Conditional Mutations Occur Predominantly in Highly Conserved Residues of RNA Polymerase II Subunits

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Conditional mutations in the *Saccharomyces cerevisiae* RNA polymerase II large subunit, *RPB1*, were obtained by introducing a mutagenized *RPB1* plasmid into yeast cells, selecting for loss of the wild-type *RPB1* gene, and screening the cells for heat or cold sensitivity. Sequence analysis of 10 conditional *RPB1* mutations and 10 conditional *RPB2* mutations revealed that the amino acid residues altered by these distinct mutations are nearly always invariant among eucaryotic *RPB1* and *RPB2* homologs. These results suggest that RNA polymerase mutants might be obtained in other eucaryotic organisms by alteration of these invariant residues.

Eucaryotic RNA polymerases I, II, and III are highly conserved enzymes that are responsible for rRNA, pre-mRNA, and small stable RNA synthesis, respectively (16, 25). These enzymes are each composed of two very large subunits, which account for much of the molecular mass of the enzyme, and 8 to 11 smaller proteins. The two large subunits of RNA polymerase I are similar in sequence to the two large subunits of RNA polymerases II and III and to the two large subunits of the procaryotic enzyme (2, 18, 26). The two large subunits of the procaryotic enzyme bind DNA and nucleoside triphosphate substrates, contain the catalytic site for RNA synthesis, and interact with the transcription factor σ (8, 29). Sequence similarity between the two large procaryotic and eucaryotic subunits and evidence that the eucaryotic large subunits can bind to DNA and nucleoside substrates (7, 10) suggest that the eucaryotic homologs play similar roles.

RNA polymerase II is highly conserved in subunit structure and sequence among eucaryotes (14, 22, 25). Comparison of the sequences of the largest RNA polymerase II subunits from *Saccharomyces cerevisiae* (2), *Caenorhabditis elegans* (5), *Drosophila melanogaster* (4, 15), and the mouse (1) reveal that almost 40% of the amino acid residues are invariant. The RNA polymerase II large subunits are also similar to their procaryotic counterparts. Much of the amino acid conservation between the large eucaryotic subunit and the procaryotic RNA polymerase β' subunit occurs in multiple segments (1, 2, 4, 5). Sequence similarities between the second largest subunits of eucaryotic and procaryotic RNA polymerases also occur in multiple segments (11, 26). The presence of multiple segments of sequence similarity may reflect the fact that these large subunits have multiple functions. Invariant amino acid residues in and around these domains probably play essential structural and functional roles.

We have begun a detailed genetic investigation of the two large RNA polymerase II subunit genes in *S. cerevisiae*. A systematic survey of mutations in the two large subunits has permitted the isolation of mutant cells that exhibit conditional and auxotrophic phenotypes. We have found that most of the amino acids altered by these mutations involve residues that are invariant among homologous subunits from a broad range of eucaryotes and discuss the implications of this observation herein.

Isolation of *RPB1* mutants. *RPB1* (also known as *RPO21*, *RPO1*, *RPB220*) encodes the largest (192 kilodaltons) subunit of *S. cerevisiae* RNA polymerase II (2, 13, 20, 28). To obtain a substantial number of *RPB1* mutants with conditional and auxotrophic phenotypes, a plasmid shuffle method was employed (6, 17). The plasmid shuffle utilized a yeast strain that carries an *RPB1* chromosomal deletion which is covered by a centromere-containing plasmid carrying *RPB1*. By replacing the plasmid containing the wild-type copy of *RPB1* with a similar plasmid that had been mutagenized in vitro, we could concentrate the mutagenesis on the plasmid-borne gene, screen for several different mutant phenotypes simultaneously, and rapidly recover the mutations on plasmids for further analysis.

