proc. Natl. Acad. Sci. USA **76:** 3532–3536.

DAHMUS, M. E., 1981 Phosphorylation of eukaryotic DNA-dependent RNA polymerase. Identification of calf thymus RNA polymerase subunits phosphorylated by two purified protein kinases, correlation with *in vivo* sites of phosphorylation in HeLa cell RNA polymerase II. J. Biol. Chem. **256:** 3332–3339.

DAHMUS, M. E., and W. S. DYNAN, 1992 Phosphorylation of RNA Polymerase II as a Transcriptional Regulatory Mechanism, pp. 109–128 in *Transcriptional Regulation,* edited by S. E. MCKNIGHT and K. R. YAMAMOTO. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

DRAPKIN, R., A. MERINO and D. REINBERG, 1993 Regulation of RNA polymerase II transcription. Curr. Opin. Cell. Biol. **5:** 469–476.

FALKENBURG, D., B. DWORNICZAK, D. M. FAUST and E. K. BAUTZ, 1987 RNA polymerase II of Drosophila. Relation of its 140,000 Mr subunit to the beta subunit of *Escherichia coli* RNA polymerase. J. Mol. Biol. **195:** 929–937.

FEAVER, W. J., O. GILEADI, Y. LI and R. D. KORNBERG, 1991 CTD kinase associated with yeast RNA polymerase II initiation factor b. Cell **67:** 1223–1230.

FEAVER, W. J., J. Q. SVEJSTRUP, L. BARDWELL, A. J. BARDWELL, S. BURATOWSKI *et al.*, 1993 Dual roles of a multiprotein complex from *S. cerevisiae* in transcription and DNA repair. Cell **75:** 1379–1387.

GREENLEAF, A. L., 1983 Amanitin-resistant RNA polymerase II mutations are in the enzyme's largest subunit. J. Biol. Chem. **258:** 13403–13406.

GREENLEAF, A. L., 1993 Positive patches and negative noodles: linking RNA processing to transcription? Trends Biochem. Sci. **18:** 117n119.

GREENLEAF, A. L., L. M. BORSETT, P. F. JIAMACHELLO and D. E. COULTER, 1979 Alpha-amanitin-resistant *D. melanogaster* with an altered RNA polymerase II. Cell **18:** 613–622.

GREENLEAF, A. L., J. R. WEEKS, R. A. VOELKER, S. OHNISHI and B. DICKSON, 1980 Genetic and biochemical characterization of mutants at an RNA polymerase II locus in *D. melanogaster.* Cell **21:** 785–792.

HAMILTON, B. J., M. A. MORTIN and A. L. GREENLEAF, 1993 Reverse genetics of Drosophila RNA polymerase II: identification and characterization of RpII140, the genomic locus for the second-largest subunit. Genetics **134:** 517–529.

JAENIKE, J., D. A. GRIMALDI, A. E. SLUDER and A. L. GREENLEAF, 1983 Alpha-amanitin tolerance in Mycophagous *Drosophila.* Science **221:** 165–167.

JOKERST, R. S., J. R. WEEKS, W. A. ZEHRING and A. L. GREENLEAF, 1989 Analysis of the gene encoding the largest subunit of RNA polymerase II in Drosophila. Mol. Gen. Genet. **215:** 266–275.

KANG, M. E., and M. E. DAHMUS, 1993 RNA polymerases IIA and IIO have distinct roles during transcription from the TATA-less murine dihydrofolate reductase promoter. J. Biol. Chem. **268:** 25033–25040.

KIM, Y. J., S. BJORKLUND, Y. LI, M. H. SAYRE and R. D. KORNBERG, 1994 A multiprotein mediator of transcriptional activation and its interaction with the C-terminal repeat domain of RNA polymerase II. Cell **77:** 599–608.

KOLESKE, A. J., and R. A. YOUNG, 1994 An RNA polymerase II holoenzyme responsive to activators. Nature **368:** 466–469.

KOLESKE, A. J., S. BURATOWSKI, M. NONET and R. A. YOUNG, 1992 A novel transcription factor reveals a functional link between the RNA polymerase II CTD and TFIID. Cell **69:** 883–894.

KOLODZIEJ, P. A., and R. A. YOUNG, 1991 Mutations in the three largest subunits of yeast RNA polymerase II that affect enzyme assembly. Mol. Cell. Biol. **11:** 4669–4678.

LAYBOURN, P. J., and M. E. DAHMUS, 1990 Phosphorylation of RNA polymerase IIA occurs subsequent to interaction with the promoter and before the initiation of transcription. J. Biol. Chem. **265:** 13165–13173.

