Isolation of Temperature-Sensitive DNA Polymerase III from *Saccharomyces cerevisiae cdc2-2*†

A. Blank* and Lawrence A. Loeb

The Joseph Gottstein Memorial Cancer Research Laboratory, Department of Pathology SM-30, University of Washington, Seattle, Washington 98195

Received February 1, 1991; Revised Manuscript Received June 3, 1991

ABSTRACT: DNA polymerase III of the yeast *Saccharomyces cerevisiae* has been reported to be encoded at the *CDC2* locus based on two observations. First, the *CDC2* gene has homology to known DNA polymerase genes [Boulet et al. (1989) *EMBO J. 8*, 1849–1854], and second, the mutants *cdc2-1* and *cdc2-2* yield little or no DNA polymerase III activity in vitro [Boulet et al. (1989); Sitney et al. (1989) *Cell 56*, 599–605]. We describe here the isolation of temperature-sensitive DNA polymerase III from *cdc2-2* strains. Our results provide direct experimental confirmation of the previously inferred gene/enzyme relationship and verify the conclusion that DNA polymerase III is required to replicate the genome. We isolated DNA polymerase III from two *cdc2-2* strains, one containing the wild-type allele for DNA polymerase I (*CDC17*) and the other a mutant DNA polymerase I allele (*cdc17-1*). Yields from *cdc2-2* cells of both DNA polymerase III activity and an associated 3′–5′-exonuclease activity [exonuclease III; Bauer et al. (1988) *J. Biol. Chem. 263*, 917–924] were decreased relative to yields from *CDC2* cells. DNA polymerase III activity from *cdc2-2* strains is thermolabile, displaying at least a 4-fold reduction in half-life at 44 °C. The activity is also labile at 37 °C, a temperature which is restrictive for growth of *cdc2-2* but not *CDC2* strains. At 23 °C, a temperature which is permissive for growth of both *cdc2-2* and *CDC2* strains, the mutant and wild-type DNA polymerase III activities display equal stability. These observations provide a demonstrable biochemical basis for the thermosensitive phenotype of *cdc2-2* cells.

DNA polymerase III of *Saccharomyces cerevisiae* is distinguished by 3′–5′-exonuclease activity which proofreads in vitro and by dependence of its processivity on proliferating cell nuclear antigen (PCNA)[1] when poly(dA)·oligo(dT) is used as template-primer (Bauer et al., 1988; Bauer & Burgers, 1988a,b). The indispensable biologic function of DNA polymerase III has been deduced from study of temperature-sensitive mutants in the *CDC2* gene, a conditionally lethal cell division cycle gene first described by Hartwell and his colleagues [reviewed in Pringle and Hartwell (1981)]. When shifted to restrictive temperature, *cdc2* mutants arrest in S phase with a terminal morphology characteristic of mutants defective in DNA replication, and several *cdc2* alleles cause arrest with one-third of the genome unduplicated (Conrad & Newlon, 1983). Moreover, DNA synthesis in permeabilized *cdc2* cells is defective at 37 °C but normal at 23 °C (Kuo et al., 1983). These and other observations on *cdc2* mutants [e.g., see Hartwell and Smith (1985)] underlie the conclusion that the *CDC2* gene product is required for S-phase DNA synthesis. Recently, it has been reported that the essential *CDC2* gene product is the 125-kDa catalytic subunit of DNA polymerase III. This conclusion is inferential and rests on the observations that the *CDC2* gene has homology to known DNA polymerase genes (Boulet et al., 1989) and that *cdc2-1* and *cdc2-2* mutants yield little, if any, DNA polymerase III activity in vitro (Boulet et al., 1989; Sitney et al., 1989). We show here that *cdc2-2* mutants harbor DNA polymerase III which is poorly recoverable and temperature-sensitive in vitro.

Experimental Procedures

Strains. Yeast strains, a gift from the laboratory of L. Hartwell, University of Washington, were 9049-14-3 (*MATa pep4-3 prb1-1122 ura3 his7 can1*), 9048-13-2 (same but *cdc2-2*), 9049-26-2 (*MATa cdc17-1 pep4-3 prb1-1122 ura3 his7 can1*), and 9055-52-2 (same but *cdc2-2 try1*).