A screen of 12,000 yeast colonies bearing hydroxylamine-mutagenized *RPB1* plasmids yielded 17 recessive mutants with conditional or auxotrophic phenotypes. The yeast strains Z23 (*MATα ura3-52 leu2-3 leu2-112 his3Δ200 rpb1Δ196::HIS3*[pRP112 *URA3 CEN4 RPB1*] and Z26 (*MATα ura3-52 leu2-3 leu2-112 his3Δ200 rpb1Δ187::HIS3* [pRP112 *URA3 CEN4 RPB1*]), which contain different deletions of the chromosomal *RPB1* gene complemented by a wild-type *RPB1* gene on the *URA3-CEN* plasmid pRP112 (21), were transformed with mutagenized *LEU2* plasmid pRP114 (21). The *rpb1Δ196::HIS3* and *rpb1Δ187::HIS3* alleles are described elsewhere (21). pRP114 was mutagenized with hydroxylamine hydrochloride as described by Nonet et al. (20). The 12,000 Leu⁺ transformants were replica plated onto medium containing 5-fluoroorotic acid (6). Approximately 2% of the transformants failed to grow on 5-fluoroorotic acid, indicating that 2% of the mutagenized pRP114 plasmids contained null alleles of the essential *RPB1* gene. The surviving clones (which had lost the pRP112 plasmid bearing a wild-type copy of *RPB1*) were then assayed for temperature sensitivity and cold sensitivity by replica plating or spot testing to rich plates at 12, 15, 24, 36, and 38°C. The 104 temperature-sensitive (*ts*) clones which failed to grow at 36 and/or 38°C were isolated, as were 45 cold-sensitive (*cs*) clones which failed to grow at 15°C and/or 12°C.

To determine whether the mutant phenotypes were caused by mutations in *RPB1* or by unlinked mutations induced by transformation, the pRP114 plasmids containing the putative
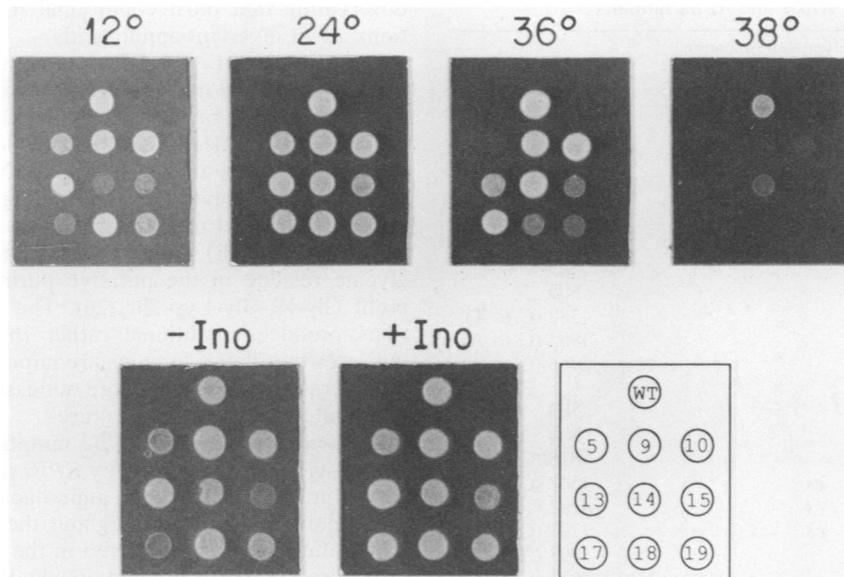
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FIG. 1. Phenotypes of *RPB1* mutants. The growth phenotypes of *RPB1* mutants were assayed by using a spot test on rich medium at various temperatures or on minimal medium with or without inositol. Media are described by Nonet et al. (20). Similar numbers of cells were dispersed in water, and equal volumes were transferred to agar plates with a 48-prong apparatus. The key contains *RPB1* allele numbers.

mutations were isolated by using the protocol of Hoffman and Winston (12). These plasmids were then reassayed in the original Z26 strain, using the plasmid shuffle procedure. Seventeen recessive, plasmid-linked *RPB1* mutants were isolated in this manner.

The growth phenotypes of a subset of the *RPB1* mutants are shown in Fig. 1. Of the 17 mutants, 4 were both *ts* and *cs* and 13 were exclusively *ts*. Some of the mutants exhibited wild-type growth rates at the permissive temperature (24°C), and others grew more slowly than wild-type cells at 24°C. The differential sensitivity of these mutants to a range of temperatures (12, 15, 24, 36, and 38°C) suggested that most represent different alleles of *RPB1*. The *RPB1* mutant alleles and their phenotypes are summarized in Table 1.

