

Prokaryotic and eukaryotic RNA polymerases have homologous core subunits

(*Saccharomyces cerevisiae*/RPB2)

DOUG SWEETSER*, MICHAEL NONET*, AND RICHARD A. YOUNG*†

*Whitehead Institute for Biomedical Research, Nine Cambridge Center, Cambridge, MA 02142; and †Department of Biology, Massachusetts Institute of Technology, Cambridge, MA 02139

Communicated by Gerald R. Fink, November 3, 1986 (received for review September 3, 1986)

ABSTRACT Eukaryotic RNA polymerases are complex aggregates whose component subunits are functionally ill-defined. The gene that encodes the 140,000-dalton subunit of *Saccharomyces cerevisiae* RNA polymerase II was isolated and studied in detail to obtain clues to the protein's function. This gene, *RPB2*, exists in a single copy in the haploid genome. Disruption of the gene is lethal to the yeast cell. *RPB2* encodes a protein of 138,750 daltons, which contains sequences implicated in binding purine nucleotides and zinc ions and exhibits striking sequence homology with the β subunit of *Escherichia coli* RNA polymerase. These observations suggest that the yeast and the *E. coli* subunit have similar roles in RNA synthesis, as the β subunit contains binding sites for nucleotide substrates and a portion of the catalytic site for RNA synthesis. The subunit homologies reported here, and those observed previously with the largest RNA polymerase subunit, indicate that components of the prokaryotic RNA polymerase "core" enzyme have counterparts in eukaryotic RNA polymerases.

Eukaryotic nuclear RNA polymerases I, II, and III are responsible for the synthesis of large ribosomal RNAs, pre-mRNAs, and small ribosomal and tRNAs, respectively (for reviews, see refs. 1, 2). Similarities in RNA polymerase subunit structure and antigenicity among eukaryotes indicate that these enzymes are highly conserved (for reviews, see refs. 3-5). Each of the three polymerases is composed of 9-14 polypeptides that copurify with transcriptional activity. Some subunits are shared by the three RNA polymerases, whereas the others are unique to each enzyme. How these components contribute to the functions of the three enzymes is not yet established, and additional factors appear to be essential for selective transcription initiation.

The *Escherichia coli* RNA polymerase has been relatively well studied biochemically and genetically (for reviews, see ref. 6 and references therein). The enzyme is composed of four different polypeptides, α (rpoA), β (rpoB), β' (rpoC), and σ (rpoD), whose molecular masses are 36,511, 150,543, 155,163 and 70,263 daltons, respectively. The core enzyme, whose subunit composition is $\alpha_2\beta\beta'$, can catalyze DNA-dependent RNA chain elongation. Selective initiation requires a holoenzyme containing the core plus a σ subunit, indicating that the σ subunit is essential for proper transcription initiation. Although multiple subunits are involved in most transcriptional activities, the individual subunits can play major roles in these activities. For example, α , β , and β' all contribute to the fidelity of transcription. However, the β' subunit appears to be largely responsible for DNA binding, and the β subunit contains binding sites for substrates and products and at least a portion of the catalytic site for RNA synthesis. β and β' also appear to play a role in promoter recognition because mutations in RpoB and RpoC have been

shown to affect selective transcription initiation *in vivo* and *in vitro* (7-9).

The roles that eukaryotic RNA polymerase subunits have in transcription are less clear. The presence of common and unique subunits in the three classes of RNA polymerase suggests one model in which common subunits are responsible for at least some of the functions shared by the RNA polymerases, and the unique subunits perform functions specific to each type of RNA synthesis. Alternatively, the eukaryotic RNA polymerases could contain a core enzyme composed of subunits that are structurally and functionally homologous to their prokaryotic counterpart but that differ sufficiently to be categorized as unique in RNA polymerases I-III. The observation that the largest subunit of yeast RNA polymerases II and III and *Drosophila* RNA polymerase II contain amino acid sequence homology with the large subunit of *E. coli* RNA polymerase (10, 11) is consistent with the latter model. To examine the roles of RNA polymerase II subunits in transcription further, we and our colleagues have begun to isolate and study the *Saccharomyces cerevisiae* genes that encode these proteins (12, 13). Here we report that the 140,000-dalton subunit of yeast RNA polymerase II is homologous to the β subunit of *E. coli* RNA polymerase and propose that the eukaryotic transcription apparatus is composed of a core enzyme that is functionally similar to that of prokaryotes.

MATERIALS AND METHODS

Yeast and Bacterial Stains. The yeast strain DB1033 (**ura3-52 SUC2*⁺) is a S288C derivative obtained from D. Botstein (M.I.T.). The *E. coli* strain Y1090 (*lacU169 proA⁺ lon100 araD139 strA supF[trpC22::Tn10]* (pMC9) is described in ref. 12.