Preparation of Lysates and Chromatography. All procedures were essentially as in Bauer et al. (1988) with modifications devised by these authors (Boulet et al., 1989). Briefly, cells were grown at 24 °C, omitting the shift to 36 °C, and were harvested at A_{660} = 0.6–1.1. Cell homogenates were treated with ammonium sulfate and poly(ethylenimine) (Sigma), and the resulting crude protein fractions were dialyzed. Subsequent DEAE-silica high-pressure liquid chromatography on a Synchrom AX-1000 column was exactly as described (Boulet et al., 1989) except that that 1.5-mL fractions were collected; the KCl gradient was measured by conducitivity.

Assays. DNA polymerase activity with activated calf thymus DNA as template-primer was measured as described (Bauer et al., 1988). 3′–5′-exonuclease activity, monitored as the release of ^{3}H from 3′ end-labeled single-stranded DNA, was also assayed as described (Bauer et al., 1988) except that the temperature was 30 °C, the reaction time was 15 min, and BSA (Promega) was acetylated. The end-labeled DNA (7.5 × 10^6 dpm/A_{260} unit) was prepared from activated DNA by chain elongation with Klenow fragment as in Bauer et al. (1988) except that the [^{3}H]TTP concentration (40 Ci/mmol) was 21 μM and dCTP was omitted from the reaction in order to confine label to, or near to, chain termini. DNA was denatured by heating at 100 °C for 6 min prior to rapid chilling in ice. Protein was determined according to Bradford (1976) using BSA as a standard.

† This work was supported in part by National Institutes of Health Grant R35-CA-39903 to L.A.L.

* To whom correspondence should be addressed.

[1] Abbreviations: BSA, bovine serum albumin; DEAE, diethylaminoethyl; DTT, dithiothreitol; EDTA, ethylenediaminetetraacetic acid; PCNA, proliferating cell nuclear antigen; Tris-HCl, tris(hydroxymethyl)aminomethane hydrochloride.

0006-2960/91/0430-8092$02.50/0

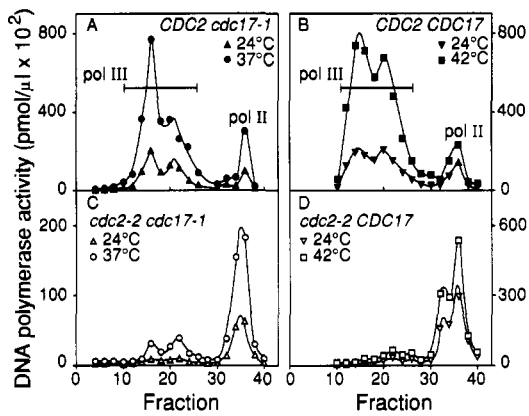

FIGURE 1: DEAE-silica high-pressure liquid chromatography of DNA polymerase activity from *CDC2* and *cdc2-2* strains. Protein preparations from cells having either the wild-type *CDC2* gene (upper panels) or the mutant *cdc2-2* allele (lower panels) were chromatographed. The strains analyzed at the right carry the wild-type gene for DNA polymerase I (*CDC17*) and the strains at the left the mutant *cdc17-1* allele. The approximate amounts of protein applied to the column were the following: *CDC2 CDC17*, 31 mg; *cdc2-2 CDC17*, 26 mg; *CDC2 cdc17-1*, 28 mg; *cdc2-2 cdc17-1*, 46 mg. Aliquots (5 μL) of individual fractions were assayed for DNA polymerase activity.

