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Cloning *DPB3*, the gene encoding the third subunit of DNA polymerase II of *Saccharomyces cerevisiae*

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ABSTRACT

DNA polymerase II purified from *Saccharomyces cerevisiae* contains polypeptides with apparent molecular masses of >200, 80, 34, 30 and 29 kDa, the two largest of which (subunits A and B) are encoded by the essential genes *POL2* and *DPB2*. By probing a λgt11 expression library of yeast DNA with antiserum against DNA polymerase II, we isolated a single gene, *DPB3*, that encodes both the 34- and 30-kDa polypeptides (subunit C and C'). The nucleotide sequence of *DPB3* contained an open reading frame encoding a 23-kDa protein, significantly smaller than the observed molecular masses, 34- or 30-kDa, which might represent post-translationally modified forms of the *DPB3* product. The predicted amino acid sequence contained a possible NTP-binding motif and a glutamate-rich region. A *dpb3* deletion mutant (*dpb3Δ*) was viable and yielded a DNA polymerase II lacking the 34- and 30-kDa polypeptides. *dpb3Δ* strains exhibited an increased spontaneous mutation rate, suggesting that the *DPB3* product is required to maintain fidelity of chromosomal replication. Since a fifth, 29-kDa polypeptide was present in DNA polymerase II preparations from wild-type cell extracts throughout purification, the subunit composition appears to be A, B, C (or C and C') and D. The 5' nontranscribed region of *DPB3* contained the *MluI*-related sequence ACGCGA, while the 0.9-kb *DPB3* transcript accumulated periodically during the cell cycle and peaked at the G1/S boundary. The level of *DPB3* transcript thus appears to be under the same cell cycle control as those of *POL2*, *DPB2* and other DNA replication genes. *DPB3* was mapped to chromosome II, 30 cM distal to *his7*.

INTRODUCTION

The yeast *Saccharomyces cerevisiae* has three distinct, essential nuclear DNA polymerases (1–4). DNA polymerase I is similar in structure and activity to mammalian DNA polymerase α (5,6), which has been proposed to replicate the lagging strand of chromosomal DNA (7). DNA polymerase III is similar to mammalian DNA polymerase δ (8), which has been proposed as the leading strand replicase (7). Although DNA polymerase II was first described in 1970 (9–11), its structure and function were until recently unknown. DNA polymerase II purified to near-homogeneity contains polypeptides with apparent molecular masses, >200, 80, 34, 30 and 29 kDa (12). The genes encoding the >200-kDa catalytic subunit A and the 80-kDa subunit B have been cloned (4,13). Both genes are essential for cell growth and their thermosensitive mutants exhibit defective DNA synthesis at the restrictive temperature, suggesting that the DNA polymerase II holoenzyme participates directly in chromosomal DNA replication (4,13; and Araki et al., in preparation). Based on these results we have proposed a new model for the replication of DNA by three DNA polymerases (4). In this model, DNA polymerase I initiates synthesis at the origin and synthesizes DNA primers on the lagging strand, DNA polymerase II elongates the leading strand, and DNA polymerase III elongates the lagging strand. Yeast DNA polymerase II shares some similarities with mammalian DNA polymerase e (previously described as δ_2 polymerase), an enzyme previously suggested to be involved in DNA repair in human cells (14).

In this report we describe the cloning, sequencing and genetic analysis of *DPB3*, the gene encoding the third subunit of DNA polymerase II. We found that both the 34- and 30-kDa polypeptides of purified DNA polymerase II are from the same gene, *DPB3*. Since 30-kDa polypeptide seemed to be a proteolytic product of 34-kDa polypeptide and the 29-kDa polypeptide also appears to be a subunit of DNA polymerase II, DNA polymerase

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II thus contains four subunits. *DPB3* is dispensable for cell growth, but is required for normal fidelity of chromosome replication, and the level of its transcript appears to be under the same cell cycle control as those of *POL2* (Araki et al., in preparation), *DPB2* (13) and other DNA replication genes (for reviews, see 15 and 16).

