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Biochemistry

DPB2, the gene encoding DNA polymerase II subunit B, is required for chromosome replication in *Saccharomyces cerevisiae*

HIROYUKI ARAKI[†‡], ROBERT K. HAMATAKE[†§], LELAND H. JOHNSTON[¶], AND AKIO SUGINO[†‖]

[†]Laboratory of Molecular Genetics, National Institute of Environmental Health Sciences, P.O. Box 12233, Research Triangle Park, NC 27709; and [¶]Laboratory of Cell Propagation, National Institute for Medical Research, The Ridgeway, Mill Hill, London NW7 1AA, England

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ABSTRACT **The *Saccharomyces cerevisiae* DNA polymerase II holoenzyme consists of five polypeptides. The largest is the catalytic subunit, whose gene (*POL2*) has been cloned and sequenced. Herein we describe the cloning and sequencing of *DPB2*, the gene for the second largest subunit of DNA polymerase II, and the isolation of temperature-sensitive *dpb2* mutations. The DNA sequence revealed an open reading frame encoding a protein of M_r 79,461 and lacking significant sequence similarity to any protein in data bases. Disruption of *DPB2* was lethal for the cell and the temperature-sensitive *dpb2-1* mutant was partially defective in DNA synthesis at the restrictive temperature, indicating that the DPB2 protein is required for normal yeast chromosomal replication. Furthermore, the DNA polymerase II complex was difficult to obtain from *dpb2-1* mutant cells, suggesting that a stable DNA polymerase II complex requires DPB2 and is essential for chromosomal replication. The *DPB2* transcript periodically fluctuated during the cell cycle and, like those of other genes encoding DNA replication proteins, peaked at the G_1/S phase boundary.**

In the yeast *Saccharomyces cerevisiae*, three distinct nuclear DNA polymerases (I, II, and III) have been purified and characterized, and the gene encoding the catalytic polypeptide of each DNA polymerase has been cloned and sequenced (1–10). Genetic studies have shown that all three DNA polymerases are essential for cell growth and that DNA polymerases I and III are required for chromosome replication. These polymerases are usually purified in association with other subunits as a complex. DNA polymerase I (a homolog of mammalian DNA polymerase α) consists of four polypeptides: the 180-kDa catalytic subunit, the 74-kDa B subunit with unidentified activity, and the 58-kDa and 48-kDa DNA primase subunits (for review, see ref. 11). DNA polymerase III (a homolog of mammalian DNA polymerase δ) consists of at least two peptides: the 125-kDa catalytic subunit and the 55-kDa B subunit of unknown function (5, 6). DNA polymerase II (a possible homolog of mammalian DNA polymerase ε) has been purified as a complex of five polypeptides of 200 kDa, 80 kDa, 34 kDa, 30 kDa, and 29 kDa (5). The largest is the catalytic subunit (5), but the other polypeptide functions are not identified. They are, however, tightly associated with the intact catalytic subunit, although not with a truncated form (9). Replicative DNA polymerases from a variety of organisms also can be purified as complexes with other subunits (for review, see ref. 12). In most cases only the catalytic subunit shows activity, and the functions of the other subunits remain unknown with the exception of the DNA primase.

The ability in yeast to augment biochemical studies with classical genetic and molecular genetic approaches makes the system attractive for analyzing the *in vivo* functions of a purified protein and for serving as a model for eukaryotic cells. In the first step of elucidating the *in vivo* functions and roles of the *S. cerevisiae* DNA polymerase II subunits, we have cloned and sequenced *DPB2*, the gene encoding subunit B.** Disruption of *DPB2* is lethal, displaying the same terminal morphology that is observed in mutants defective in DNA replication (13). Furthermore, temperature-sensitive mutations in this gene have been isolated. DNA synthesis in these mutant cells is partially defective at the restrictive temperature, consistent with our model (9) to explain how three DNA polymerases may function at the replication fork. The amount of *DPB2* transcript fluctuated periodically during the cell cycle and increased at the G_1/S phase boundary, when several other transcripts of DNA replication genes are coordinately expressed (14). These results, and the previous results with the catalytic subunit (9), strongly suggest that DNA polymerase II is required for DNA replication and that its holoenzyme participates in *S. cerevisiae* chromosome replication.

MATERIALS AND METHODS

Bacterial and Yeast Strains. *Escherichia coli* DH5α (15), Y1090 (16), and MC1066 (17) were used for propagating plasmids, λgt11 phages, and mutated plasmids, respectively. *S. cerevisiae* YHA1 (*MATa/MATα leu2-3,-112/leu2-3,-112 trp1-289/trp1-289 ura3-52/ura3-52 his7-2/++/can1*) (9) and BJ3501 (*MATα pep4::HIS3 prb1-Δ1.6 his3-Δ200 ura3-52 can1 gal7*) (from E. W. Jones, Carnegie Mellon University) were used for constructing gene disruptants. CG378 (*MATa ade5 leu2-3,112 ura3-52*) (from C. Giroux, Wayne State University) and SB155 (*MATα trp1 cdc7-1*) (from Robert A. Sclafani, University of Colorado) were used for constructing strains. L155-1B (18) was used for preparing RNA.