Recombinant DNA Libraries and Clones. The construction of the yeast genomic DNA library in λ gt11 has been described (12, 14). The λ EMBL3a phage library of yeast genomic DNA was a gift of M. Snyder (Stanford University School of Medicine); it contains 10⁵ individual recombinants. Both DNA libraries were constructed with yeast strain S288C DNA. The libraries were screened with radiolabeled DNA probes (15) or with antibodies (16). DNA was manipulated as described (15).

Tetrad Analysis. Genetic manipulation of yeast was performed as described (17).

DNA Sequence Analysis. DNA was subcloned into vector M13mp18 or M13mp19 (New England Biolabs) as suggested by the supplier. Dideoxynucleotide chain-termination reactions and gel electrophoresis of the sequenced products were as described (18). DNA sequences were determined for both strands of DNA. Computer analysis of sequences with UWGCG programs was according to Devereux *et al.* (19).

RESULTS

RNA Polymerase Subunit Gene Isolation. DNA clones containing the gene for the 140,000-dalton subunit of *S. cerevisiae* RNA polymerase II (*RPB2*) were isolated. Two DNA clones were previously isolated from a λ gt11 library with anti-RNA polymerase II polyclonal antibodies and shown to encode a portion of the second largest RNA polymerase II subunit (12). Clones representing the entire gene were obtained from two different λ phage genomic DNA libraries. Four additional λ gt11 clones were isolated by screening with antibodies and a λ EMBL3a clone containing a large genomic DNA fragment was isolated using labeled insert DNA from the λ gt11 clone Y3015. DNA purified from each of these phages was mapped with restriction endonucleases (Fig. 1). The restriction map determined for the 17-kb genomic DNA insert of the EMBL3a clone Y3053 is identical to that found by Southern analysis in the yeast genome. All of the λ gt11 clones contain inserts whose restriction maps overlap with Y3053 DNA, consistent with the observation that *RPB2* DNA sequences occur in single copy in the haploid genome (12).

The *RPB2* Gene Specifies 4- and 4.2-kb mRNAs. The limits of the RNA polymerase subunit gene were determined by S1 nuclease mapping of the 5' and 3' termini of *RPB2* mRNA (not shown). *RPB2* is transcribed into two poly(A) mRNAs of about equal abundance. These mRNAs are 4 and 4.2 kb, differ in the length of their 3' untranslated region, and do not appear to be spliced.

***RPB2* Is Essential for Cell Viability.** The single chromosomal *RPB2* gene was disrupted to assess whether the gene is essential for cell viability (20). Diploid cells were transformed with the plasmid Y2059 (Fig. 1), a YIp5 recombinant plasmid (21) containing the 1.4-kb *HindIII*-*Bgl* II restriction fragment of *RPB2*, which includes only sequences internal to the protein coding region of the gene. Integration of the recombinant plasmid can occur at loci homologous to either *RPB2* or *URA3* sequences. DNA was prepared from six of the diploid transformants and studied by Southern analysis; four of the transformants had integrations at the *RPB2* locus (Y146–Y149) and the remaining two at the *URA3* locus (Y145, Y150). Disruption of an essential gene should produce a recessive lethal mutation that is detected by standard tetrad analysis. The six independent transformants were sporulated

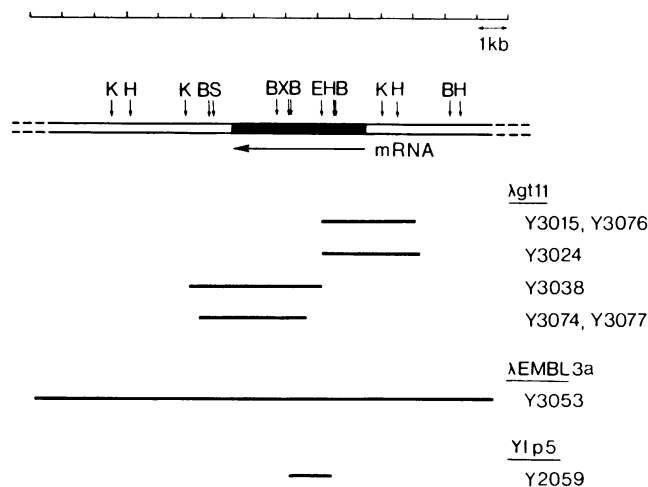


FIG. 1. Organization of *RPB2* DNA. The heavy horizontal line depicts the *RPB2* gene in the yeast genome. The light horizontal lines represent the DNA inserts of the recombinant DNA clones. The DNA clones were mapped with the restriction enzymes *Bgl* II (B), *Eco*RI (E), *Hind*III (H), *Kpn* I (K), and *Sal* I (S). The presence of these restriction sites in the yeast genome was confirmed by Southern analysis of S288C DNA. kb, Kilobase.