MATERIALS AND METHODS

Bacterial and yeast strains

Escherichia coli DH5α (17) was used for the propagation of plasmids. Y1090 (18) and LE392 (17) were used for λgt11 and λCharon28A phages, respectively. *S. cerevisiae* strains were YHA1 (*MATa/MATα leu2-3,-112/leu2-3,-112 trp1-289/trp1-289 ura3-52/ura3-52 his7-2/+ +/can1*) (4), BJ3501 (*MATα pep4::HIS3 prb1*-Δ1.6 his3-Δ200 ura3-52 can1 gal7) (from E. W. Jones, Carnegie-Mellon University, PA), L155-1B (19), YHA3 (*MATa ade5 leu2 met8-1 trp1 ura3-52*), CG379(*MATα ade5 his7-2 leu2-3,112 ura3-52*) (from C. Giroux, Wayn State University, MI), XS803-2C (*MATα his1-7 hom3-10 leu2-3, 112 ura3-52 can1*), and AMY50-1B (*MATα lys1-1 his1-7 ade2-1 hom3-10 leu2-1 ura3-52*).

Yeast genomic libraries and plasmid DNAs

λgt11 and λCharon28A *S. cerevisiae* genomic DNA libraries were described previously (4,20). Plasmid pBS(SK+) (Stratagene, La Jolla, CA) was used for subcloning and DNA sequencing. Plasmid pGB310 contains the *S. cerevisiae URA3* gene, excisable by *Eco*RI, *Bam*HI, *Hind*III, *Sma*I, and *Sac*I (obtained from C. Giroux).

Biochemical and genetical methods

Antibody screening of λgt11 yeast genomic library (18), screening of λCharon 28A library with a DNA probe (17), double-stranded DNA sequencing by dideoxy-termination method (21), Western and Northern blottings were described previously (4). Partial purification of DNA polymerase II activity was as described (4,12). Synchronization of yeast cells by mating pheromone α, feed-starve, and elutriator centrifugation and the preparation of total RNA from the yeast cells followed by Northern blotting were described (19). The gene disruption mutants were constructed by the one-step gene replacement method (22) with a specific fragment containing the disrupted gene, followed by spore dissection. Yeast transformation, and other materials and methods, were as described previously (23,4,12,13).

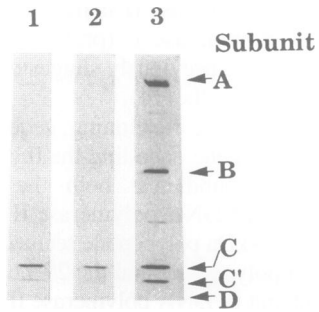

Figure 1. The purified DNA polymerase II (12) was separated by 4–20% SDS-PAGE and transferred to a Immobilon membrane filter as published (12). The filter was incubated either with 1000-fold diluted mouse antiserum against polymerase II (lane 3) or with the antiserum purified with antigens produced by λgt11-2 (lane 1) or λgt11-7 (lane 2) clones. The immuno-reacted polypeptides were visualized with alkaline-phosphatase conjugated anti-mouse IgG (Promega). On the right the polypeptide of DNA polymerase II is shown. Mouse antiserum against polymerase II did not recognize the D subunit.

Rabbit antibodies against yeast DNA polymerase II

The mouse antiserum against yeast DNA polymerase II (12) did not recognize the 29-kDa polypeptide of DNA polymerase II (12). Therefore, we have tried to raise rabbit antiserum against DNA polymerase II (12) as described (24). This rabbit antiserum recognizes not only the >200-kDa, 80-kDa, 34-kDa and 30-kDa polypeptides previously recognized by the mouse antiserum, but also the 29-kDa polypeptide (see Fig. 6) by Western blotting.

Measurement of spontaneous mutation rates

In preliminary experiments, spontaneous reversion frequencies were monitored by a patch test, in which patches of cells (about 10 cm^2) grown on YPDA were replica plated onto omission media and revertants counted after five to ten days. Patches of *DPB3*$^+$ cells yielded an average of one Lys$^+$ revertant, and 40 His$^+$ revertants. Subsequently, spontaneous reversion rates were quantitated using the Leningrad test (25). Yeast cells grown in YPDA medium to stationary phase were washed, suspended in water at densities of approximately 10^6-to-10^7 cells/ml, and applied to plates using a multipronged replicator. Five-to-six hundred compartments were replicated for each determination. The plates contained synthetic medium either lacking or containing a limiting amount of either lysine, adenine or histidine. Limiting concentrations were 2 μg/ml histidine, 5 μg/ml lysine,