Yeast Genomic Libraries and Plasmid DNAs. The λgt11 *S. cerevisiae* genomic DNA library was described (9). Plasmid pBS(SK+) (Stratagene) was used for subcloning and DNA sequencing. YCplac22, YCplac33, and YIplac211 (19) were used for cloning the *DPB2* gene and YEp13 (20) was for preparing the 2.3-kilobase (kb) *Hpa* I *LEU2* fragment.

Isolation of Thermosensitive Mutants. The *Kpn* I–*Nsi* I fragment containing the *DPB2* gene from λgt11-31 was cloned in the *Kpn* I and *Pst* I sites of both YCplac22 and YCplac33 DNAs, resulting in plasmids pDPB2-4 and pDPB2-5, respectively (Fig. 1). A diploid strain containing a disrupted *DPB2* gene on one chromosome (see text) was transformed with pDPB2-5 and the resultant Ura$^+$ transformants were sporulated and dissected. One Ura$^+$ Leu$^+$ segregant, YHA3, was used for further study. pDPB2-4 was treated with 1 M hydroxylamine at 75°C for 1.5 hr and used for *E. coli* MC1066

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[‡]Present address: Department of Fermentation Technology, Faculty of Engineering, Osaka University, Suita, Osaka, Japan.
[§]Present address: Department of Virology, Bristol-Myers Squibb Pharmaceutical Research Institute, Princeton, NJ 08540.
[‖]To whom reprint requests should be addressed.
**The sequence reported in this paper has been deposited in the GenBank data base (accession no. M61710).

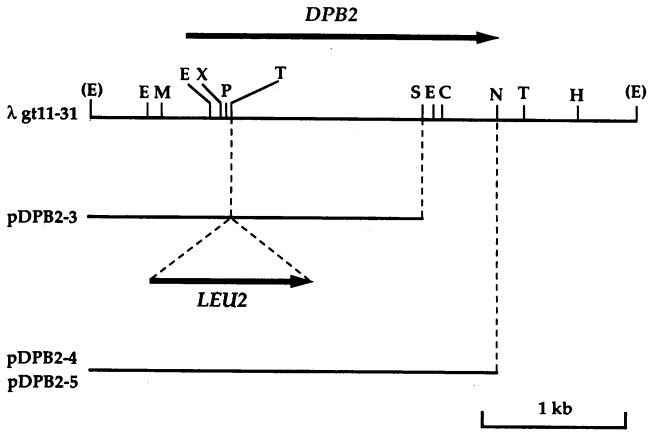

FIG. 1. Structure of the *DPB2* gene and construction of a gene disruption. λgt11-31 was the representative clone after screening a λgt11 yeast genomic library with mouse antiserum against DNA polymerase II* (5). A 2.8-kb *Kpn* I–*Sst* I fragment of λgt11-31, which contains a 1-kb *Kpn* I–*Eco*RI λ DNA fragment and a 1.8-kb yeast DNA fragment, was subcloned on pBS(SK+). The 2.3-kb *Hpa* I *LEU2* fragment from YEp13 was inserted at the *Stu* I site of pBS(SK+) yeast DNA. The resulting plasmid (pDPB2-3) was cleaved by *Mlu* I and *Sst* I and used to transform YHA1. The construction of pDPB2-4 and pDPB2-5 is described in text. A thick arrow indicates the open reading frame of the *DPB2* gene and its orientation predicted from the DNA sequence. B, *Bam*HI; Bs, *Bst*EII; C, *Cla* I; E, *Eco*RI; H, *Hin*dIII; M, *Mlu* I; N, *Nsi* I; S, *Sst* I; T, *Stu* I; P, *Pst* I; X, *Xho* I.

transformation. Approximately 10,000 ampicillin-resistant transformants were obtained and plasmid DNA was extracted from them and used for YHA3 transformation. Approximately 2000 Ura^+-Trp^+ transformants grown at 25°C were duplicated onto plates lacking uracil and tryptophan (SD-UT plates) and incubated at 25°C or 37°C for 3 days. Each colony was replica-plated onto two plates containing 0.1% 5-fluoroorotic acid (FOA plates) (21) and onto two SD-UT plates, and sets of plates were incubated at 25°C or 37°C for 3 days. Colonies grown on FOA plates at 37°C were transferred to new FOA plates by replica plating and were incubated at 25°C for 3 days. Colonies grown on FOA plates at 25°C were transferred to new FOA plates and incubated at 37°C for another 3 days. Seven colonies grew at 25°C but not at 37°C and four colonies grew at 37°C but not at 25°C. From these colonies, plasmid DNA was isolated and used to transform YHA3 cells. Then, pDPB2-3 was cured from the transformed YHA3 cells by plating on FOA plates. The resultant FOA-resistant cells showed thermosensitivity on YPDA plates. Therefore, either heat-sensitive or cold-sensitive phenotypes could result from *DPB2* gene mutations. Among these mutants, *dpb2-1* showed the most stringent temperature-sensitive growth and was chosen for further study. To replace *DPB2* with the *dpb2-1* mutation on the chromosome, the *Kpn* I–*Nsi* I DNA fragment containing the *dpb2-1* mutation was transferred to YIplac211. The resultant plasmid pDB2-6 was cleaved with *Xho* I and used to transform BJ3501. Ura^+ transformants were grown in YPDA medium at 25°C and spread onto FOA plates and a temperature-sensitive colony was selected. The resultant strain (YHA6) was crossed with CG378 and one segregant (YHA7) (*MATa dpb2-1 Ura3-52 his3-Δ200 trp1-289 can1*) from this cross was used. The wild-type *DPB2* strain (YHA8) was constructed from YHA7 by transferring the *DPB2* gene to the chromosome just as YHA6 was constructed.