RESULTS

Isolation of DNA Polymerase III from cdc2-2 Mutants. In previous work (Boulet et al., 1989), DNA polymerase III activity was not detected in *cdc2-1* and *cdc2-2* mutants grown at permissive temperature (23 °C) and shifted to restrictive temperature (36 °C) prior to processing. Whether inactivation of the polymerase occurred at elevated temperature in vivo or during isolation in vitro was uncertain. In other work (Sitney et al., 1988), *cdc2-1* cells grown at 23 °C also yielded little or no DNA polymerase III activity; in this study, cell lysis was at 30 °C, and extracts were subjected to more extensive and perhaps inactivating procedures. To promote recovery of putatively temperature-sensitive DNA polymerase III from *cdc2-2* strains, we used cells which were not subjected to elevated temperature. In addition, we used protease-deficient strains carrying *pep4* and *prb1* mutations, from which recovery of wild-type DNA polymerase III is enhanced (Bauer et al., 1988). To ensure comparability of our results with the benchmark work characterizing DNA polymerase III, all procedures including cell breakage with glass beads, preparation of a crude protein fraction, and high-pressure liquid chromatography on an AX-1000 DEAE-silica column adhered closely to established protocols (Bauer et al., 1988; Boulet et al., 1989). To increase reliability of our findings, we analyzed the *cdc2-2* allele in two different strains, comparing each in pairwise fashion with a corresponding *CDC2* strain. One pair of strains carried the wild-type *CDC17* gene for DNA polymerase I, another the mutant *cdc17-1* allele which yields no DNA polymerase I activity in vitro (Sitney et al., 1988). As shown in Figure 1, activity having the chromatographic behavior of DNA polymerase III (Bauer et al., 1988) was observed in *CDC2* strains, as expected, and was also observed in *cdc2-2* strains in greatly reduced yield. Figure 1A,B depicts the elution profiles of DNA polymerase activity from the two strains having the wild-type *CDC2* allele. The elution profiles of activity from these strains are essentially the same in two cardinal respects as previously observed by Bauer et al. (1988). First, each has an early peak at 0.07 M KCl (fraction 15 or 16), which corresponds to the elution position of DNA polymerase III. Second, each has a late peak at 0.30 M KCl (fraction 36), which is the position of DNA polymerase II. In addition, both profiles have an intermediate peak at 0.13 M KCl (fraction 21 or 22), not previously observed by Bauer et al. in profiles of activity from protease-deficient cells processed with protease inhibitors. Such an intermediate elution position is expected for DNA polymerase I, but the activity in our profiles is not likely to be this enzyme, for two reasons. First, little if any DNA polymerase I has been obtained from either protease-deficient strains (Bauer et al., 1988) or protease-competent strains grown to low density (Boulet et al., 1989) when cells were processed with protease inhibitors according to the protocol we followed. With this protocol, DNA polymerase I has been recovered in significant quantity only from cells having a normal complement of proteases or when inhibitors were omitted (Bauer et al., 1988). Second, and very important, mutant *cdc17-1* cells have yielded no active DNA polymerase I in vitro (Sitney et al., 1989). The intermediate peak in our DEAE profiles is likely another form of DNA polymerase III, as indicated by the profiles of activity from the *cdc2-2* mutants. As shown in Figure 1C,D, both the early peak at fraction 16 and the overlapping, intermediate peak at fraction 22 are greatly reduced in *cdc2-2* strains, while activity in the DNA polymerase II peak at fraction 36 is not significantly affected. That the early and intermediate peaks are different forms of DNA polymerase III is supported by the finding of Hamatake et al. (1990) that DNA polymerase III activity elutes from a Mono Q column in two overlapping peaks.

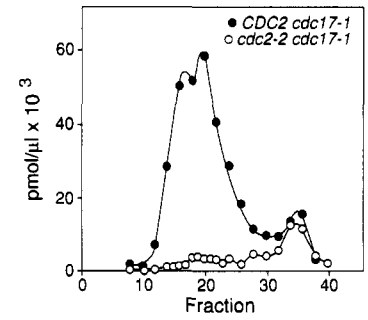

FIGURE 2: DEAE-silica high-pressure liquid chromatography of 3′–5′-exonuclease from *CDC2 cdc17-1* and *cdc2-2 cdc17-1* strains. Aliquots (0.1–1.0 μL) of fractions from the chromatographic profiles shown in Figure 1A,C were assayed for 3′–5′-exonuclease with 3′-³H-end-labeled single-stranded DNA as substrate.