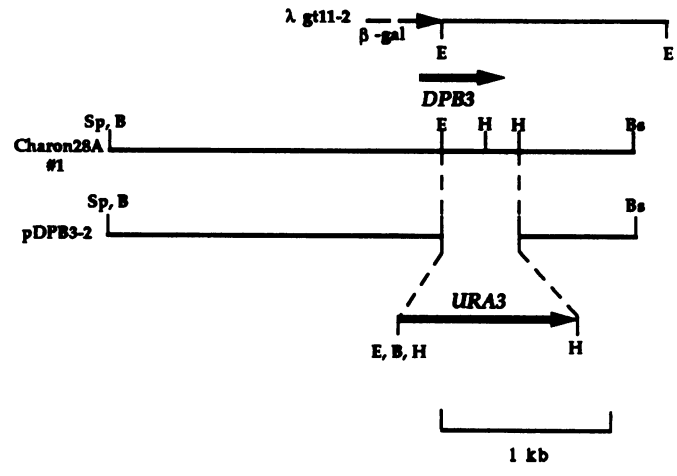

Figure 2. Structure of the *DPB3* gene and construction of the *dpb3*Δ gene disruption. λgt11-2 is a representative of the positives after screening a λgt11 yeast genomic DNA library with mouse antiserum against DNA polymerase II. λCharon28A # 1 was isolated from the λCharon28A yeast genomic DNA library by plaque hybridization with the 1.3-kb *Eco*RI insert of λgt11-2. For construction of *dpb3*Δ, the 3.8-kb *Bam*HI-*Bst*EII fragment of λCharon28A # 1 was first ligated to the pBS(SK+) DNA treated with *Bam*HI and *Kpn*I, the remaining ends were blunt-ended by T4 DNA polymerase and ligated by T4 DNA ligase. Then, the 1.2-Kb *Eco*RI-*Hin*dIII *URA3* fragment from pGB310 plasmid was inserted into the above plasmid DNA after 0.32 Kb *Eco*RI-*Hin*dIII and 0.22-Kb *Hin*dIII fragments were removed (pDPB3-2). This pDPB3-2 was further digested with *Spe*I and *Bst*EII and was used for transformation of diploid strain (YHA1) to make the *dpb3*Δ disruptant. A thick arrow indicates the open reading frame of the gene and its orientation predicted from the DNA sequence. Abbreviations used here are; β-gal :β-galactosidase, B:*Bam*HI, Bs:*Bst*EII, E:*Eco*RI, H:*Hin*dIII, Sp:*Spe*I.

or 1 μg/ml or 2.5 μg/ml adenine. Plates were incubated for five-to-ten days at 30°C, and spontaneous reversion rates were calculated by the P_o method (25).

RESULTS

Cloning the gene for DNA polymerase II subunit C

It was very difficult to obtain enough DNA polymerase II to raise specific antibodies against each subunit of the polymerase (see ref. 12). Therefore, a λgt11 library of yeast genomic DNA was probed with mouse antiserum against DNA polymerase II (12). From approximately 6×10^5 recombinant plaques, 29 positive λgt11 phages have been isolated. Antibody was affinity-purified using the positives as described by Snyder *et al.* (18) and used to probe a Western blot of DNA polymerase II to identify which of the subunits was being expressed by the recombinant λgt11 phage. Seventeen λgt11 phages had part of the gene for subunit B, as described previously (13). Eleven other positives generated affinity-purified antibody that reacted with both the 34- and 30-kDa polypeptides of DNA polymerase II but not with subunits, A and B. Figure 1 shows examples of such results. And one positive was not able to be assigned to any specific polypeptide of DNA polymerase II.

Since Western blotting indicated that each of these eleven lgt11 clones produced a fusion protein between β-galactosidase and either 34- or 30-kDa polypeptide, the clones seemed likely to have only a part of the gene for either 34- or 30-kDa polypeptide. Thus intact gene was cloned from a λCharon28A yeast genomic DNA library by plaque lift hybridization using the 1.3-kb *Eco*RI insert of λgt11−2 (Fig. 2) as a probe. From approximately 10,000 recombinant plaques, twenty positives were obtained. All generated DNA containing a common 5-kb *Bam*HI insert that hybridized to the 1.3-kb *Eco*RI fragment (Fig. 2). The restriction map of this *Bam*HI fragment was identical to that of chromosomal DNA, suggesting that no rearrangement had occurred during cloning. Since the 5-kb *Bam*HI fragment hybridized only to the same size *Bam*HI fragment of yeast chromosomal DNA, this is a single-copy gene. The 5-kb *Bam*HI fragment was subcloned into pBS(SK+) and the region encompassing the 1.3 kb *Eco*RI fragment was sequenced. The nucleotide sequence revealed one open reading frame encoding a protein of calculated molecular weight 23,005 (Fig. 3). We named this gene *DPB3* (*D*NA *P*olymerase *B* subunit *3*). The calculated molecular weight of the *DPB3* gene product is substantially less than the observed molecular masses of the 34- or 30-kDa polypeptides, suggesting that the gene product either migrates anomalously in gel electrophoresis or is post-translationally modified. The 30-kDa polypeptide might be modified differently than the 34-kDa polypeptide, or might simply be a proteolytic product of the 34-kDa polypeptide.