Some *RPB1*, *RPB2*, and *RPB4* mutants are inositol auxotrophs (3, 21a, 24, 27), and this auxotrophy appears to be due to a defect in the ability of mutant RNA polymerase II to transcribe *INO1* (3, 24). Of the 17 conditional *RPB1* mutants isolated in this study, 5 exhibited a requirement for inositol (Fig. 1), as did five *RPB1* mutants that were isolated previously (*rpb1-1*, *rpb1-2*, *rpb1Δ101*, *rpb1Δ103*, and *rpb1Δ104*) (20, 21a). The precise mechanism responsible for this gene-specific transcriptional defect is not yet clear.

Mapping the RNA polymerase II gene mutations. The *RPB1* gene of the *S. cerevisiae* A364A has been sequenced (2), but differences between this strain and the S288C strain used in our study have been noted, including differences in the *RPB1* gene itself (21). In order to be certain that nucleotide differences observed in the mutated *RPB1* genes were not due to strain differences, we sequenced the wild-type S288C *RPB1* gene. Several differences between the S288C and the A364A *RPB1* sequence were observed (S288C *RPB1* nucleotide 420 is A, 518 is T, 783 is T, 1506 is G, 2214 is G, 3657 is C, 3852 is G, 4620 is C, 4853 is C, and 4883 is G). None of these differences in DNA sequence altered the predicted amino acid sequence of the *RPB1* subunit.

Ten of the mutant *RPB1* genes were sequenced by using oligonucleotide primers. Plasmid DNA sequencing was carried out as described by Chen and Seeburg (9), using a set of

25 nucleotide primers that were spaced by 200 bases and that spanned the entire *RPB1* gene. A single nucleotide change was observed in nine of the mutant genes, while two point mutations were found in *rpb1-10*. The point mutations observed in the 10 mutant *RPB1* genes are shown in Fig. 2A. All but one of these mutations are G→A or C→T transitions, which are expected to occur through hydroxylamine mutagenesis. The exception, which occurs in *rpb1-15*, may have arisen during yeast transformation, the process of which has been shown to be mutagenic (17).

Conditional *RPB1* mutations alter highly conserved amino acids throughout the protein. Single amino acid changes occur in nine of the RPB1 mutant proteins, and two amino acid alterations occur in the subunit encoded by *rpb1-10*. Examination of the amino acid alterations responsible for the conditional phenotypes of these mutants reveals that most of the affected residues are highly conserved among RPB1 proteins from a variety of eucaryotic species (Fig. 2A). Seven of the nine single point mutations (*rpb1-1*, *rpb1-5*, *rpb1-9*, *rpb1-13*, *rpb1-14*, *rpb1-17*, and *rpb1-18*) alter amino acid residues that are invariant among the large RNA polymerase II subunits of yeast, *Drosophila*, nematode, and mouse cells. Three of the point mutations (*rpb1-1*, *rpb1-5*, and *rpb1-17*) affect residues that are also shared with the *Escherichia coli* β' subunit. Some of these mutations produce amino acid changes that are conservative and some do not.

Conditional *RPB2* mutations also affect highly conserved amino acids. To determine whether conditional mutations tend to affect highly conserved amino acid residues in another RNA polymerase II subunit, the positions of *RPB2* conditional mutations, whose isolation and characterization is described elsewhere (24), were investigated. Ten of the *RPB2* mutant genes were sequenced by using a set of oligonucleotide primers. A single nucleotide change was observed in all 10, and all were G→A or C→T transitions. The amino acid alterations produced by these mutations are shown in Fig. 2B and are summarized in Table 1. All of the *RPB2* mutations alter amino acid residues that are identical