To verify that the early and intermediate peaks in Figure 1 are DNA polymerase III, we documented two distinctive properties of the enzyme: 3′–5′-exonuclease activity and stimulation by PCNA. Each of these characteristics distinguishes DNA polymerase III from DNA polymerase I. A 3′–5′-proofreading exonuclease remains associated with DNA polymerase III through extensive purification (Bauer et al., 1988) and subsequent glycerol gradient sedimentation in ethylene glycol (Bauer & Burgers, 1988a,b). Figure 2 illustrates the chromatographic profile of 3′–5′-exonuclease activity from *CDC2 cdc17-1* and *cdc2-2 cdc17-1* cells, assayed in the same DEAE-silica fractions as shown in Figure 1A,C. Exonuclease activity from *CDC2 cdc17-1* cells elutes in two overlapping peaks at fractions 16 and 21, and in a late peak at fraction 36, as for DNA polymerase activity. A similar profile was found for exonuclease activity from the *CDC2 CDC17* cells analyzed in Figure 1B (data not shown). These results corroborate identification of the early and intermediate peaks in Figure 1 as DNA polymerase III and the late peak as DNA polymerase II. The latter enzyme also possesses 3′–5′-exonuclease activity and elutes from DEAE-silica after DNA polymerases III and I (Bauer et al., 1988). In the case of *cdc2-2 cdc17-1* cells, Figure 2 shows that exonuclease activity in the early and intermediate peaks is diminished relative

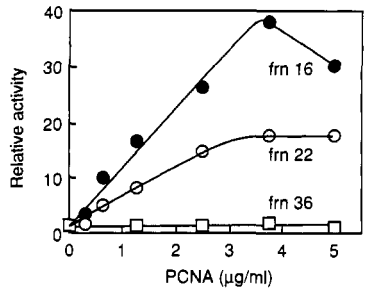

FIGURE 3: Effect of PCNA on the activity of DEAE-silica fractions. Incorporation assays with poly(dA)·oligo(dT) as template-primer were carried out according to a minor modification of the procedure of Bauer and Burgers (1988a). Assay mixtures (15 μL) contained 20 mM Tris-HCl (pH 7.8), 4% glycerol, 100 μg/mL BSA, 5 mM DTT, 8 mM $Mg(OAc)_2$, 10 μg/mL poly(dA)·$(dT)_{12}$ (53:1 by weight), 20 μM [α-^{32}P]TTP (18 910 cpm/pmol), and 1 μL of DEAE-silica eluate. Incubation was for 10 min at 37 °C. Incorporation without added PCNA was 36×10^{-3}, 15×10^{-3}, and 9.9×10^{-3} pmol for fractions 16, 22, and 36, respectively. Calf thymus PCNA (Tan et al., 1986) was a gift from Dr. C.-K. Tan.

to *CDC2 cdc17-1* cells, while that of the late peak is unchanged. A comparable profile was obtained for the exonuclease activity from the *cdc2-2 CDC17* cells analyzed in Figure 1D (data not shown). These results demonstrate that the *cdc2-2* mutation is associated with reduction in recovery of the exonuclease activity as well as the polymerase activity of DNA polymerase III. This observation is consistent with the previous work of Boulet et al. (1989) in which *cdc2-2* cells yielded neither the polymerase nor the exonuclease activities associated with DNA polymerase III.

The activity of DNA polymerase III with poly(dA)·oligo(dT) as template-primer is stimulated by PCNA from either yeast or mammalian cells, whereas the activities of DNA polymerases I and II are not enhanced (Bauer & Burgers, 1988a). The effect of calf thymus PCNA on the activity of the DEAE-silica peaks of Figure 1 is illustrated in Figure 3. The first two peaks show a 38- and 18-fold enhancement of incorporation, respectively, with a maximum effect at ca. 4 μg/mL PCNA, while the late peak eluting as DNA polymerase II is unresponsive. An 8-fold stimulation of highly purified DNA polymerase III by calf thymus PCNA has been observed previously (Bauer & Burgers, 1988a), with a maximum effect at 8 μg/mL.

The foregoing results demonstrate that the early and intermediate DEAE-silica peaks at fractions 16 and 22 are DNA polymerase III. In summary, the evidence is (1) characteristic early elution from the column, (2) elution together with 3′–5′-exonuclease activity, (3) stimulation by PCNA, and (4) poor recovery of both polymerase and exonuclease activities from *cdc2-2* cells, a phenomenon observed by others in more extreme form (Boulet et al., 1989).