The deduced amino acid sequence of *DPB3* has a region of thirty-five residues (residues 120−154) containing 63% acidic residues—primarily glutamate—including a run of fourteen residues that contains eleven glutamate and one aspartate residues. Also, a possible nucleoside triphosphate-binding consensus sequence (26) occurs in the amino-terminal half of the predicted sequence (Fig.4). However, the amino acid sequence of *DPB3* had no significant similarity to any protein appearing in GenBank, NBRF-Nucleic acid and -protein Bank, EMBL Gene Bank, or SwissProt Bank, except for a kind of heat-shock proteins, such as chicken *Hhch08* (27), hamster glucose-regulated protein grp94 (28) and *Trypanosoma cruzi* heat-shock protein 90-kDa homolog (29). These similarities are limited to the acidic region of *DPB3*.

DPB3 is dispensable

The disrupted *DPB3* gene, *dpb3*Δ, was constructed by replacing the 0.32-kb *Eco*RI-*Hin*dIII and 0.22-kb *Hin*dIII DNA fragments of *DPB3* with a 1.2 kb fragment containing the *URA3* gene from plasmid pGB310 (Fig. 2). One wild-type *DPB3* copy of a homozygous diploid strain (YHA1) was replaced with the disrupted gene using the one-step gene replacement method (22). After confirming this construction by Southern blotting, a *DPB3/dpb3*Δ diploid was sporulated and the resulting tetrads were dissected. Out of twenty tetrads, twelve tetrads showed four viable spores, seven showed three viable spores and one gave two viable spores. The total numbers of Ura^+ and Ura^- spores were 35 and 36, respectively, and therefore any defect in

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   1 TTCTTCTTGTACTTTTCTTTTTGAACCTTAAAAACTCTTGCAATTATGTTTCCATGATGC
  61 TTTTGAAGTAATAATTTATTTTTATTACGCGAAATTAATGGCCTTAACAACTGCCTGTCC
 121 CAACTGATAAAAACAAGCAAGGGTCAACCGTGTTGCAAAAAAAATGTCCAATTTAGTTAA
                                          M  S  N  L  V  K     6
 181 AGAAAAAGCACCTGTCTTTCCTATATCTAAAGTAAAGAAGATTGCCAAATGCGATCCCGA
      E  K  A  P  V  F  P  I  S  K  V  K  K  I  A  K  C  D  P  E  26
 241 ATACGTAATTACATCTAATGTAGCTATATCAGCGACCGCATTCGCTGCTGAGTTATTTGT
      Y  V  I  T  S  N  V  A  I  S  A  T  A  F  A  A  E  L  F  V  46
 301 ACAGAATCTCGTCGAAGAATCGCTGGTCTTAGCACAACTGAATTCGAAAGGAAAGACAAG
      Q  N  L  V  E  E  S  L  V  L  A  Q  L  N  S  K  G  K  T  S  66
 361 CCTACGATTAAGCCTAAATTCTATAGAAGAATGTGTCGAAAAAAGAGATAATTTCAGGTT
      L  R  L  S  L  N  S  I  E  E  C  V  E  K  R  D  N  F  R  F  86
 421 TCTAGAGGATGCCATTAAACAACTGAAGAAGAATAGCGCACTCGACAAGAAGAGAGAACT
      L  E  D  A  I  K  Q  L  K  K  N  S  A  L  D  K  K  R  E  L 106
 481 AAACATGCAACCGGGTCGGAGCGATCAAGAGGTTGTTATAGAAGAGCCTGAATTGCATGA
      N  M  Q  P  G  R  S  D  Q  E  V  V  I  E  E  P  E  L  H  E 126
 541 GGATGATGGTGTAGAGGAAGAAGAAGAAGAAGACGAGGTATCCGAAGAAGAAGAGCCCGT
      D  D  G  V  E  E  E  E  E  E  D  E  V  S  E  E  E  E  P  V 146
 601 ACACAATGAAGAACTTCTTGATGATAGTAAAGATCAACAAAATGATAAATCCACGCGCAG
      H  N  E  E  L  L  D  D  S  K  D  Q  Q  N  D  K  S  T  R  S 166
 661 TGTGGCAAGCTTGCTGTCGAGATTCCAGTATAAATCCGCACTAGACGTAGGAGAACACTC
      V  A  S  L  L  S  R  F  Q  Y  K  S  A  L  D  V  G  E  H  S 186
 721 CGACTCTTCTGATATCGAAGTTGACCATATGAAAAGCACCGATCCTTAGTGTTATTGCTA
      D  S  S  D  I  E  V  D  H  M  K  S  T  D  P              201
 781 TTATTGCTATGTAATTACTATTCGATGCAATATGTTATACATATACATATACGCAATGAG
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Figure 3. Nucleotide sequence of *DPB3*. The nucleotide sequence was determined for both strands by the dideoxy-termination method using plasmid DNA, Sequenase (United States Biochemical Co.), and specific oligonucleotide primers synthesized on a Vega Coder 300 DNA synthesizer. The predicted amino acid sequence of the ORF is given below the nucleotide sequence. Numbers on the left and right sides of the figure indicate nucleotide and amino acid residues, respectively. The putative cell cycle dependent regulatory sequence, ACGCGA, is underlined.