TABLE 1. *RPB1* and *RPB2* mutants

Allele	Phenotype determination			Mutation
	ts	*cs*	Inositol[a]	
RPB1				
rpb1-1	*ts*		−	4622 (G to A)
rpb1-2	*ts*		−	ND[b]
rpb1-4	*ts*	*cs*	+	ND
rpb1-5	*ts*		+	1315 (C to T)
rpb1-6	*ts*		+	ND
rpb1-7	*ts*		+	ND
rpb1-8	*ts*		+	ND
rpb1-9	*ts*		+	382 (C to T)
rpb1-10	*ts*		+	1985 (G to A)
				2766 (G to A)
rpb1-11	*ts*		+	ND
rpb1-12	*ts*		−	ND
rpb1-13	*ts*		+	826 (C to T)
rpb1-14	*ts*	*cs*	+	1547 (G to A)
rpb1-15	*ts*	*cs*	−	4292 (T to A)
rpb1-17	*ts*	*cs*	−	1773 (G to A)
rpb1-18	*ts*		+	808 (G to A)
rpb1-19	*ts*		+	4031 (G to A)
rpb1-20	*ts*		−	ND
rpb1-21	*ts*		−	ND
rpb1Δ101	*ts*	*cs*	−	5139[c]
rpb1Δ103	*ts*	*cs*	−	5125[c]
rpb1Δ104	*ts*	*cs*	−	5143[c]
RPB2				
rpb2-1	*ts*	*cs*	−	4233 (G to A)
rpb2-2	*ts*	*cs*	−	4233 (G to A)
rpb2-3	*ts*	*cs*	+	4125 (G to A)
rpb2-4	*ts*	*cs*	−	3854 (G to A)
rpb2-5	*ts*		+	4011 (G to A)
rpb2-6	*ts*		+	3378 (G to C)
rpb2-7	*ts*	*cs*	+	ND
rpb2-8	*ts*	*cs*	+	4253 (G to A)
rpb2-9	*ts*	*cs*	+	3946 (G to A)
rpb2-10		*cs*	−	3860 (C to T)
rpb2-11	*ts*	*cs*	−	3294 (G to A)
rpb2-12	*ts*		+	ND

[a] Growth (+) or absence of growth (−) in medium lacking inositol.
[b] ND, Not determined.
[c] Deletion endpoint.

in *S. cerevisiae* and *Drosophila* sp. Thus, as we observed for *RPB1*, there is a strong bias for conditional mutations to affect invariant amino acid residues in *RPB2*.

We considered the possibility that these observations are a consequence of a bias inherent in hydroxylamine mutagenesis. Because hydroxylamine mutagenesis produces C to T transitions, we investigated whether highly conserved amino acids have codons that are relatively rich in G and C residues. For *RPB1* and *RPB2*, the codons for conserved amino acids do not contain significantly more G and C residues than those that encode nonconserved amino acids. Thus, the mutagen does not appear to be responsible for the

observation that most conditional *RPB1* and *RPB2* mutations affect invariant amino acids.

The *rpb2-8* and *rpb2-9* mutations alter conserved amino acids in sequence motifs associated with metal binding and purine nucleotide binding, functions known to be carried out by RNA polymerase II. The *rpb2-8* allele changes one of the cysteine residues in a Cys-X_2-Cys-X_{15}-Cys-X_2-Cys motif that may be involved in zinc binding (14) and that occurs near the carboxyl terminus in yeast and *Drosophila RPB2* subunits (Fig. 2B). The *rpb2-9* allele alters an invariant glycine residue in the putative purine nucleotide binding motif Gly-X_5-Gly-Lys-Thr (26). The fact that these mutations produce conditional rather than lethal phenotypes indicates that these domains are important for normal RNA polymerase II function but are not essential for cell survival in a limited range of temperatures.

Positions of *RPB1* and *RPB2* mutations. The positions of amino acid residues altered by *RPB1* and *RPB2* mutations is shown in Fig. 3. The *RPB1* amino acid changes are distributed relatively evenly throughout the protein, whereas the *RPB2* alterations are clustered in the carboxy-terminal half of the protein. We are uncertain why this has occurred but it may reflect the relatively nonuniform distribution of conserved amino acids in *RPB2*. It is interesting to note that the mutations that lead to inositol auxotrophy tend to cluster at the carboxy terminus of the *RPB1* protein and occur among the mutations at the carboxy terminus of *RPB2* (Table 1).

Segments of the two large subunits are highly conserved among procaryotic and eucaryotic RNA polymerases. A minority of the *RPB1* conditional mutations occur in these homology segments, whereas most of the *RPB2* mutants occur within these regions of sequence similarity. The presence of the majority of *RPB1* mutations outside of the homology segments indicates that portions of the eucaryotic RNA polymerase II large subunit that are not similar in sequence to its procaryotic counterpart are important for function, assembly, or stability.

Implications. Analysis of 10 conditional *RPB1* and 10 conditional *RPB2* mutants revealed that the amino acid residues altered by the mutations are nearly always invariant among eucaryotic *RPB1* and *RPB2* subunit homologs. Thus, conditional mutations in the two large subunits of RNA polymerase generally affect highly conserved amino acid residues. While one might expect that mutations in the highly conserved residues would tend to produce defects more often than mutations in less-conserved amino acids, it is striking that so few of the conditional mutations affected less-conserved amino acid residues.