Table 1. Tetrad analysis of *RPB2* gene disruption

Transformant	Locus of integration	Viable spores/tetrad				Ratio of spores URA ⁺ :URA ⁻
		4	3	2	1	
Y145	<i>URA3</i>	14	1	0	0	30:29
Y146	<i>RPB2</i>	0	0	7	0	0:14
Y147	<i>RPB2</i>	0	0	5	1	0:11
Y148	<i>RPB2</i>	0	0	17	2	0:36
Y149	<i>RPB2</i>	0	0	6	0	0:12
Y150	<i>URA3</i>	10	0	0	0	20:20

To determine whether the *RPB2* is an essential gene, the *RPB2* coding sequence was disrupted in one of the two chromosomes in a diploid cell, and the viability of the meiotic products was ascertained.

and subjected to tetrad analysis (Table 1). As expected, transformants Y145 and Y150 generally produced four viable spores in which the Ura⁺ phenotype segregated 2:2. In contrast, transformants Y146–Y149 showed the pattern of viability expected for the segregation of a recessive lethal mutation (2 live:2 dead), and all viable spores had a Ura⁻ phenotype. We conclude that the *RPB2* gene is essential for yeast cell viability.

***RPB2* Protein Is Homologous to *E. coli* RNA Polymerase Subunit β .** The amino acid sequence of the *RPB2* protein was deduced from DNA sequence analysis of the 6-kb *Kpn* I fragment of Y3053 (Fig. 2). The single large open reading frame predicts a 1224 amino acid polypeptide of 138,750 daltons, smaller than the NaDodSO₄/polyacrylamide gel estimate of 150,000 daltons (22).

The amino acid sequence deduced for the RNA polymerase subunit was compared with that of other proteins in the National Biomedical Research Foundation protein sequence database. The *E. coli* RNA polymerase subunit β exhibited a striking degree of sequence homology with the 140,000-dalton subunit (Fig. 3). The nine major segments of homology occur in nearly identical relative positions within the two polypeptides. Within these segments, the amino acid sequences of the proteins align without introducing any spacing (Fig. 4). One-third of the amino acids in the segments are identical, and conservative amino acid replacements account for many of the nonidentical residues. Less significant homology exists between the DNA sequences of the prokaryotic and eukaryotic genes, reflecting different codon usage in the two organisms.

RPB2 protein sequences were analyzed for motifs that are conserved in nucleotide-binding and metal-binding domains of various proteins. The sequence G-X-X-X-G-K-(T), where X is any amino acid, is a common motif among purine nucleotide-binding or processing proteins (24). This sequence is found in a conserved region in both yeast and *E. coli* subunits (Fig. 4), although the yeast sequence varies from the consensus by having five rather than four amino acids in the X positions. Nucleic acid binding proteins often contain sites that complex with zinc ions, and a sequence of the form C-X₂-C-X₉₋₁₅-C-X₂-C has been implicated in binding metals (25). This sequence motif occurs in the 140,000-dalton yeast subunit (encoded by nucleotides 4295–4363 in Fig. 2), although it is not found in the prokaryotic subunit.

DISCUSSION

The molecular genetic techniques available in yeast provide a powerful approach to study the functions of the highly conserved eukaryotic RNA polymerases. We have investigated the gene for the second largest RNA polymerase II subunit, *RPB2*. This gene encodes a 138,750-dalton protein and is essential for cell viability. Most important, the RNA polymerase II subunit contains amino acid sequences that have been implicated in binding purine nucleotides and zinc