S.c. Dpb3	54-71	L V L A Q L N S K G K T - S L R L S L
S.c. Rad3	38-55	S I L E M P S G T G K T V S L - L S L
S.c. RadH	31-49	L Q V I A G P G T G K T K V L T S R V
S.c. Ras1	13-31	I V V V G G G G V G K S A L T I Q F I
S.c. Yp2	11-29	L L L I G N S G V G K S C L L L R F S
S.c. EF-Tu	51-68	I G T I G H V D H G K T - T L T A A I
SV40 Tag	422-439	W L F K G P I D S G K T - T L A A A L
H.s. Gi α	43-60	L L L L G A G E S G K S - T I V K Q M
E.c. RecA	62-79	V E I Y G P E S S G K T - T L T L Q V
E.c. DnaA	168-185	L F L Y G G T G L G K T - H L L H A V
E.c. DnaB	226-243	I I V A A R P S M G K T - T F A M N L
E.c. UvrD	25-43	L L V L A G A G S G K T R V L V H R I
E.c. UvrA	636-653	T C I T G V S G S G K S - T L I N D T
E.c. ATPase α	165-183	E L I I G D R Q T G K T R L A I D A I
E.c. ATPase β	146-164	V G L F G G A G V G K T V N M M E L I
E.c. rho	174-192	G L I V A P P K A G K T M L L Q N I A
E.c. MalK	32-49	V V F V G P S G C G K S - T L L R M I
E.c. Rep	18-36	C L V L A G A G S G K T R V I T N K I
E.c. Era	11-28	I A I V G R P N V G K S - T L L N K L

Figure 4. Alignment of part of the predicted amino acid sequence of Dpb3 protein and proteins with NTPase or NTP-binding activities. Characteristic identical or similar residues are boxed. Sequences were obtained from the GenBank or NBRF databases.

germination of the *dpb3Δ* spores is unlikely. No growth rate difference was detected between wild type and *dpb3Δ* cells. Furthermore, as the homoallelic diploid (*dpb3Δ/dpb3Δ*) sporulated normally at 30°C and spore viability was more than 80%, *DPB3* is also dispensable for sporulation.

Mapping of *DPB3*

Yeast chromosomes were separated by transverse alternating field electrophoresis, transferred to a nitrocellulose filter, and hybridized to ^{32}P-labeled *DPB3* DNA. Only chromosome II hybridized to the probe (data not shown). To map the *DPB3* gene genetically, *URA3* in the *dpb3Δ* disruptant was followed as described (30). The *DPB3* gene was located 30 cM from *his7* (the distribution of parental ditype : tetratype : non-parental ditype tetrads was 36:1:41) and 52 cM from *met8* (the tetrad distribution was 12:3:27) on the right arm of chromosome II . Since no gene maps at this locus (31), *DPB3* is a newly identified gene.

dpb3Δ is a modest mutator

We initially used a semi-quantitative patch test to assess the effect of the *dpb3Δ* allele on spontaneous reversion. Three independent *dpb3Δ* derivatives of AMY50-1B were compared with YCp50 (30) transformants of AMY50-1B obtained in the same transformation. *dpb3Δ* increased the average frequency of *lys1-1* revertants about six-fold (three experiments) and that of *his1-7* about two-fold (two experiments). We observed no significant effect of *dpb3Δ* on growth, lethality of UV irradiation or methyl methane sulfonate, or on UV-induced reversion of *lys1-1* or *his1-7* (data not shown). Spontaneous reversion rates were quantitated using the Leningrad test (25) for a *dpb3Δ* derivative of AMY50-1B for reversion of *lys1-1* and *ade2-1*, and for a *dpb3Δ* derivative of XS803-2C for reversion of *his1-7*. The results, shown in Table 1, indicate that *dpb3Δ* elevated the spontaneous reversion rate by factors of 2.2, 2.6 and 20 at *his1-7*, *ade2-1* and *lys1-1*, respectively.