Biochemical and Genetical Methods. Antibody screening of the λgt11 yeast genomic library (16), double-stranded DNA sequencing by the dideoxynucleotide-termination method (22), Western and Northern blot analyses (9), and measurement of DNA synthesis *in vivo* (23) were as described. Partial purification of DNA polymerase II was as published (5, 9). Synchronization of yeast cells by mating factor α and preparation of total cellular RNA followed by Northern blot analysis were described (14, 18). The gene disruption mutants were constructed by the one-step gene replacement method (24) with a specific fragment containing the disrupted gene, followed by spore dissection. Yeast cell transformation was as described (19, 25). Other materials and methods have been described (5, 9).

RESULTS

Cloning the Gene for DNA Polymerase II Subunit B. To clone the genes for *S. cerevisiae* DNA polymerase II subunits B and C, we screened a λgt11 yeast genomic DNA library (9) with mouse antiserum against DNA polymerase II* (5). Among 6×10^5 recombinant plaques, 28 plaques reacted. These were classified into two groups in two ways, by Southern blot hybridization using a specific insert DNA probe isolated from several λgt11 DNAs and by Western blots of the proteins produced in λgt11-infected cells probed with the mouse antiserum affinity-purified with subunit A, B, or C. Seventeen λgt11 phages contained at least part of the gene for subunit B and 11 phages contained DNA for subunit C, whereas none had the gene for subunit A, which was later cloned independently by an improved method (9). Antibody affinity-purified by each kind of phage reacted only with either subunit B or subunit C polypeptide as determined by Western blot analysis. These results strongly suggest that each phage class has at least part of the gene encoding either the subunit B or C polypeptide.

Among the clones containing DNA for subunit B, λgt11-31 had the largest insert and contained a 1.5-kb *Eco*RI DNA fragment common to most of the other reacting clones (Fig. 1). The restriction map of the insert was the same as for chromosomal DNA, suggesting that no rearrangement had occurred during cloning. The DNA nucleotide sequence revealed a large open reading frame encoding 698 amino acids (Fig. 2). The calculated molecular weight of 79,461 is in good agreement with the observed molecular mass of the polypeptide (80 kDa). We have named the gene *DPB2* (*DNA polymerase B* subunit *2*). The analysis of the gene for subunit C will be published elsewhere.

The deduced amino acid sequence of *DPB2* had no significant similarities with genes in GenBank, National Biomedical Research Foundation Nucleic Acid and Protein Bank, European Molecular Biology Laboratory Gene Bank, or Swiss-Prot Bank. Moreover, it lacked sequence-specific DNA binding protein consensus sequences, such as zinc-finger and leucine-zipper consensus sequences, and also lacked a nucleoside triphosphate binding domain. However, it is noteworthy that *DPB2* exhibited some similarity to the second largest subunit of yeast DNA polymerase I (D. Hinkle, personal communication) by using the BESTFIT program of the University of Wisconsin Genetics Computer Group (similarity 41% and identity 20%).

***DPB2* Is Essential for Cell Growth.** To disrupt the *DPB2* gene, the 2.3-kb *LEU2* fragment was inserted at the *Stu* I site in *DPB2* (Fig. 1). One of the wild-type genes in a diploid strain was replaced with the disrupted gene by the one-step gene replacement method (24). After confirming the chromosomal gene disruption by Southern blot analysis, the Leu^+ transformant was sporulated and dissected. Of 20 tetrads dissected, 17 showed two viable spores and three showed one viable spore. All viable spores were Leu^- and thus possessed an intact *DPB2* gene. This indicates that *DPB2* is essential for cell growth. About 80% of the inviable spores exhibited a dumbbell shape and the remaining spores went through the cell cycle twice before arresting. These disrupted cells were fixed, stained with 4′,6-diamino-2-phenylindole, and ob-