Thermolability of DNA Polymerase III from cdc2-2 Mutants. To test the inference that the *cdc2-2* gene encodes temperature-sensitive DNA polymerase III, we assessed the heat stability of activity from the early and intermediate chromatographic peaks in Figure 1A–D. As shown in Figure 4, the stability at 44 °C of DNA polymerase III activity from *CDC2* strains is greater than that of activity from *cdc2-2* mutants. The heat inactivation curves for the enzymes from *CDC2* strains are linear, except for the biphasic curve for the first peak from *CDC2 cdc17-1* cells (Figure 4, upper left). The half-lives calculated from the slopes of the inactivation curves for the wild-type enzyme preparations are 14 and 4.1 min, respectively, for the two portions of the biphasic curve for the first peak from *CDC2 cdc17-1* cells (Figure 4, upper left), 5.5 min for the second peak from *CDC2 cdc17-1* cells (lower left),

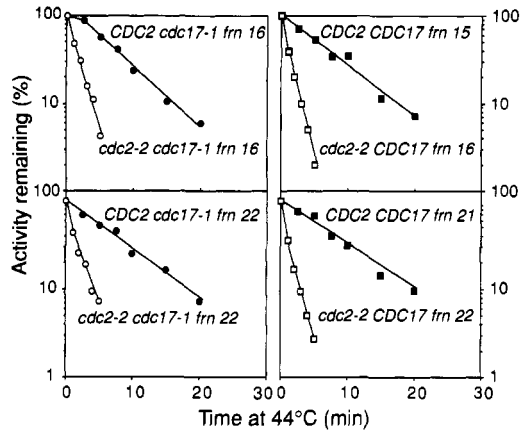

FIGURE 4: Inactivation of DNA polymerase III from *CDC2* and *cdc2-2* strains at 44 °C. Aliquots of peak fractions from the DEAE-silica profiles in Figure 1 were heated at 44 °C for increasing time prior to assay. The aliquots (0.5 and 5 μL, respectively, for the enzymes from *CDC2* and *cdc2-2* cells) were heated in assay mixture minus DNA [20 mM Tris-HCl (pH 7.8), 8 mM $MgCl_2$, 5 mM DTT, 2 mM spermidine, 4% glycerol, 0.5 mg/mL BSA, 50 μM each of dATP, dCTP, and dGTP, and 20 μM [α-^{32}P]TTP (2055–2242 cpm/pmol)], transferred to ice, and assayed immediately. Assays were started by addition of DNA and transfer to 37 °C. One hundred percent activity corresponds to 0.49–0.93 pmol of [α-^{32}P]TTP incorporated. The curves drawn are the lines of best fit calculated by linear regression analysis.

5.4 min for the first peak from *CDC2 CDC17* cells (upper right), and 6.0 min for the second peak from *CDC2 CDC17* cells (lower right). The curves for the activities in the corresponding peaks from *cdc2-2* cells are biphasic, showing an initial rapid loss followed by a slower decay. To obtain a minimum estimate of the relative stability of the mutant *cdc2-2* polymerase, half-lives were calculated for the slow decay observed between 1 and 5 min. These half-lives are 1.3 and 1.5 min, respectively, for the two peaks from *cdc2-2 cdc17-1* cells and 1.0 min for each of the two peaks from *cdc2-2 CDC17* cells. The reduced half-lives of the mutant DNA polymerase activities at 44 °C provide direct evidence of a destabilizing structural alteration conferred by the *cdc2-2* mutation. The data thereby verify encoding of DNA polymerase III by the *CDC2* gene. The structural alteration caused by the *cdc2-2* lesion also reduces stablity in vitro at 37 °C, a temperature which is permissive for growth of *CDC2* but not *cdc2-2* strains. As shown in Figure 5 (lower panel), the mutant enzyme undergoes relatively rapid denaturation at 37 °C under our assay conditions. In contrast, the mutant and wild-type activities show equal stability at 23 °C (Figure 5, upper panel), a temperature which is permissive for growth of both *CDC2* and *cdc2-2* strains. These observations, though limited in applicability by the nonphysiological conditions, exemplify a simple mechanism to account for the temperature-sensitive growth of *cdc2-2* cells.