The abundance of the *DPB3* transcript fluctuates during the cell cycle

A Northern blot of polyA-RNA separated by formaldehyde-agarose gel electrophoresis was probed with the 0.3 kb *Eco*RI-*Hin*dIII fragment of *DPB3*. A 0.9-kb transcript hybridized to the probe (data not shown). The steady-state levels of this 0.9-kb transcript were followed before and after synchronization of yeast cells with the α-factor mating pheromone. As shown in Fig. 5, the abundance of *DPB3* mRNA fluctuated in a cell-cycle dependent manner and peaked at the same time as the transcript of *CDC9* (32) as well as other DNA synthesis related gene transcripts (32). We also used two other methods of synchronizing yeast cells, namely the Feed-Starve protocol (33) and elutriator centrifugation (32). The results with these two synchronizations were essentially the same as with mating factor synchronization (data not shown). Therefore, it is unlikely that the periodic accumulation of the *DPB3* transcript during the cell cycle is a synchronization artifact.

DNA polymerase II from *dpb3Δ* cells

DNA polymerase II (DNA polymerase II* in ref. 12) activity was partially purified from cells bearing the *dpb3Δ* gene. The activity eluted reproducibly earlier (at 0.29 M NaCl) than wild-type polymerase II (at 0.34 M NaCl) from a MonoQ column and later (at 0.32 M NaCl) than wild-type polymerase II (at 0.27 M NaCl) from a MonoS column (data not shown). The activity recovered in polymerase II fraction from *dpb3Δ* cells were reproducibly less than 50% of that from wild-type cells. The active fractions from the MonoS column (Fig. 6A) were subjected to Western blotting followed by probing with rabbit antiserum against DNA polymerase II. As shown in Fig. 6B, subunits A and B coeluted with the DNA polymerase II activity. However, the 34-kDa (C) and 30-kDa (C') polypeptides found in DNA polymerase II from wild-type cells (12 and Fig. 6C) were not detected. Note that the membrane filter of Fig. 6B was developed twice longer than that of Fig. 6C in order to make sure absence of C and C' polypeptides. Thus, the catalytic subunit A of DNA polymerase II (>200 kDa polypeptide) forms a stable complex with subunit B in the absence of both the 34- and 30-kDa polypeptides, while the 29 kDa polypeptide seemed to be dissociated from the complex (Fig. 6B). The control experiment with wild-type cells (Fig. 6C) showed that, while the 29-kDa polypeptide was copurified with the polypeptides corresponding to subunits A, B and C on a MonoS column, the amount of the 30-kDa polypeptide (C') was considerably less than that of the most purified DNA polymerase II (12). Thus, it is highly possible that the 30-kDa polypeptide is a degradation product of the 34-kDa subunit C. The result strongly suggests that the 29 kDa polypeptide is a subunit of DNA polymerase II (subunit D).

Table 1. Spontaneous reversion rates of *dpb3Δ* mutant cells

Parent strain	Revertible Marker	Spontaneous Reversion Rate ($\times 10^8$) *DPB3*	*dpb3Δ*
AMY50-1B	*lys1-1*	0.28 ± 0.18	5.7 ± 1.2
AMY50-1B	*ade2-1*	1.8 ± 0.1	4.7 ± 0.3
XS803-2C	*his1-7*	4.5 ± 0.8	9.8 ± 2.2

Numbers are the mean ± standard deviation of three determinations.