The presence of amino acid residues that are invariant among eucaryotic RNA polymerase II large subunits and the observation that mutations in some of these residues produce conditional and auxotrophic phenotypes suggests a rapid means to obtain RNA polymerase mutants in other organisms. Mutations comparable to those in the *S. cerevisiae* RNA polymerase II large subunits could be constructed in the RNA polymerase II subunit genes of other organisms.

FIG. 2. Conditional mutations occur in highly conserved residues. Positions of amino acid alterations caused by *RPB1* (A) and *RPB2* (B) mutations are compared with homologous sequences of RNA polymerase subunits from other organisms. The dots represent amino acid sequence identity, and the dashes indicate gaps in the sequence. The mutational alterations are shown above the amino acid sequences, and the presence of these sequences in a homology region (see Fig. 3 for regions that exhibit strong sequence similarity between procaryotic and eucaryotic subunits) is indicated below the sequences. The numbers to the right of the yeast sequences indicate the position of the last amino acid residue within the *RPB1* (2) or the *RPB2* (26) amino acid sequence. The *rpb1-10* allele consists of two different mutations, which alter *RPB1* nucleotides 1985 and 2766 (Table 1). The mutation at nucleotide 2766 alters an amino acid residue immediately amino terminal to region E.

A

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                    rpb1-9                              rpb1-18   rpb1-13
                      S                                   S         S
Yeast       VKEVQFGLFSPEEVRAISVAK 34        DDPT------QLVSRGGCGNTQPTIRKDGLKL 181
Drosophila  ..R....IL..D.I.RM..TE           QQ.DPN----KKPGH....HY..S..RT..D.
Mouse       I.R....VL..D.LKRM..TE           EQ.EGDEDLTKEKGH....RY..R..RS..E.
Nematode    .SR....ILG...IKRM...H           ...DDPMNDGKK.A-....RY..SY.RV.IDI


                    rpb1-5                              rpb1-14
                      C                                   H
Yeast       ARLKGKEGRIRGNLMGKRVDF 347       AKYVIRDSGDRIDLRYSKRAG 422
Drosophila  .....................           ...IV..N.E.....FHPKSS
Mouse       Q........V...........           ...I...N.......FHPKPS
Nematode    Q....................           ..----EN.A.V....HP..A
E. coli     DMI...Q..F.Q..L......
               Region C


                    rpb1-17                             rpb1-10
                      I                                   D
Yeast       PYNADFDGDEMNLHVPQSEET 497       NMLYWVPDWDGVIPTPAIIKP 568
Drosophila  .................M..            .L.MFL.T..AKM.Q.C.L..
Mouse       ..............L...L             .L.MFLST...KV.Q...L..
Nematode    ..............L...L.            DL.MYL.T...KV.Q...L..
E. coli     A........Q.AV...LTL.A           VL------------.GPKEAE
               Region D                        Region E


                    rpb1-10                             rpb1-19
                      I                                   Y
Yeast       LTPQEFFFHAMGGREGLIDTA 828       EDNDEKLIIRCRV--VRPKSL 1248
Drosophila  ...S..Y.............            D..AD..VL.I.IMNNEENKF
Mouse       ...T...............             D..A...VL.I.IMNSDENKM
Nematode    ...S...............            D..A...VF.L.I--AGEDKG
E. coli     .NVLQY.ISTH.A.K..A...
               Region F


                    rpb1-15                             rpb1-1
                      N                                   D
Yeast       MTVPGIDPTRIYTNSFIDIME 1337      NVILGQMAPIGTGAFDVMIDE 1447
Drosophila  LSERDV..I.TSS.DICE.FQ           .I.M..LPKM...C..LLL.A
Mouse       LSEKDV..V.TTS.DIVE.FT           .IM...L..A...C..LLL.A
Nematode    LSERQ...V.TTS.DICE.F.           .IM...L.RC...C..LVL--
E. coli                                     ...V.RLI.A...--------
                                               Region H
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B