Discussion

DNA polymerase III of *Saccharomyces cerevisiae* has been considered essential for viability, based on the strong inference that it is encoded by the *CDC2* gene (Boulet et al., 1989; Sitney et al., 1989). The present work affords direct biochemical evidence that a mutation in the *CDC2* gene causes a deleterious structural alteration in DNA polymerase III. The isolation of thermolabile DNA polymerase III from *cdc2-2* cells confirms the inferred gene/gene product relationship and verifies the important conclusion that DNA polymerase III is necessary for chromosomal replication. Moreover, thermolability of the *cdc2-2* DNA polymerase in vitro can mimic the thermosensitivity of *cdc2-2* cell growth. This finding provides a

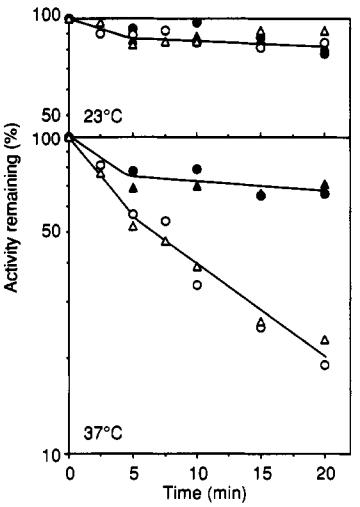

FIGURE 5: Stability of DNA polymerase III from *CDC2* and *cdc2-2* strains at 23 and 37 °C. Aliquots of peak fractions from the DEAE-silica profiles in Figure 1A,C were held at 23 or 37 °C for increasing times prior to assay. The aliquots were held in assay mixture (see legend to Figure 4) minus DNA and nucleoside triphosphates, transferred to ice, and assayed immediately. Assays were started by the addition of DNA, dATP, dGTP, dCTP, and [α-^{32}P]TTP (1917 cpm/pmol) and transfer to 30 °C. One hundred percent activity corresponds to 0.41–0.71 pmol incorporated. The amounts of DEAE-silica eluate assayed were as follows: *CDC2 cdc17-1* fraction 16 (●) 0.25 μL; *CDC2 cdc17-1* fraction 22 (▲), 0.5 μL; *cdc2-2 cdc17-1* fraction 16 (○), 5 μL; *cdc2-2 cdc17-1* fraction 22 (△), 5 μL.

demonstrable biochemical basis for the temperature-sensitive phenotype.

In our experiments, as in those of Hamatake et al. (1990), DNA polymerase III has more than one chromatographic form. We believe that proteolysis is the source of the heterogeneity we observed, despite our precautions.

DNA polymerase III copurifies with a 3′–5′-exonuclease which catalyzes proofreading in vitro (exonuclease III; Bauer et al., 1988). Whether this exonuclease activity also resides in the 125-kDa subunit encoded by the *CDC2* gene, or in a tightly associated 55-kDa subunit, is not known. Initial work indicated that the exonuclease and polymerase activities of DNA polymerase III are dissociable in glycerol gradients (Bauer et al., 1988). However, these studies have not been extended, and it has been suggested that the *CDC2* gene contains an exonuclease domain near its 5′-terminus [Morrison and Sugino, unpublished work; see also Boulet et al. (1989) and Bernad et al. (1989)]. In a previous study of *cdc2* mutants, neither DNA polymerase III nor associated exonuclease activities were recovered from *cdc2-1* or *cdc2-2* cells held at restrictive temperature prior to being processed (Boulet et al., 1989). The *cdc2-2* mutants we examined yielded reduced exonuclease activity in the DNA polymerase III region of our chromatographic profiles (Figure 2). To estimate the ratio of exonuclease to polymerase activities recovered from *CDC2 cdc17-1* and *cdc2-2 cdc17-1* cells, we summed the activities in fractions 10–26 of the profiles in Figure 3 and in Figure 1A,C (24 °C). For *CDC2* cells, the ratio of exonuclease to polymerase activities is 3.9×10^{-2}, and for *cdc2-2* cells, we calculated a ratio of 3.3×10^{-2}. The similarity of these ratios suggests that the activity in *cdc2-2* cells, like that in *CDC2* cells, is functionally associated with DNA polymerase III and is not due to an unrelated, contaminating nuclease. Moreover, the exonuclease activity from *cdc2-2* mutants is temperature-sensitive relative to the activity from *CDC2* cells, though much less affected than DNA polymerase activity (data not shown). Yet neither thermolability, even if it were extreme, nor reduced recovery of activity, such as we observed, permits assignemt of 3′–5′-exonculease activity to the 125-kDa protein encoded by the *CDC2* gene, for several reasons. Crucial among them is that thermolability and poor recovery could arise either from instability of an exonuclease domain in the *cdc2-2* protein or from failure of the defective *cdc2-2* protein to interact normally with an associated exonuclease subunit.