GGTAC2AATTTTTTCATAGACACAAAACAAAGTTCAGCGCACGAAACACAAAATGCCATATATATTTACGCATATGTATGCATTCTAGGAAACAGAAAAATAAATAATATAGTTCC
10 20 30 40 50 60 70 80 90 100 110 120
TTCTTGACTTCTCTCCAAACCCCGGATAATCGTCGGCATGTTAAACATGCTGTATCGGCTATTAAGGAAAGAAAGTATACATAAAAGAAATGAAATAAAGTTGCCAAATGGTGAA
130 140 150 160 170 180 190 200 210 220 230 240
GAATTTAAAAAATGCTACTTCGAGCTGCTACTAGTGTATAGGCTGAACATAGCATATAGCATTTTATAACTGCACATAAATGAGGTTTCTCTGTTTAAAAAGCATAATCCCAAAT
250 260 270 280 290 300 310 320 330 340 350 360
AATCTGAGTTGACAAAATAGCGGCCATCTAAAATAATTTGTTACCTGCTGGCGTCAAATGATTTTTCACTTTTCTATTTTTTCATCCTCCTCAGCTTCGTAGTAGCTTTTGAA
370 380 390 400 410 420 430 440 450 460 470 480
AAAGTGGGCAACAAAGAGTGAATTCATGCAATAAGGAACGACCACTTTTAGCAACCAATAAAGCTAGTGATTGTCTAAGTAGGGAGCAGCTTATAAAGTAGGG
490 500 510 520 530 540 550 560 570 580 590 600
TACTTGGATTTGCACCTATCTCTCCAGGTTACAGATAGTTCGAATAATTTTGCCTATCGTATATTTCTTAAAGAAGAACCCACAGTAAGGAAACAAACATAAGGAACACGCAC
610 620 630 640 650 660 670 680 690 700 710 720
M S D L A N S E K Y Y
AGCGAATAAGCACACTGTAGAAGCTCCGTATTATAAAAAATTAGGACCAGAAAAAGAACACAAACAGTATAGAAAAATAATATAAGGATGTCAGACCTTGCAAACTCAGAAAAATGATTA
730 740 750 760 770 780 790 800 810 820 830 840
D E D P Y G F E D E S A P I T A E D S W A V I S A F F R E K G L V S Q Q L D S F
TGATGAGGCCATATGGATCGAGGATGAAAGTGCACCAATCTCGAGAAGTCTGGGCTGTATATCGGCTTTTTTTCGCGAAGGGGCTAGTTTCACAACTGACTCTTT
850 860 870 880 890 900 910 920 930 940 950 960
N Q F V D Y T L Q D I I C E D S T L I L E Q L A Q H T T E S D N I S R K Y E I S
CAATCAATCGTGTATTACTTTACAAGCATTATTTGCGAGGATCCACCTTGATTTAGAGCAGTTGGCTCAACATACTACCGAATCAGACAACATCAGTAGAAGTATGAGATTAG
970 980 990 1000 1010 1020 1030 1040 1050 1060 1070 1080
F G K I Y V T K P M V N E S D G V T H A L Y P Q E A R L R N L T Y S S G L F V D
TTTTGTAATAATGATGTTTACAAGCAATGGTAAATGAATCGTGGTACCCTGCTGTATCCCAAGAAGCAGTTACGTAATTTGACATATTCGCTGGTTTATTTGTTGA
1090 1100 1110 1120 1130 1140 1150 1160 1170 1180 1190 1200
V K K R T Y E A I D V P G R E L K Y E L I A E E S E D D S E S G K V F I G R L P
TGTAAGAAGACACATATGAGCTATTGATGTTCCAGTAGGGAACGAAATGAAATTAATGCGGAAGAAATCTGAAGATGACAGCGAAAGCGGAAAGTTTTCATTTGCCGTTTACC
1210 1220 1230 1240 1250 1260 1270 1280 1290 1300 1310 1320
I M L R S K N C Y L S E A T E S D L Y K L K E C P F D M G G Y F I I N G S E K V
GATTATGTTAAGATCAAGAATGTTACCTAAGTGAAGCTACAGATCAGATTTATATAAGTGAAGAATGTCCTTTGATGGGTGTTATTTTCATCAATTAAGTTTCTGAAAAGT
1330 1340 1350 1360 1370 1380 1390 1400 1410 1420 1430 1440
L I A Q E R S A G N I V Q V F K K A A P S P I S H V A E I R S A L E K G S R F I
TTTGATTCGACAGGAGCTTCGAGGTAATTTGTTCAAGTGTAAAAAAGCGCCCTCTCAATTTCTCATGTAGCAGAAATAGATCTGCCCTGAAAAGGTTCTAGTATTAT
1450 1460 1470 1480 1490 1500 1510 1520 1530 1540 1550 1560
S T L Q V K L Y G R E G S S A R T I K A T L P Y I K Q D I P I V I F R A L G I
CAGTACCCTCAAGTCAAGCTTTTGGTGTGAGGAGTTCAGCTCGTACTATTAAGCCACATACCATATATCAACAGGATATTCCTATTGTAATCATATTCAGAGCTTTAGGTAT
1570 1580 1590 1600 1610 1620 1630 1640 1650 1660 1670 1680
I P D G E I L E H I C Y D V N D W Q M L E M L K P C V E D G F V I Q D R E T A L
TATTCAGACGGTAAAATTTAGAACATATCTGCTACGACGTAATGATGGCAATGCTGAAATGCTTAAGCCTTGTTGTAAGACGGGTTGTTATTCAAGATCGTAACTGCATT
1690 1700 1710 1720 1730 1740 1750 1760 1770 1780 1790 1800
D F I G R R G T A L G I K K E K R I Q Y A K D I L Q K E F L P H I T Q L E G F E
AGACTTATTTGGTCTGCTGGTACTGCCCTTGATTAAGAAAGAAAAAGATCCAATATGCAAAAAGACATTTTACAAAAGAAATCTCTACCTCATATTACTCAATTAGAAGGTTTGA