LEE, J. M., and A. L. GREENLEAF, 1989 A protein kinase that phosphorylates the C-terminal repeat domain of the largest subunit of RNA polymerase II. Proc. Natl. Acad. Sci. USA **86:** 3624–3628.

LEE, J. M., and A. L. GREENLEAF, 1991 CTD kinase large subunit is encoded by CTK1, a gene required for normal growth of *Saccharomyces cerevisiae.* Gene Expr. **1:** 149–167.

LEFEVRE, G., JR., 1971 Salivary chromosome bands and the frequency of crossing over in *Drosophila melanogaster.* Genetics **67:** 497–513.

LIAO, S. M., I. C. TAYLOR, R. E. KINGSTON and R. A. YOUNG, 1991 RNA polymerase II carboxy-terminal domain contributes to the response to multiple acidic activators *in vitro.* Genes Dev. **5:** 2431–2440.

LU, H., O. FLORES, R. WEINMANN and D. REINBERG, 1991 The nonphosphorylated form of RNA polymerase II preferentially associates with the preinitiation complex. Proc. Natl. Acad. Sci. USA **88:** 10004–10008.

LU, H., L. ZAWEL, L. FISHER, J. M. EGLY and D. REINBERG, 1992 Human general transcription factor IIH phosphorylates the C-terminal domain of RNA polymerase II. Nature **358:** 641–645.

MORTIN, M. A., and T. C. KAUFMAN, 1982 Developmental genetics of a temperature-sensitive RNA polymerase II mutation in *Drosophila melanogaster.* Mol. Gen. Genet. **187:** 120–125.

MORTIN, M. A., and G. LEFEVRE, JR., 1981 An RNA polymerase II mutation in *Drosophila melanogaster* that mimics ultrabithorax. Chromosoma **82:** 237–247.

MOZER, B., R. MARLOR, S. PARKHURST and V. CORCES, 1985 Characterization and developmental expression of a Drosophila ras oncogene. Mol. Cell. Biol. **5:** 885–889.

MUNRO, S., and H. R. PELHAM, 1984 Use of peptide tagging to detect proteins expressed from cloned genes: deletion mapping functional domains of Drosophila hsp 70. EMBO J. **3:** 3087–3093.

NONET, M. L., and R. A. YOUNG, 1989 Intragenic and extragenic suppressors of mutations in the heptapeptide repeat domain of *Saccharomyces cerevisiae* RNA polymerase II. Genetics **123:** 715–724.

NONET, M., D. SWEETSER and R. A. YOUNG, 1987 Functional redundancy and structural polymorphism in the large subunit of RNA polymerase II. Cell **50:** 909–915.

O'BRIEN, T., S. HARDIN, A. GREENLEAF and J. T. LIS, 1994 Phosphorylation of RNA polymerase II C-terminal domain and transcriptional elongation. Nature **370:** 75–77.

O'HARE, K., and G. M. RUBIN, 1983 Structures of *P* transposable elements and their sites of insertion and excision in the *Drosophila melanogaster* genome. Cell **34:** 25–35.

OTTER, T., S. M. KING and G. B. WITMAN, 1987 A two-step procedure for efficient electrotransfer of both high-molecular-weight (greater than 400,000) and low-molecular-weight (less than 20,000) proteins. Anal. Biochem. **162:** 370–377.

PAYNE, J. M., and M. E. DAHMUS, 1993 Partial purification and characterization of two distinct protein kinases that differentially phosphorylate the carboxyl-terminal domain of RNA polymerase subunit IIa. J. Biol. Chem. **268:** 80–87.

PAYNE, J. M., P. J. LAYBOURN and M. E. DAHMUS, 1989 The transition of RNA polymerase II from initiation to elongation is associated with phosphorylation of the carboxyl-terminal domain of subunit IIa. J. Biol. Chem. **264:** 19621–19629.

PIRROTTA, V., 1988 Vectors for P-Mediated Transformation in Drosophila, pp. 437–456 in *Vectors, A Survey of Molecular Cloning Vectors and Their Uses,* edited by R. L. RODRIGUEZ and D. T. DENHARDT. Butterworths, Boston.

ROBERTSON, H. M., C. R. PRESTON, R. W. PHILLIS, D. M. JOHNSON-SCHLITZ, W. K. BENZ *et al.,* 1988 A stable genomic source of *P* element transposase in *Drosophila melanogaster.* Genetics **118:** 461–470.

SACHS, A. B., 1993 Messenger RNA degradation in eukaryotes. Cell **74:** 413–421.

SAMBROOK, J., E. F. FRITSCH and T. MANIATIS, 1989 *Molecular Cloning, A Laboratory Manual.* Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

SCAFE, C., D. CHAO, J. LOPES, J. P. HIRSCH, S. HENRY *et al.,* 1990 RNA polymerase II C-terminal repeat influences response to transcriptional enhancer signals. Nature **347:** 491–494.