The identity and functions of the DNA polymerases which catalyze eukaryotic DNA replication are important current questions. Genetic and molecular biological studies have demonstrated that DNA polymerase I of *S. cerevisiae*, whose 167-kDa catalytic subunit is the product of the *CDC17* gene (Carson, 1987; Johnson et al., 1985; Lucchini et al., 1985), and its mammalian homologue DNA polymerase α (Wong et al., 1988) are required for cell viability (Johnson et al., 1985; Murakami et al., 1985). Biochemical data indicate that mammalian DNA polymerase α, together with tightly associated DNA primase, catalyzes initiation of DNA synthesis on both strands and replication of some or all of the lagging strand [see references compiled in Davey and Faust (1990)]. Early studies of *cdc2* mutants indicated that the *CDC2* gene product is required for S-phase DNA synthesis but the exact role of this gene product—now known to be DNA polymerase III—is uncertain. DNA polymerase III and its putative mammalian homologue DNA polymerase δ have been postulated to catalyze leading-strand synthesis in a reaction whose processivity depends on PCNA (Burgers, 1989; Thommes & Hubscher, 1990). A model system illustrating the functional potential of mammalian DNA polymerases α and δ—and by extension, of yeast DNA polymerases I and III—is provided by in vitro reconstitution of bidirectional DNA replication from the simian virus 40 origin (Tsurimoto et al., 1990). In this model system, DNA polymerase α is required for lagging-strand synthesis, and DNA polymerase δ is utilized for leading-strand synthesis, although disparate DNA polymerases which are processive can substitute for DNA polymerase δ. Recently, a third DNA polymerase in *S. cerevisiae*, DNA polymerase II (Wintersberger, 1978; Chang, 1977; Hamatake et al., 1990), has been reported to be necessary for viability, and initial data indicate that its essential function may also be in genome replication (Morrison et al., 1990). DNA polymerase II, which is highly processive, has been suggested as an alternative candidate for leading-strand synthesis, with DNA polymerase III catalyzing lagging-strand synthesis following initiation of both Okazaki fragments and leading-strand synthesis by DNA polymerase I and its associated primase (Morrison et al., 1990). DNA polymerase II shows weak similarity of sequence to DNA polymerases I and III (Morrison et al., 1990). It is similar catalytically to mammalian DNA polymerase ϵ (Syvaoja & Linn, 1990). The sequence of the latter is unknown.

There are available a number of *cdc2* alleles with varying phenotypic characteristics (Hartwell et al., 1973) which can be utilized to further study altered forms of DNA polymerase III and to examine the role of DNA polymerase III and its associated 3′–5′-exonuclease in S-phase DNA synthesis and in the repair of DNA damage which occurs during the G_2 phase of the cell cycle (Weinert & Hartwell, 1988). Some of these *cdc2* alleles, such as *cdc2-1* and *cdc2-2*, cause arrest during the first S phase following shift to restrictive temperature. At permissive but elevated temperature (30 °C), the *cdc2-2* mutation we examined appears to result in accumulation of incomplete or defective replication products (Hartwell & Smith, 1985) whose analysis may be revealing of the functions of DNA polymerase III. Other *cdc2* alleles, such as *cdc2-6* and *cdc2-7*, permit one or more division cycles prior

to S-phase arrest, presumably because the lesion in DNA polymerase III is less detrimental. Our confirmation of the relationship between the *CDC2* gene and the 125-kDa subunit of DNA polymerase III lends greater confidence to interpretation of future work on *cdc2* mutants and on the role of DNA polymerase III in DNA replication.