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   1 TTTCGTATCCCCAATGTCTTTTCCTGAATTCCCATTGTGATCTTCAATGCCAACCCAACCTTCTTTTTACTTTGTTGGGTAATAAATTATGTTACTTTGTCATTTCATCTCTTTTCAAGA
 121 TGGAATCTAAAAGAAGAAAAACGACAAAAGAACAAAAGTGATGTGACGCGTTATTTTCGCGTGACATAATAGTAAAATATTTCAACTGGGCATCTTTAACACATGTTACAATAAAGTTGA
 241 GATGACTTGTTTATGCCTTTAAAGTTTGTAAATGGAACTAGAAGCGTCTATGTGTGAAATGTTTGGCTCTGGGAATGTTCTGCCTGTTAAAATTCAGCCTCCGCTGCTTAGGCCACTGGC
                                  M  E  L  E  A  S  M  C  E  M  F  G  S  G  N  V  L  P  V  K  I  Q  P  P  L  L  R  P  L  A  30
 361 GTATAGAGTTTTATCAAGAAAATATGGTTTATCAATTAAATCTGATGGCCTGTCTGCTCTTGCAGAATTCGTTGGCACTAATATAGGTGCTAACTGGAGACAAGGGCCTGCTACAATAAA
      Y  R  V  L  S  R  K  Y  G  L  S  I  K  S  D  G  L  S  A  L  A  E  F  V  G  T  N  I  G  A  N  W  R  Q  G  P  A  T  I  K  70
 481 ATTTCTCGAGCAATTTGCTGCAGTATGGAAACAACAAGAAAGAGGCCTTTTCATCGACCAGAGCGGGGTTAAAGAAGTTATACAGGAGATGAAAGAACGTGAGAAAGTCGAATGGAGTCA
      F  L  E  Q  F  A  A  V  W  K  Q  Q  E  R  G  L  F  I  D  Q  S  G  V  K  E  V  I  Q  E  M  K  E  R  E  K  V  E  W  S  H  110
 601 TGAACACCCTATTCAACATGAGGAGAATATCTTGGGACGGACAGATGACGATGAGAACAACAGTGACGATGAAATGCCGATCGCTGCTGATTCATCCTTACAAAATGTTTCATTATCTTC
      E  H  P  I  Q  H  E  E  N  I  L  G  R  T  D  D  D  E  N  N  S  D  D  E  M  P  I  A  A  D  S  S  L  Q  N  V  S  L  S  S  150
 721 ACCCATGCGACAGCCCACCGAAAGAGATGAATATAAACAGCCTTTCAAGCCAGAAAGCTCCAAGGCGTTGGACTGGAGGGACTACTTCAAAGTTATCAATGCTTCTCAGCAACAAAGATT
      P  M  R  Q  P  T  E  R  D  E  Y  K  Q  P  F  K  P  E  S  S  K  A  L  D  W  R  D  Y  F  K  V  I  N  A  S  Q  Q  Q  R  F  190
 841 CTCATACAATCCGCACAAAATGCAATTTATTTTCGTCCCCAATAAAAAGCAGAATGGGCTAGGAGGCATTGCGGGTTTTCTACCAGATATAGAGGACAAAGTTCAAATGTTCTTGACAAG
      S  Y  N  P  H  K  M  Q  F  I  F  V  P  N  K  K  Q  N  G  L  G  G  I  A  G  F  L  P  D  I  E  D  K  V  Q  M  F  L  T  R  230
 961 ATATTACCTCACGAACGACAGAGTCATGAGAAATGAAAACTTCCAAAACAGTGACATGTTTAATCCATTATCATCTATGGTGTCTTTACAAAATGAACTGTCCAATACTAACCGACAGCA
      Y  Y  L  T  N  D  R  V  M  R  N  E  N  F  Q  N  S  D  M  F  N  P  L  S  S  M  V  S  L  Q  N  E  L  S  N  T  N  R  Q  Q  270
1081 GCAATCCAGCAGTATGAGTATCACCCCAATAAAAAACCTACTAGGTAGGGATGCTCAAAACTTTCTACTATTGGGGCTCTTAAATAAGAACTTTAAGGGCAATTGGTCACTGGAAGATCC
      Q  S  S  S  M  S  I  T  P  I  K  N  L  L  G  R  D  A  Q  N  F  L  L  L  G  L  L  N  K  N  F  K  G  N  W  S  L  E  D  P  310
1201 GTCTGGATCTGTCGAAATTGACATTTCCCAAACTATCCCTACACAAGGCCATTACTACGTGCCAGGTTGCATGGTTCTTGTGGAAGGAATATACTATTCTGTAGGAAATAAATTCCATGT
      S  G  S  V  E  I  D  I  S  Q  T  I  P  T  Q  G  H  Y  Y  V  P  G  C  M  V  L  V  E  G  I  Y  Y  S  V  G  N  K  F  H  V  350
1321 TACCTCCATGACTTTACCCCCTGGTGAGAAGAAGAAATTACATTAGAAACGATAGGTAATCTCGATCTCTTAGGAATACATGGCATTTCTAATAACAATTTCATTGCTCGTTTGGATAA
      T  S  M  T  L  P  P  G  E  R  R  E  I  T  L  E  T  I  G  N  L  D  L  L  G  I  H  G  I  S  N  N  N  F  I  A  R  L  D  K  390