1810 1820 1830 1840 1850 1860 1870 1880 1890 1900 1910 1920
S R K A F F L G Y M I N R L L L C A L D R K D Q D D R D H F G K K R L D L A G P
AAGTAGAAAGGCAATTTCTTAGGTTATGATAACAGATTTACTGTGTCTTAGATCGTAAAGACCAAGATGATCGTATCATTTTCGGGAAAAAAGATAGATTGGCAGGCTCC
1930 1940 1950 1960 1970 1980 1990 2000 2010 2020 2030 2040
L L A Q L F K T L F K K L T K D I F R Y M Q R T V E E A H D F N M K L A I N A K
ATTATGGCCCACTTTCAAGACATTTGTCAAAATACTAAAGATTTTTCCGTTATATGCAAGAAGCTGAGAGGAGCCATGACTTTAACTGAAATAGCGATTAAACGAAA
2050 2060 2070 2080 2090 2100 2110 2120 2130 2140 2150 2160
T I T S G L K Y A L A T G N W G E Q K K A M S S R A G V S Q V L N R Y T Y S S T
AACCATAACATCGGCTCTAAGTACGCTTTGGCTACTGGAATCGGGTGAACAAAAAAGCCATGCTCTTAGGGCAGGTGTTTCTCAGGTTTGAACCGTTACACTATTATCCACC
2170 2180 2190 2200 2210 2220 2230 2240 2250 2260 2270 2280
L S H L R R T N T P I G R D G K L A K P R Q L H N T H W G L V C P A E T P E G Q
CTTATCACATTTAAGAAGAAACAAATCCCTTATGGCTGATGTTAAATAGCCAAACCGTCAATTCGATAATACACATTTGGGGTGGTCTGCTCGAAGAACTCCTGAGGGCA
2290 2300 2310 2320 2330 2340 2350 2360 2370 2380 2390 2400
A C G L V K N L S L M S C I S V G T D P M P I I T F L S E W G M E P L E D Y V P
AGCGTGGTGGTTAGTAAAGATCTGCACTGATGCTTGTATTTCTGTGGTACAGATCCGATGCCATCATCACTTTTTGAGTGAATGGGGTATGGAAACCGTGAAGATTACGATCC
2410 2420 2430 2440 2450 2460 2470 2480 2490 2500 2510 2520
H Q S P D A T R V F V N G V W H G V H R N P A R L M E T L R T L R R K G D I N P
ACATCAATCACTGACGGCAAGGGTCTTCGTCAATGGTGTGGCAGGGTTCACAGAAACCCAGCAAGATTAATGAAACCTTAGAACATTGAGAAGAAAGGGTATATCAATCC
2530 2540 2550 2560 2570 2580 2590 2600 2610 2620 2630 2640
E V S M I R D I R E K E L K I F T D A G R V Y R P L F I V E D D E S L G H K E L
GGAAGTTCTGATTAGAGATATTCGTAAAAGGAGCTAAAATCTTTACAGATGCCGTAGAGTTTATAGACCATTATTTGTTGAAGACGATGAATCACTAGGCCATAAGGAAT
2650 2660 2670 2680 2690 2700 2710 2720 2730 2740 2750 2760
K V R K G H I A K L M A T E Y Q D I E G G F E D V E E Y T W S S L L N E G L V E
AAAGTGAAGAAGGGTCATATTTGCCAACTGATGGCTACCGAATATCAAGATTTGAAGTGGATTTGAAGATGAGAAGAATATACATGGTCATCATTATGAATGAGGGTTTATGGA
2770 2780 2790 2800 2810 2820 2830 2840 2850 2860 2870 2880
Y I D A E E E S I L I A M Q P E D L E P A E A N E E N D L D V D P A K R I R V
ATACATTGATGCTGAAGAAGAAGAACTATATTTGATCGCAATGCAACCGGAAGATCTCGAGCCTGCAGAGGCAATGAGGAAACGATCTCGATGTTGATCCTGCCAACGTATAAGAT
2890 2900 2910 2920 2930 2940 2950 2960 2970 2980 2990 3000
S H H A T T F T H C E I H P S M I L G V A A S I I P F P D H N Q S P R N T Y Q S
ATCACAATCATGCTACAACATTTACACATTTGAGATTTCTCTTCTGATTTCTCGGTGTCGAGCATCCATTTTCCATTCCTGACCATAATCAATCTCCGCTAACACTTACCAATC
3010 3020 3030 3040 3050 3060 3070 3080 3090 3100 3110 3120
A M G K Q A H G V F L T N Y N V R M D T M A N I L Y P Q K P L G T T R A M E Y
TGCGATGGTAAGCAAGCTATGGGTGTTTTAACAACATAATGTCGCTATGGATCTACTGGCCAAATTTCTATATTCTCAAAAACCATTTGGGTACTACCGTGCATGGAATA
3130 3140 3150 3160 3170 3180 3190 3200 3210 3220 3230 3240
L K F R E L P A G Q N A I V A I A C Y S G Y N Q E D S M I M N Q S S I D R G L F
TTTGAGTTTAGAGAATTAACCGCTGGTCAAAATGCAATTTGCTATTGCAATGTTACTCCGGTTATAATCAAGAAGATTTCTATGATTGAACCACTTCAATTTGATCGTGTCTATT
3250 3260 3270 3280 3290 3300 3310 3320 3330 3340 3350 3360
R S L F F R S Y M D Q E K Y G M S I T E T F E K P Q R T N T L R M K H G T Y D
CAGATCTCTGTTTTAGATCTTATGATGAGAAAGAAAGTACGGTATGCTATAACGGAGACTTTGAGAAACCAACAGTACAAACACCTTAAAGATGAACATGTTACATACGA
3370 3380 3390 3400 3410 3420 3430 3440 3450 3460 3470 3480