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                    rpb2-11                             rpb2-6
                      Y                                   K
Yeast       GQNAIVAIACYSGYNQEDSM 838        DRGLFRSLFFRSYMDQEKKYG 867
Drosophila  .D.S....L..T.......V            E..F...Y.Y...K.S.N.RV
E. coli     ...MR..FMPWN...F...I
               Region F


                    rpb2-9                              rpb2-4    rpb2-10
                      D                                   T         S
Yeast       GLIAPGVRVSGEDVIIGKTT 916        IVPDLIINPHAIPSRMTVAHLIECLLS 1032
Drosophila  .I....I....D..V....I            LA..I............IG......QG
E. coli     .IVYI.AE.T.G.ILV..V.            TPV.IVL..LGV....NIGQIL.THLG
               Region G                        Region H


                    rpb2-5                              rpb2-3
                      E                                   K
Yeast       LLREHGYQSRGFEVMYNGHTG 1078      LRHMVDDKIHARARGPMQVLT 1115
Drosophila  F.Q.Y..HL..N.........           .K........S.....V.I.V
E. coli     ..KLGDLPTS.QIRLYD.R..           .N.L....M...ST.SYSLV.
               Region I                        Region I


                    rpb2-2                              rpb2-8
                      D                                   Y
Yeast       GEMERDCMIAHGAASFLKERL 1151      RVHICGICGLMTVIAKLNHNQFECKGCDNK 1188
Drosophila  .......Q.S....Q..R...           .....NF...I-A..N.RN.T......K..
E. coli     ....VWALE.Y...YT.Q.M.
               Region I
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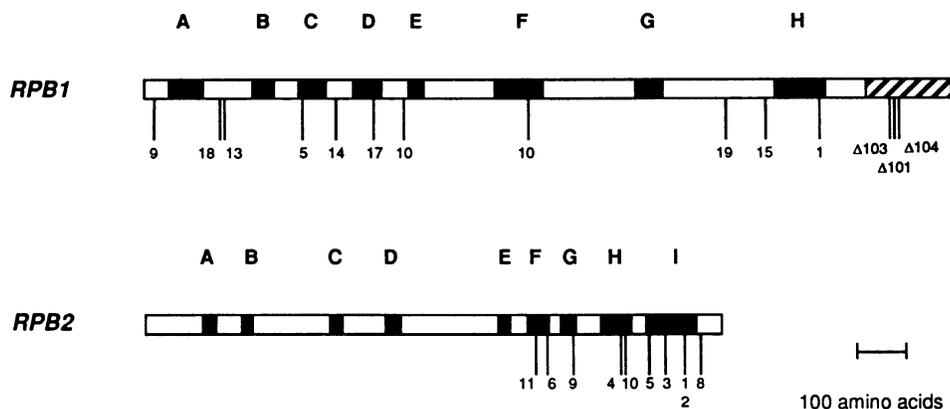
FIG. 3. Distribution of *RPB1* and *RPB2* conditional mutations. The positions of *RPB1* and *RPB2* alleles described here and elsewhere (20–21a) are indicated relative to segments of procaryotic and eucaryotic homology and the heptapeptide repeat domain. The eight regions of substantial homology between *RPB1* protein and the β' subunit of *E. coli* RNA polymerase (2) and the nine regions conserved between *RPB2* and the β subunit of *E. coli* RNA polymerase (26) are shown as black boxes in this diagram. The hatched box represents the heptapeptide repeat domain (2).

Because some RNA polymerase II mutations can produce developmental defects (19, 23), it is possible that some of the mutations described here may have some utility for investigating developmental problems in higher eucaryotes.