1441 AGATTTGAAGATTAGATTACACCTTTGGAGAAAGAATTAACAGATCATAAATTTGTAATTCTCGGCGCAAATTTGTTCCTAGATGATTTGAAAATTATGACTGCACTCAGCAAAATTTT
      D  L  K  I  R  L  H  L  L  E  K  E  L  T  D  H  K  F  V  I  L  G  A  N  L  F  L  D  D  L  K  I  M  T  A  L  S  K  I  L  430
1561 GCAAAAATTAAATGATGACCCACCGACCCTATTAATTTGGCAAGGTTCTTTCACTTCAGTTCCCGTTTTCGCATCAATGAGTAGCCGGAATATAAGTAGTTCCACTCAATATAAGAATAA
      Q  K  L  N  D  D  P  P  T  L  L  I  W  Q  G  S  F  T  S  V  P  V  F  A  S  M  S  S  R  N  I  S  S  S  T  Q  Y  K  N  N  470
1681 TTTCGATGCCTTGGCGACGCTTCTGTCAAGATTTGACAATTTGACCGAAAATACCACAATGATATTTATTCCAGGTCCTAACGATTTATGGGGGTCGATGGTGTCATTGGGGGCAAGTGG
      F  D  A  L  A  T  L  L  S  R  F  D  N  L  T  E  N  T  T  M  I  F  I  P  G  P  N  D  L  W  G  S  M  V  S  L  G  A  S  G  510
1801 GACATTACCGCAAGATCCAATTCCTAGTGCGTTTACCAAAAAAATCAACAAGGTCTGTAGAAACGTTGTATGGAGCTCAAATCCAACTAGAATAGCATACTTATCCCAAGAAATAGTCAT
      T  L  P  Q  D  P  I  P  S  A  F  T  K  K  I  N  K  V  C  R  N  V  V  W  S  S  N  P  T  R  I  A  Y  L  S  Q  E  I  V  I  550
1921 TTTCAGGGACGATTTATCCGGAAGATTCAAAAGACACCGTTTGGAATTCCCATTCAACGAGAGCGAAGATTTTTATACTGAAAACGATAATATGATGTCTAAAGACACCGATATTGTACC
      F  R  D  D  L  S  G  R  F  K  R  H  R  L  E  F  P  F  N  E  S  E  D  F  Y  T  E  N  D  N  M  M  S  K  D  T  D  I  V  P  590
2041 AATCGATCAATTAGTTAAAGAACCAGACCAGTTACCACAAAAGGTTCAAGAAACAAGAAAACTCGTTAAAACAATACTAGACCAGGGTCATTTATCGCCATTTCTTGATTCCTTGCGCCC
      I  D  Q  L  V  K  E  P  D  Q  L  P  Q  K  V  Q  E  T  R  K  L  V  K  T  I  L  D  Q  G  H  L  S  P  F  L  D  S  L  R  P  630
2161 AATTTCATGGGATTTGGACCACACTTTGACACTTTGCCCAATACCATCGACAATGGTTCTTTGCGACATTACTTCTGCACAATTTGATTTGACATACAACGGTTGTAAAGTAATTAACCC
      I  S  W  D  L  D  H  T  L  T  L  C  P  I  P  S  T  M  V  L  C  D  I  T  S  A  Q  F  D  L  T  Y  N  G  C  K  V  I  N  P  670
2281 TGGAAGTTTCATTCATAATAGACGTGCTAGGTATATGGAGTACGTTCCATCCTCAAAGAAAACTATACAAGAAGAGATATATATCTAAATAATGCATCAAAATAAGAACACTTAAACAAG
      G  S  F  I  H  N  R  R  A  R  Y  M  E  Y  V  P  S  S  K  K  T  I  Q  E  E  I  Y  I                                    698
2401 CACGGTAGTTTGGTGCTCTGCCATCTATTTGTATGGATACTTTTGAATTTGATGTAACGGATTTCGGCATACAATATATGGGATCTATGGGGACTAAATTGTCGTATCCCATTAAACT
2521 TAAACCAGCAACACCAAAAACGCTCGGAAGACATCCACTTCATTTTCAGGCCT 2573
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FIG. 2. Nucleotide sequence of *DPB2*. The nucleotide sequence of *DPB2* was determined in both strands by the dideoxynucleotide-termination method using double-stranded plasmid DNA, Sequenase (United States Biochemical), and synthetic oligonucleotide primers synthesized by a Vega coder model 300 DNA synthesizer. The predicted amino acid sequence of the open reading frame 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 consensus sequence ACGCGT is underlined.