ACKNOWLEDGMENTS

We are grateful to Lee Hartwell for his invaluable help, to Barbara Garvik for construction of yeast strains, to Dr. C.-K. Tan for his generous gift of PCNA, and to Keith Cheng for his clear-sighted comments.

REFERENCES

Bauer, G. A., & Burgers, P. M. J. (1988a) *Proc. Natl. Acad. Sci. U.S.A. 85*, 7506–7510.

Bauer, G. A., & Burgers, P. M. J. (1988b) *Biochim. Biophys. Acta 951*, 274–279.

Bauer, G. A., Heller, H. M., & Burgers, P. M. J. (1988) *J. Biol. Chem. 263*, 917–924.

Bernad, A., Blanco, L., Lazaro, J. M., Martin, G., & Salas, M. (1989) *Cell 59*, 219–228.

Boulet, A., Simon, M., Faye, G., Bauer, G. A., & Burgers, P. M. J. (1989) *EMBO J. 8*, 1849–1854.

Bradford, M. M. (1976) *Anal. Biochem. 72*, 248–254.

Burgers, P. M. J. (1989) *Prog. Nucleic Acid Res. Mol. Biol. 37*, 235–280.

Carson, M. (1987) Ph.D. Thesis, University of Washington.

Chang, L. M. S. (1977) *J. Biol. Chem. 252*, 1873–1880.

Conrad, M. N., & Newlon, C. S. (1983) *Mol. Cell. Biol. 3*, 1000–1012.

Davey, S. K., & Faust, E. (1990) *J. Biol. Chem. 265*, 3611–3414.

Eki, T., Enomoto, T., Murakami, Y., Hanaoka, F., & Yamada, M. (1988) *Arch. Biochem. Biophys. 260*, 552–560.

Hamatake, R. K., Hasegawa, H., Clark, A. B., Bebenek, K., Kunkel, T. A., & Sugino, A. (1990) *J. Biol. Chem. 265*, 4072–4083.

Hartwell, L. H., & Smith, D. (1985) *Genetics 110*, 381–395.

Johnson, L. M., Snyder, M., Chang, L. M. S., Davis, R. W., & Campbell, J. L. (1985) *Cell 43*, 369–377.

Kuo, C. L., Huang, N. H., & Campbell, J. L. (1983) *Proc. Natl. Acad. Sci. U.S.A. 80*, 6465–6469.

Lucchini, G., Mazza, C., Scacheri, E., & Plevani, P. (1988) *Mol. Gen. Genet. 212*, 459–465.

Morrison, A., Araki, H., Clark, A. B., Hamatake, R. K., & Sugino, A. (1990) *Cell 62*, 1143–1151.

Murakami, Y., Yasuda, H., Miyazawa, H., Hanaoka, F., & Yamada, M. (1985) *Proc. Natl. Acad. Sci. U.S.A. 82*, 1761–1765.

Pringle, J. R., & Hartwell, L. H. (1981) in *The Molecular Biology of the Yeast Saccharomyces: Life Cycle and Inheritance* (Strathern, J., Jones, E., & Broach, J., Eds.) pp 97–142, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Sitney, K. C., Budd, M. E., & Campbell, J. L. (1989) *Cell 56*, 599–605.

Syvaoja, J., Suomensaari, S., Nishida, C., Goldsmith, J. S., Chui, G. S. J., Jain, S., & Linn, S. (1990) *Proc. Natl. Acad. Sci. U.S.A. 87*, 6664–6668.

Tan, C.-K., Castillo, C., So., A. G., & Downey, K. M. (1986) *J. Biol. Chem. 261*, 12310–12316.

Thommes, P., & Hubscher, U. (1990) *Eur. J. Biochem. 194*, 699–712.

Tsurimoto, T., Melendy, T., & Stillman, B. (1990) *Nature 346*, 534–539.

Weinert, T. A., & Hartwell, L. H. (1988) *Science 241*, 317–322.

Wintersberger, E. (1978) *Eur. J. Biochem. 84*, 167–172.

Wong, S. W., Wahl, A. F., Yuan, P. M., Arai, N., Pearson, B. E., Arai, K., Korn, D., Hunkapillar, M. W., & Wang, T. S. F. (1988) *EMBO J. 7*, 37–47.