FIG. 2. (Figure continues on the opposite page.)

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K L D D D G L I A P G V R V S G E D V I I G K T T P I S P D E E E L G Q R T A Y
TAAGTTGGACGATGATGGTCTAATTCGGCTGTTAGAGTTTCAGGAGAAGATGTGATATCGGTAACCAACCACTCTCACCAGATGAAGAGGAACCTCGGTCAAGAACAGCATA
3490 3500 3510 3520 3530 3540 3550 3560 3570 3580 3590 3600
H S K R D A S T P L R S T E N G I V D Q V L V T T N Q D G L K F V K V R V R T T
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3610 3620 3630 3640 3650 3660 3670 3680 3690 3700 3710 3720
K I P Q I G D K F A S R H G Q K G T I G I T Y R R E D M P F T A E G I V P D L I
AAAGATTCCTCAAATTTGGTACAAATTTGCTTCTCGTCCAGGTCAAAAGGGTACTATGGTATCAGATATCGTAGAGAAGATATGCCATTTACCCGAGAAGGTATTTGCCAGATTTGAT
3730 3740 3750 3760 3770 3780 3790 3800 3810 3820 3830 3840
I N P H A I P S R M T V A H L I E C L L S K V A A L S G N E G D A S P F T D I T
TATTAACCTCATGCTATCCATCTCGTATGACTGTTGCCATTTAATTTGAATGTTTGTGAGTAAAGTCGGCCACTATCTGTAATGAAGGTGACGCCCTCTCTTTCCAGGACATTAC
3850 3860 3870 3880 3890 3900 3910 3920 3930 3940 3950 3960
V E G I S K L L R E H G Y Q S R G F E V M Y N G H T G K K L M A Q I F F G P T Y
TGTAGAAGTATATCCAACTACTCGGTGAGCATGTTATCAATCTCGTGGTTGAAGTTATGTATAATGGTCACACAGGTAACCAACTAATGGCTCAAATTTCTTTGGTCTACATA
3970 3980 3990 4000 4010 4020 4030 4040 4050 4060 4070 4080
Y Q R L R H M V D D K I H A R A R G P M Q V L T R Q P V E G R S R D G G L R F G
TTATCAACGACTAAGACACATGGTGGATGACA/GATACATGCCAGAGCAGCTGGTCCAAGTATTGACGAGACAGCCGTAGAGGGTAGATCAGAGACGGTGGTTAAGATTCGG
4090 4100 4110 4120 4130 4140 4150 4160 4170 4180 4190 4200
E M E R D C M I A H G A A S F L K E R L M E A S D A F R V H I C G I C G L M T V
TGAGATGGAACGTGACTGTATGATGCTCATGGTCTGCTCATTCTTGAAGGAGAGATTAATGGAAGCATCCGATGCCTTAGAGTCCATATTTGGTATTTCGGGCTGATGACAGT
4210 4220 4230 4240 4250 4260 4270 4280 4290 4300 4310 4320
I A K L N H N Q F E C K G C D N K I D I Y Q I H I P Y A A K L L F Q E L M A M N
TATCGGAAATTAATCATAACCAATTTGAATGAAGGGATGTATAAAGATTGATATTTACCAAAATTCATATCCATACGCCGGAAGTATTATTCCAAGAATTTATGGCTATGAA
4330 4340 4350 4360 4370 4380 4390 4400 4410 4420 4430 4440
I T P R L Y T D R S R D F
CATTACACCAGTTTATATACCGATCGTTCGAGAGATTTTAAAGAAATATGTTGTAAGTCTAAGAAAGTAAAATAATAAAAAACATTTTCATTTGTCTGATCTTCCAAAAAAGGGTA
4450 4460 4470 4480 4490 4500 4510 4520 4530 4540 4550 4560
ACAAGTAGGCAATAGTAATAAAATAAGAATGAAGCATTTCTTTAATAAGCAGAGAGAAGTCTCAAGCATTCAAACACACAAAAAATGATGAATAAAATGGGACTCACAGTGTTA
4570 4580 4590 4600 4610 4620 4630 4640 4650 4660 4670 4680
TTTATAGTCTGTTTCGCATTTACCAAAATAAAATTTAATTTTATCAAGACATATTAATGAACCTTAGCGGAAACCTTAAATATCCAAGTCATAAATAATGACTTATGTAAGT
4690 4700 4710 4720 4730 4740 4750 4760 4770 4780 4790 4800
    