served by epifluorescent microscopy. Their nuclei were located near or in the isthmus (data not shown). This terminal morphology is observed in mutants defective in DNA replication under nonpermissive conditions (13).

A Thermosensitive Mutant of *DPB2* with Defective DNA Synthesis. To elucidate the *in vivo* function of subunit B, seven temperature-sensitive and four cold-sensitive *DPB2* mutations were isolated from 2000 colonies harboring mutagenized *DPB2* plasmids by using the plasmid-shuffling method (21, 26). The most stringent temperature-sensitive mutation, *dpb2-1*, was used to replace the chromosomal copy of *DPB2*. Cells containing the *dpb2-1* mutation exhibited slow growth even at 24°C (doubling time, 4 hr) and stopped cell division within 4 hr after a temperature shift-up to 38°C. The terminal morphology

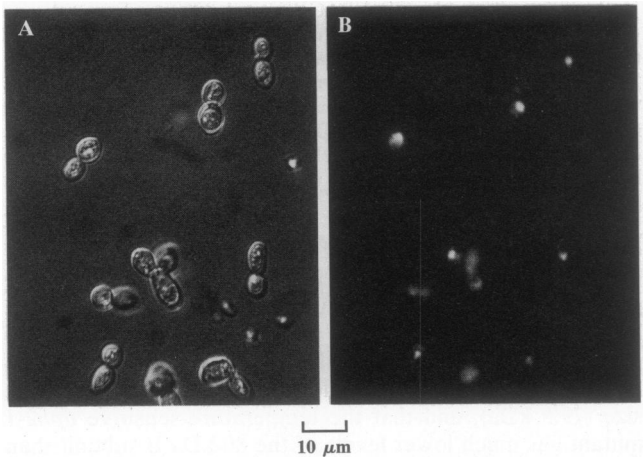

FIG. 3. Terminal morphology of the *dpb2-1* mutant. *dpb2-1* cells grown in YPDA to 5×10^6 cells per ml at 24°C were transferred to 37°C and incubated for 6 hr. Cells were collected, fixed with 80% ethanol, and stained with 4',6-diamino-2-phenylindole. Cells were observed by differential interference contrast (*A*) or by epifluorescence (*B*) microscopy.

of *dpb2-1* cells at the restrictive temperature (Fig. 3) was the same as the *dpb2::URA3* disruptant cells.

The effect of *dpb2-1* on DNA synthesis was determined by synchronizing cells grown at 24°C and measuring DNA synthesis at both 24°C and 38°C after release from mating factor. Because initiation of DNA synthesis does not occur in *cdc7-1* cells at the restrictive temperature (13), DNA synthesis was also measured in a *dpb2-1 cdc7-1* double mutant to determine the residual mitochondrial DNA synthesis. The cell shape was monitored microscopically to determine progression from START to late G_1 phase (appearance of budding cells) and the terminal phenotype (dumbbell-shaped cells). As shown in Fig. 4, budding of *dpb2-1* mutant cells started at the same time at both 24°C and 38°C. However, at 38°C the cells remained dumbbell shaped and did not re-enter S phase. The amount of DNA synthesized at the restrictive temperature in *dpb2-1* mutant cells was significantly less than at the permissive temperature. The residual DNA synthesis in the *dpb2-1 cdc7-1* double mutant is most likely mitochondrial and suggests that a substantial amount of the DNA synthesis seen in the *dpb2-1* mutant cells at the restrictive temperature is also due to mitochondria. RNA and protein synthesis were also measured but were unaffected in *dpb2-1* mutant cells at either temperature (data not shown). Thus, the *dpb2-1* mutation primarily affects chromosome replication.

DNA Polymerase II Holoenzyme in *dpb2-1* Mutant Cells. DNA polymerase II was partially purified from *dpb2-1* mutant cells grown at 24°C by using S-Sepharose, Mono Q, and Mono S columns as described (9). As shown in Fig. 5*A*, DNA polymerase II activity eluted from the Mono Q column in two peaks. The first peak was a proteolytic product of the DNA polymerase II catalytic subunit and the second peak was similar to the DNA polymerase II complex observed in wild-type cells (5, 9) by Western blot analysis followed by probing with antibody against DNA polymerase II* (Fig. 5*B*). However, the amount of activity in the complex was substantially lower than that from wild-type cells and the stoichiometry of subunits in this complex differed from polymerase II* from