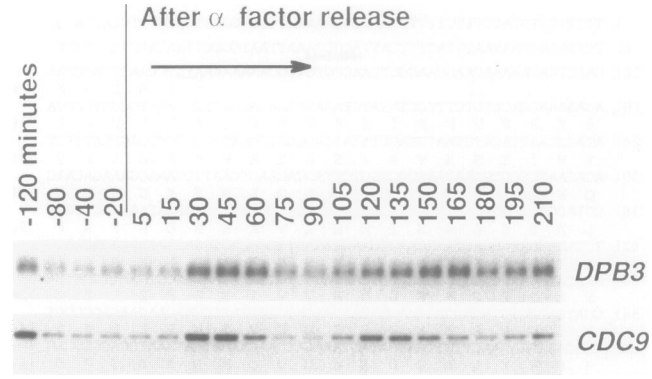

Figure 5. The steady-state levels of the *DPB3* transcript during the cell cycle. *S. cerevisiae* cells grown in YPDA to 1×10^7 cells/ml were synchronized with α-factor as described (19). After releasing the cells from the α-factor treatment, cells were incubated in fresh YPDA medium. At 15-min intervals, 10-ml aliquots were withdrawn and total RNA was extracted (19). Five μg of total RNA from each sample was denatured with glyoxal, separated by agarose gel electrophoresis. and transferred to a GeneScreen membrane (DuPont). The filters were hybridized with ^{32}P-labeled 0.3-Kb *Eco*RI-*Hin*dIII fragment (Fig.2). After autoradiography, ^{32}P-label was stripped off and the filter was reprobed with ^{32}P-labeled *CDC9* DNA (19).

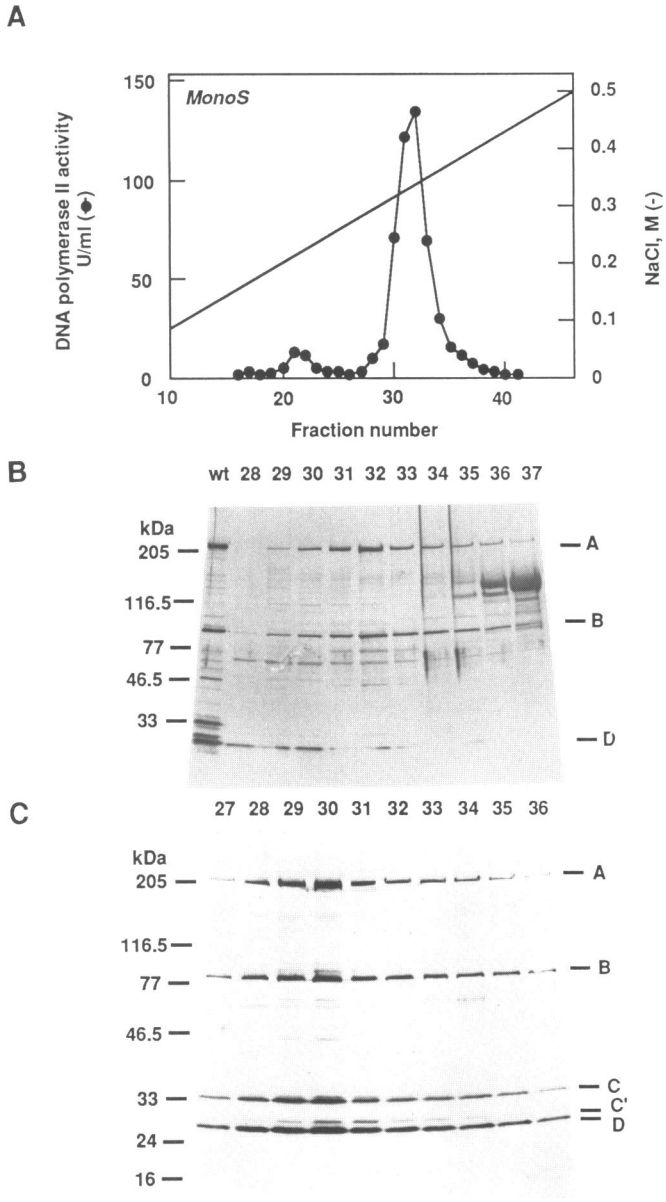

Figure 6. Western blot of DNA polymerase II from *dpb3Δ* cells. (A) The *dpb3Δ* derivative of BJ3501 was grown in YPDA medium (60 L) at 30°C to 2×10^7 cells/ml, collected, and the ammonium sulfate fraction prepared as described (12). This fraction was applied onto an S-Sepharose column (5×19 cm) and proteins eluted by 0.1M and 0.5M NaCl in Buffer A (12). The 0.5 M NaCl fractions, which contained DNA polymerases I, II, and III (12), were applied onto a MonoQ column (HR16/10) and the polymerase activities were eluted with a 400-ml linear gradient of 0.1−0.5 M NaCl in Buffer A. Fractions containing DNA polymerase II activity were pooled, dialyzed against Buffer A containing 0.1 M NaCl for 4 hrs at 0°C, and subjected to MonoS column (HR5/5) chromatography as described previously (12). (B) The active fractions (20 μl each) were analyzed by 4−20% SDS-PAGE, and proteins were electrophoretically transferred to Immobilon-P filters (Millipore). The filter was incubated with 5,000-fold diluted rabbit antiserum against DNA polymerase II and the polymerase polypeptides were visualized with alkaline phosphatase-conjugated anti-rabbit IgG (Promega). Numbers on the left show the molecular masses in kDa of prestained marker proteins (Bethesda Research Laboratories), while the polypeptide of DNA polymerase II is shown on the right. The strong signal migrated approximately 140 kDa is a non-specific band reacted with rabbit antiserum. (C) The DNA polymerase II was partially purified from BJ3501 cells (wild type) by S-Sepharose, MonoQ and MonoS columns as described in (A) and the active fractions (20 μ each) in MonoS were analyzed by Western blotting as (B) except for the filter being less developed. The experiment was repeated several times and the the result was very reproducible.