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Fig. 2. Sequence of RPB2 DNA and its protein product. The DNA sequence of 4800 base pairs of the 6232-base-pair *Kpn* I DNA fragment (Fig. 1) containing RPB2 is shown. The sequence is numbered from the *Kpn* I restriction site nearest the beginning of RPB2 (Fig. 1). The sites of RPB2 mRNA initiation [nucleotide 540 (± 25)] and polyadenylation [nucleotides 4507 and 4787 (± 25)] are underlined. The first AUG in the RPB2 mRNA sequence occurs at nucleotide 809, and the protein sequence terminates at a UUA at nucleotide 4481.

ions and is homologous to the *E. coli* RNA polymerase subunit (β) that accommodates a portion of the catalytic site for RNA synthesis.

The homology between the RPB2 protein and the prokaryotic β subunit and the presence of nucleotide-binding sequence motifs in these proteins indicate they share similar functions. The β subunit of *E. coli* RNA polymerase plays several roles in transcription. The binding sites for substrates and products and at least a portion of the catalytic site for RNA synthesis appear to reside on this subunit (7, 26–28). Although the σ subunit is essential for proper transcription initiation, RNA polymerases with mutant β or β' subunits can

exhibit altered promoter selection *in vivo* and *in vitro*, and some β subunit mutations can suppress the phenotype of some σ mutants (7–9). The β and σ subunits are probably in close proximity in the initiation complex, as they can be crosslinked to DNA sequences only five nucleotides apart in the *lac* UV5 promoter (29). Several lines of evidence suggest that the yeast 140,000-dalton subunit is responsible for at least some of these roles in eukaryotic mRNA synthesis. The presence of a purine nucleotide-binding motif in the sequence of the 140,000-dalton subunit (Fig. 4) indicates that the yeast subunit is involved in substrate binding. Similarly, a sequence found in zinc-binding domains occurs in the yeast subunit; the Zn ion associated with the β subunit coordinates with ATP and is thought to be involved in RNA catalysis (30). Finally, the ability to affinity label the 140,000-dalton subunit of wheat germ RNA polymerase II with a purine nucleoside analogue (31) indicates that substrate-binding activity is a conserved feature of this subunit in eukaryotes. Whether the 140,000-dalton subunit performs other functions that have been attributed to the prokaryotic subunit is not yet clear. It is tempting to speculate that eukaryotic transcription control factors may interact with the 140,000-dalton subunit, in a manner analogous to that suggested for the prokaryotic β and σ subunits.

Eukaryotic polymerases I–III are each composed of 2 large and 5–10 distinctly smaller subunits. The prokaryotic core RNA polymerase is composed of 2 large and 2 smaller but identical subunits. This conservation of overall structure, the sequence homologies between the 2 large subunits of eukaryotic and prokaryotic RNA polymerases, and the similarities in DNA and substrate-binding properties of the subunits all indicate that eukaryotic RNA polymerases have structural and functional homologues of prokaryotic RNA polymerase core subunits. We propose that eukaryotic RNA polymerases contain subunits homologous to all 3 core subunits of the *E. coli* enzyme. The yeast RNA polymerase II 45,000-dalton subunit is a candidate for such a homologue; subunits of similar size occur in all eukaryotic RNA polymerase II enzymes investigated thus far.