LITERATURE CITED

1. **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.
2. **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.
3. **Arndt, K., C. Styles, and G. R. Fink.** 1989. A suppressor of a *HIS4* transcriptional defect encodes a protein with homology to the catalytic subunit of protein phosphatases. Cell **56:**527–537.
4. **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.
5. **Bird, D. M., and D. L. Riddle.** 1989. Molecular cloning and sequencing of *ama-1*, the gene encoding the largest subunit of *Caenorhabditis elegans* RNA polymerase II. Mol. Cell. Biol. **9:**4119–4130.
6. **Boeke, J. D., J. Truehart, G. Natsoulis, and G. R. Fink.** 1987. 5-Fluoroorotic acid as a selective agent in yeast molecular genetics. Methods Enzymol. **154:**164–175.
7. **Carroll, S. B., and B. D. Stollar.** 1983. Conservation of a DNA-binding site in the largest subunit of eukaryotic RNA polymerase II. J. Mol. Biol. **170:**777–790.
8. **Chamberlin, M. J.** 1982. Bacterial DNA-dependent RNA polymerases, p. 61–86. *In* P. Boyer (ed.), The enzymes. Academic Press, Inc., New York.
9. **Chen, E. Y., and P. H. Seeburg.** 1985. Supercoil sequencing: a fast and simple method for sequencing plasmid DNA. DNA **4:**165–170.
10. **Cho, J. M., and A. P. Kimball.** 1982. Probes of eukaryotic DNA-dependent RNA polymerase II. I. Binding of 9-β-D-arabinofuranosyl-6-mercaptopurine to the elongation subsite. Biochem. Pharmacol. **31:**2575–2581.
11. **Falkenburg, D., B. Dworniczak, D. Faust, and E. K. F. Bautz.**
12. 1987. RNA polymerase II of *Drosophila*: relation of its 140kd subunit to the β subunit of *E. coli* RNA polymerase. J. Mol. Biol. **195:**929–937.
12. **Hoffman, C. S., and F. Winston.** 1987. A ten minute DNA preparation from yeast efficiently releases autonomous plasmids for transformation of *Escherichia coli*. Gene **57:**267–272.
13. **Ingles, J. C., H. J. Himmelfarb, M. Shales, A. L. Greenleaf, and J. D. Friesen.** 1984. Identification, molecular cloning, and mutagenesis of *Saccharomyces cerevisiae* RNA polymerase genes. Proc. Natl. Acad. Sci. USA **81:**2157–2161.
14. **Jendrisak, J. J., P. W. Petranyi, and R. Burgess.** 1976. Wheat germ DNA-dependent RNA polymerase II: purification and properties, p. 779–792. *In* R. Losick and M. Chamberlin (ed.), RNA polymerase. Cold Spring Harbor Laboratories, N.Y.
15. **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.
16. **Lewis, M. K., and R. R. Burgess.** 1982. Eukaryotic RNA polymerases, p. 109–153. *In* P. Boyer (ed.), The enzymes. Academic Press, Inc., New York.
17. **Mann, C., J.-M. Buhler, I. Treich, and A. Sentenac.** 1987. *RPB40*, a unique gene for a subunit shared between yeast RNA polymerase A and C. Cell **48:**627–637.
18. **Memet, S., W. Saurin, and A. Sentenac.** 1988. RNA polymerases B and C are more closely related to each other than to RNA polymerase A. J. Biol. Chem. **263:**10048–10051.
19. **Mortin, M. A., and T. C. Kaufman.** 1984. Developmental defects of a temperature sensitive RNA polymerase II mutation in *Drosophila melanogaster*. Dev. Biol. **103:**343–354.
20. **Nonet, M., C. Scafe, J. Sexton, and R. A. Young.** 1987. Eucaryotic RNA polymerase conditional mutant that rapidly ceases mRNA synthesis. Mol. Cell. Biol. **7:**1601–1611.
21. **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.
21a.**Nonet, M., 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.
22. **Paule, M. R.** 1981. Comparative subunit composition of the eukaryotic nuclear RNA polymerases. Trends Biochem. Sci. **6:**128–131.
23. **Rogalski, T. M., and D. L. Riddle.** 1988. A *Caenorhabditis elegans* RNA polymerase II gene, *ama-1 IV*, and nearby essential genes. Genetics **118:**61–74.
24. **Scafe, C., M. Nonet, and R. A. Young.** 1990. RNA polymerase II mutants defective in transcription of a subset of genes. Mol. Cell. Biol. **10:**1010–1016.

25. Sentenac, A. 1985. Eukaryotic RNA polymerases. Crit. Rev. Biochem. 18:31–91.
26. 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.
27. Woychik, N. A., and R. A. Young. 1989. RNA polymerase II subunit RPB4 is essential for high- and low-temperature yeast cell growth. Mol. Cell Biol. 9:2854–2859.
28. Young, R. A., and R. W. Davis. 1983. Yeast RNA polymerase II genes: isolation with antibody probes. Science 222:778–782.
29. Yura, T., and A. Ishihama. 1979. Genetics of bacterial RNA polymerases. Annu. Rev. Genet. 13:59–97.