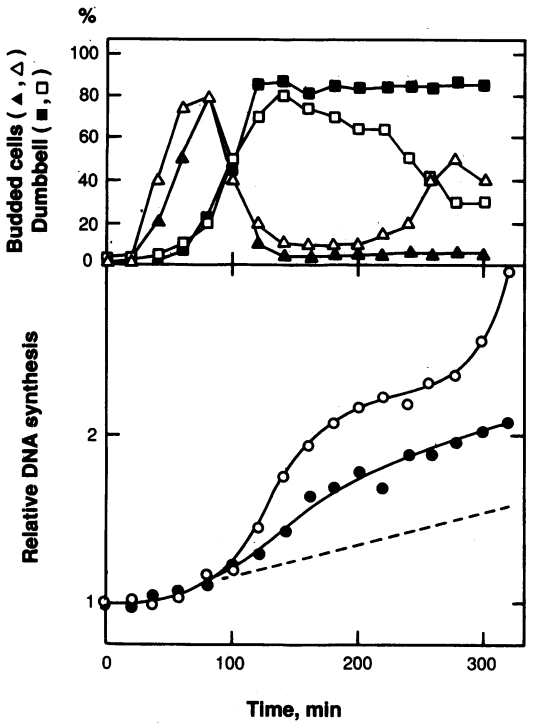

FIG. 4. *In vivo* DNA synthesis in synchronized *dpb2-1* and *dpb2-1 cdc7-1* double mutant cells. Cells grown at 24°C and prelabeled with [^{3}H]uracil were synchronized by treatment with mating type pheromone α factor (4 μg/ml) for 4 hr. After removing α factor, the cells were suspended in YPDA medium containing the same specific activity of [^{3}H]uracil as before α factor treatment and held at 24°C (open symbol) or 38°C (solid symbol). At the indicated times, 1 ml of culture was withdrawn and DNA synthesis was measured as described (23). At the same time cell morphology at 24°C and 38°C was monitored by microscopy. Since DNA synthesis at 24°C in *dpb2-1 cdc7-1* double-mutant cells was almost the same as that of *dpb2-1* cells, the data were not included. The dashed line is DNA synthesis in *dpb2-1 cdc7-1* mutant cells at 38°C.

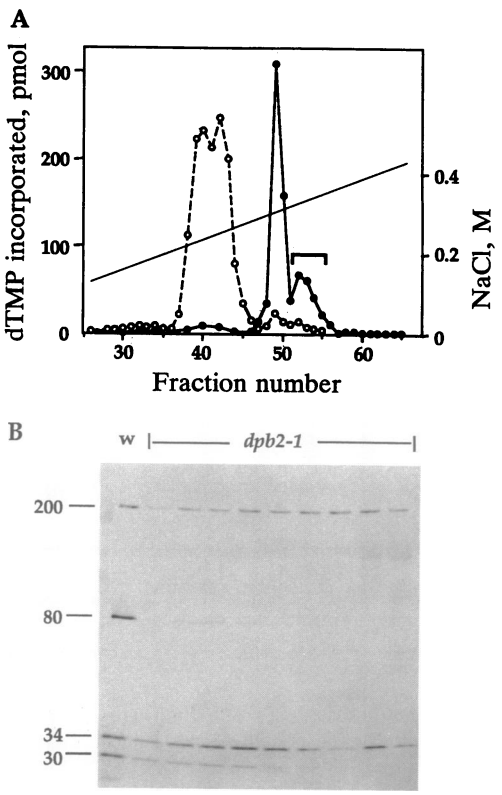

FIG. 5. DNA polymerase II activity from *dpb2-1* cells. (*A*) DNA polymerase II activity in Mono Q fractions. The cells were grown in YPDA medium (60 liters) at 24°C to 2 × 10^7 cells per ml and collected, and the ammonium sulfate fraction was prepared as described (5). The fraction was applied to a S-Sepharose column (5 × 19 cm) and the proteins were eluted by 0.1 M and 0.3 M NaCl in buffer A (5). The 0.3 M NaCl fractions containing DNA polymerases I, II, and III (5) were applied to a Mono Q column (HR10/10) and the polymerase activities were eluted with a 400-ml linear gradient of 0.1–0.5 M NaCl in buffer A. DNA polymerase activity was measured using as template primer either activated calf thymus DNA (open circle), which measures primarily DNA polymerases I and III, or $(dA)_n$·$(dT)_{10}$ (solid circle), which measures almost exclusively DNA polymerase II. (*B*) Western blots of peak fractions of DNA polymerase II activity. Fractions containing DNA polymerase II activity in Mono Q, indicated by the bar, were pooled, dialyzed against buffer A for 4 hr at 0°C, and subjected to Mono S column (HR5/5) chromatography as described (5). The active fractions (20-μl samples) were analyzed by SDS/PAGE in a 4–20% gradient gel, and proteins were electrophoretically transferred to Immobilon-P filters (Millipore). The filters were incubated with mouse antiserum against DNA polymerase II* and the polymerase subunit polypeptides were visualized with alkaline phosphatase-conjugated anti-mouse IgG (Promega). ω and *dpb2-1* indicate DNA polymerase II from wild-type cells (5) and the mutant *dpb2-1* cells, respectively. Numbers on the left show the molecular masses in kDa of each subunit of DNA polymerase II*

wild-type cells. Although subunit A was tightly associated with subunits C and D, only a trace amount of subunit B was detected. Thus, the association of subunits A, C, and D does not appear to be affected by the mutation. However, DNA polymerase II* from *dpb2-1* mutant cells containing the mutated subunit B is unstable *in vivo* and/or *in vitro*.