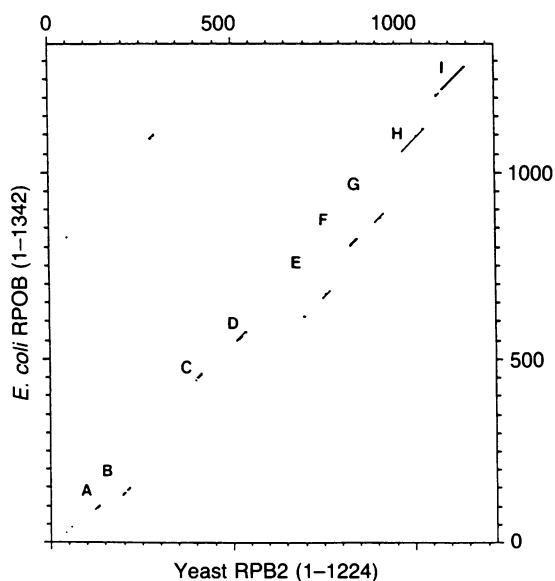


Fig. 3. Yeast RPB2 and *E. coli* RPOB amino acid sequence comparison. RPB2 amino acid sequences are compared with those of the *E. coli* RNA polymerase β subunit using a dot matrix computer program (19), with the window set at 25 and the stringency at 40.

REGION	GENE	AMINO ACIDS	SEQUENCE
A	RPB2	115-138	QEARLRNLTYSGLFVDVKKRTYE
	RPOB	83-106	QECQIRGVITYSAPLRVKLRLLVYE -- ** - * - - - - - - - - ** --
B	RPB2	201-215	GYFIINGSEKVLIAQ
	RPOB	134-148	GTFVINGTERVIVSQ - - - - - - - - - - - - - - -
C	RPB2	391-406	DRKDQDDRDFHGKRL
	RPOB	438-453	GKGEVDDIDHLGNRRI ** * - - - - - - - - -
D	RPB2	512-541	RQLHNTHWGLVCPAETPEGQACGLVKNLSL
	RPOB	548-577	RDVHPHTHYGRVCPLETPEGPNIGLINSLSV - - - - - - - - - - - - - - -
E	RPB2	748-766	ILGVAASIIFFPDHNQSPR
	RPOB	660-678	VVSVGASLIPFLEHDDANR *** - - - - - - - - -
F	RPB2	816-851	ELPAGQNAIVAIACYSYGNQEDSMIMNQSSIDRGLF
	RPOB	793-828	ELALGQNMRFVAFMPWNGYNFEDSILVSRVQEDRF - - * - - - - - * - - - - - - - - - * * * -
G	RPB2	887-917	HGTYDKLDDDGLIAPGVRVSGEDVITIGKTP
	RPOB	859-889	EAALSKLDESGIVYIGAEVTGGDILVGVKTP *** - - - - - - - - - - - - - - -
H	RPB2	961-1032	LKFVKVVRVTKIPQIGDKFASRHGQKGTIGITYRREDMPFTAEGIVPDLIINPHAIPSRMTVAHLIECLLS
	RPOB	1047-1118	LKIVKVYLAVKRRIQPGDKMAGRHNKGVISKINPIEDMPYDENGTPVDIVLNPLGVPSRMNIGQILETHLG - - - - - * * - - - - - - - - - * -
I	RPB2	1058-1156	LLREHGYSRSGFEVYMGHTGKKLMAQIFFGPTYQRLRHMVDDKIHARGMPQVLTQRPVEGRSRDGLRFGEMERDCHIAHGAASFLKERLMEASD
	RPOB	1198-1296	LLKLGDLPTSGQIRLYDGRTEQFERPVTVMYMLKLNHLVDDKMHARSTGSYSLVTQQLGGKAFQGGQRFGEMEVWALEAYGAAAYTLQEMLTVKSD - - * * * - - * - - - - - * * - - - * - - - - - - - - - - - * -

FIG. 4. Subunit amino acid sequence homologies. The nine segments of amino acid sequence homology in Fig. 3 are shown in detail. Identical sequences are underlined. Conservative amino acid replacements, those with a value of at least +1 (23), are starred. The purine nucleotide-binding motif (24) is overlined.

If eukaryotic RNA polymerases contain a core enzyme whose components are homologous to those found in *E. coli*, then what are the functions of the additional subunits found associated with the three eukaryotic RNA polymerases? Some of these polypeptides are unique to one class of RNA polymerase and others are shared by two or all three of the enzymes. It seems likely that some of these additional subunits fine tune the specificity and efficiency of the transcription reaction and others are involved in regulation of the relative levels of active RNA polymerases I-III. Detailed genetic and biochemical analysis of these components should reveal their contribution to the transcription apparatus and may enhance our understanding of the molecular mechanisms involved in the regulation of gene expression.

We are grateful to Gerry Fink and Robert Roeder for discussion and comments on the manuscript and thank J. Sexton for technical assistance and C. Carpenter for preparing the manuscript. This work was supported by a grant from the National Institutes of Health (GM34365).

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