SEARLES, L. L., A. L. GREENLEAF, W. E. KEMP and R. A. VOELKER, 1986 Sites of *P* element insertion and structures of *P* element deletions in the 5′ region of *Drosophila melanogaster* RpII215. Mol. Cell. Biol. **6:** 3312–3319.

SERIZAWA, H., R. C. CONAWAY and J. W. CONAWAY, 1992 A carboxy-terminal domain kinase associated with RNA polymerase II transcription factor delta from rat liver. Proc. Natl. Acad. Sci. USA **89:** 7476–7480.

SHYU, A. B., J. G. BELASCO and M. E. GREENBERG, 1991 Two distinct destabilizing elements in the *c-fos* message trigger deadenylation as a first step in rapid mRNA decay. Genes Dev. **5:** 221–231.

SOUTHERN, E. M., 1975 Detection of specific sequences among DNA fragments separated by gel electrophoresis. J. Mol. Biol. **98:** 503–517.

STONE, N., and D. REINBERG, 1992 Protein kinases from *Aspergillus nidulans* that phosphorylate the carboxy-terminal domain of the largest subunit of RNA polymerase II. J. Biol. Chem. **267:** 6353–6360.

SWEETSER, D., M. NONET and R. A. YOUNG, 1987 Prokaryotic and eukaryotic RNA polymerases have homologous core subunits. Proc. Natl. Acad. Sci. USA **84:** 1192–1196.

THOMPSON, N. E., T. H. STEINBERG, D. B. ARONSON and R. R. BURGESS, 1989 Inhibition of *in vivo* and *in vitro* transcription by monoclonal antibodies prepared against wheat germ RNA polymerase II that react with the heptapeptide repeat of eukaryotic RNA polymerase II. J. Biol. Chem. **264:** 11511–11520.

THOMPSON, C. M., A. J. KOLESKE, D. M. CHAO and R. A. YOUNG, 1993 A multisubunit complex associated with the RNA polymerase II CTD and TATA-binding protein in yeast. Cell **73:** 1361–1375.

USHEVA, A., E. MALDONADO, A. GOLDRING, H. LU, C. HOUBAVI *et al.,* 1992 Specific interaction between the nonphosphorylated form of RNA polymerase II and the TATA-binding protein. Cell **69:** 871–881.

VOELKER, R. A., A. L. GREENLEAF, H. GYURKOVICS, G. B. WISELY, S. M. HUANG *et al.,* 1984 Frequent imprecise excision among reversions of a *P* element-caused lethal mutation in Drosophila. Genetics **107:** 279–294.

VOELKER, R. A., B. B. WISELY, S. M. HUANG and H. GYURKOVICS, 1985 Genetic and molecular variation in the *RpII215* region of *Drosophila melanogaster.* Mol. Gen. Genet. **201:** 437–445.

WEEKS, J. R., S. E. HARDIN, J. SHEN, J. M. LEE and A. L. GREENLEAF, 1993 Locus-specific variation in phosphorylation state of RNA polymerase II *in vivo:* correlations with gene activity and transcript processing. Genes Dev. **7:** 2329–2344.

YOUNG, R. A., 1991 RNA polymerase II. Annu. Rev. Biochem. **60:** 689–715.

ZAWEL, L., and D. REINBERG, 1992 Advances in RNA polymerase II transcription. Curr. Opin. Cell. Biol. **4:** 488–495.

ZEHRING, W. A., and A. L. GREENLEAF, 1990 The carboxyl-terminal repeat domain of RNA polymerase II is not required for transcription factor Sp1 to function *in vitro.* J. Biol. Chem. **265:** 8351–8353.

ZEHRING, W. A., J. M. LEE, J. R. WEEKS, R. S. JOKERST and A. L. GREENLEAF, 1988 The C-terminal repeat domain of RNA polymerase II largest subunit is essential *in vivo* but is not required for accurate transcription initiation *in vitro.* Proc. Natl. Acad. Sci. USA **85:** 3698–3702.

ZHANG, J., and J. L. CORDEN, 1991a Identification of phosphorylation sites in the repetitive carboxyl-terminal domain of the mouse RNA polymerase II largest subunit. J. Biol. Chem. **266:** 2290–2296.

ZHANG, J., and J. L. CORDEN, 1991b Phosphorylation causes a conformational change in the carboxyl-terminal domain of the mouse RNA polymerase II largest subunit. J. Biol. Chem. **266:** 2297–2302.

Communicating editor: A. G. HINNEBUSCH