Increased *DPB2* Transcripts at the Boundary of G_1 and S Phases During the Cell Cycle. Northern blot analysis of poly(A) RNA separated in a formaldehyde/agarose gel and probed with *DPB2* (1.5-kb *Eco*RI fragment) identified a 2.5-kb transcript (data not shown). We also examined the amount of *DPB2* transcript during the cell cycle. As shown in Fig. 6, DPB2 mRNA fluctuated during the cell cycle. The amount of transcript peaked at the same time as the transcripts for *CDC9* and several other genes involved in DNA replication (14, 18). This peak time was clearly different from that for the histone H2A transcript. Similar results were obtained from cells synchronized by two other methods, the feed-starved protocol and elutriator centrifugation methods (14) (data not shown). Therefore, it is likely that the expression of *DPB2* is coordinately regulated by the same mechanism applicable to the other DNA replication genes (14), including the *POL2* gene encoding the catalytic subunit of DNA polymerase II and the *DPB3* gene encoding subunit C of DNA polymerase II (L.H.J., unpublished results).

DISCUSSION

The gene for the 80-kDa B subunit of the DNA polymerase II holoenzyme complex has been isolated and shown to be essential for cell viability and for chromosome replication. The evidence supporting the identification of *DPB2* as the gene encoding the 80-kDa B subunit is that the recombinant bacteriophage expressing the *DPB2* gene product affinity-purifies IgG that reacts with the 80-kDa B subunit, that the *DPB2* open reading frame codes for a protein of the correct size (79.5 kDa), and that the temperature-sensitive *dpb2-1* mutant has much lower levels of the 80-kDa B subunit than an isogenic wild-type strain.

We have previously shown that DNA polymerase II can be isolated as a multisubunit complex (5) and that the C-terminal portion of the catalytic subunit is important for the stability of this complex (9). Its stability appears to be crucial to the cell because there is a strong correlation between cell growth

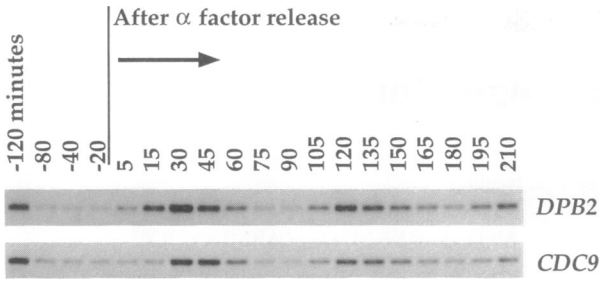

FIG. 6. Amount of *DPB2* transcript during the cell cycle. *S. cerevisiae* cells grown in minimal medium to 1×10^7 cells per ml were synchronized with α factor as described (18). After releasing the cells from α-factor treatment, they were incubated in fresh minimal medium and, at 15-min intervals, 10 ml of culture was withdrawn and total RNA was extracted. Total RNA (5 μg) from each sample was denatured with glyoxal, separated by agarose gel electrophoresis, and transferred to a GeneScreen membrane (DuPont). The filters were hybridized with a ^{32}P-labeled 1.5-kb *Eco*RI fragment of λgt11-31 (Fig. 1). After autoradiography of the filter, the ^{32}P label was stripped off and the filter was reprobed with *CDC9* (27).

and the presence of an isolatable DNA polymerase II complex consisting of all the subunits (ref. 9 and this work). Although the 80-kDa subunit is an integral part of the DNA polymerase II complex, we cannot rule out the possibility that the *DPB2* gene product has other essential functions unrelated to its role as a polymerase subunit. Nevertheless, the analysis of the temperature-sensitive mutant argues for a role for *DPB2* in DNA synthesis. Although DNA synthesis in the *dpb2-1* mutant was not completely temperature-sensitive, the rate of DNA synthesis at 38°C was ≈50% of that at 24°C. Bud appearance, an indicator of cell cycle progression from START to late G_1 phase, was not affected, suggesting that initiation and/or elongation of DNA synthesis partially depends on the product of the *DPB2* gene. The cell cycle regulation of the *DPB2* transcription and the terminal morphology of the disruptant are similar to other genes known to be involved in DNA synthesis and is consistent with a replicative function for *DPB2*. The phenotype of *dpb2-1* suggests that the complexes of DNA polymerases with their subunits are the functional entities *in vivo* and that protein–protein interactions are critical for biological activity.

We have proposed (9) a model to explain how three DNA polymerases function at the replication fork in yeast. In this model, DNA polymerase I is responsible for initiating synthesis of the leading and lagging strands while elongation of the leading and lagging strands is performed by DNA polymerases II and III, respectively. If synthesis on the leading and lagging strand can occur independently, then we can predict from the model that DNA polymerase II or III mutants would show a partial defect in DNA synthesis. The data shown in Fig. 4 are consistent with this interpretation as is the observation that *cdc2-1* mutant cells [*CDC2* encodes the catalytic subunit of DNA polymerase III (10, 28)] also exhibit a partial defect in DNA synthesis (29).

Because of the strong conservation of structural and functional similarities between the DNA polymerases of yeast and mammalian cells, our results (refs. 5 and 9 and this study) suggest that mammalian DNA polymerase ε (30) may exist as a complex form that also plays an essential function during chromosomal replication.

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