DISCUSSION

Our previous studies have shown that *POL2* and *DPB2*, the genes encoding the catalytic and second largest subunits, respectively, of *S. cerevisiae* DNA polymerase II, are essential for cell growth (4,13). In contrast, *DPB3* which encodes the third largest subunit was dispensable for cell growth, although cells bearing the *dpb3Δ* gene showed increases in rates of spontaneous reversion of between two- and twenty-fold, depending on the test allele (Table 1). The *pol2-1* and *dpb2-1* mutants also show higher rates of spontaneous mutation and an unstable complex of DNA polymerase II holoenzyme (4,13; and our unpublished observations). These data suggest that stable complex formation of the polymerase II is important for normal fidelity of chromosome replication. This is not the first demonstration of a subunit of an essential enzyme being dispensable for yeast cell growth. For example *RPB4*, which encodes the fourth largest subunit of yeast RNA polymerase II, is dispensable for cell growth at 24−30°C (34).

λgt11 fusions of *lacZ* and the *DPB3* gene expressed proteins coantigenic with both the 34- and 30-kDa polypeptides (Fig. 1), and these polypeptides were absent from DNA polymerase II from *dpb3Δ* mutant cells (Fig. 6B), strongly suggesting that both polypeptides are the product of *DPB3*. The observed molecular mass of each polypeptide was significantly greater than predicted from the nucleotide sequence (Fig. 3). These polypeptides, therefore, might migrate abnormally in SDS-polyacrylamide gel electrophoresis, with the 30-kDa polypeptide (C′) possibly being a proteolytic product of the 34-kDa polypeptide (subunit C). Alternatively, they might be modified post-translationally and the difference in migration may reflect the extent and/or the type of modification. Since the amount of the 30-kDa polypeptide in the DNA polymerase II varied considerably from preparation to preparation and varied at the stage of the purification, the polypeptide is likely a degradation product of the 34-kDa subunit C. On the other hand, the 29-kDa polypeptide (subunit D) copurified with the subunits A, B and C throughout the purification. Thus, the subunit structure of the DNA polymerase II is A, B, C and D.

A possible nucleoside triphosphate-binding consensus sequence is identified in the *DPB3* gene. However, there was no detectable NTPase, or DNA (or RNA) helicase activity in the nearly homogeneous DNA polymerase II (12). Thus, it is less likely that the *DPB3* polypeptide is either a NTPase or helicase, although it is still possible that other DNA polymerase II subunits mask the activity. Alternatively, the *DPB3* polypeptide may bind to NTP to promote the DNA polymerase II formation *in vivo*. Availability of each DNA polymerase II subunit gene (*POL2* (4), *DPB2* (13), and *DPB3* (this study)) now makes a detailed study on complex formation of DNA polymerase II possible.

The abundance of the *DPB3* transcript fluctuated during the cell cycle and peaked at the same time as *CDC9* (Fig. 5). The 5′ upstream region of *DPB3* contains the $^{5'}$ACGCGT$^{3'}$-related sequence, ACGCGA (Fig. 3). It has been strongly suggested that the sequence ACGCGT (the recognition sequence of restriction enzyme *Mlu*I) has an important role in the coordinate expression during the cell cycle of genes involved in DNA replication (15, 16). Included within this group are the genes for all four subunits of DNA polymerase I (6,19,35; and Hinkle, D., personal communication), DNA polymerase III catalytic subunit (*CDC2*) and yeast PCNA (*POL30*) (36), and the three subunits of DNA polymerase II (13; this study and Araki et al., in preparation),

suggesting that expression of the genes for nuclear DNA polymerases and their associated subunits are coordinately regulated during the cell cycle and turned on prior to the initiation of chromosomal DNA replication.

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