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Structure and function of the *Saccharomyces cerevisiae* *CDC2* gene encoding the large subunit of DNA polymerase III

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***Saccharomyces cerevisiae* cdc2 mutants arrest in the S-phase of the cell cycle when grown at the non-permissive temperature, implicating this gene product as essential for DNA synthesis. The *CDC2* gene has been cloned from a yeast genomic library in vector YEp13 by complementation of a *cdc2* mutation. An open reading frame coding for a 1093 amino acid long protein with a calculated mol. wt of 124 518 was determined from the sequence. This putative protein shows significant homology with a class of eukaryotic DNA polymerases exemplified by human DNA polymerase α and herpes simplex virus DNA polymerase. Fractionation of extracts from *cdc2* strains showed that these mutants lacked both the polymerase and proofreading 3′–5′ exonuclease activity of DNA polymerase III, the yeast analog of mammalian DNA polymerase δ. These studies indicate that DNA polymerase III is an essential component of the DNA replication machinery.**

Key words: *cdc2* mutants/*CDC2* gene/DNA polymerase

Introduction

Eukaryotic DNA polymerase δ has recently been hypothesized as a component of the DNA replication fork, primarily because of its interaction with proliferating cell nuclear antigen (PCNA) [reviewed by So and Downey (1988) and Burgers (1989)]. PCNA is required for *in vitro* replication of simian virus 40 (SV40) DNA (Prelich *et al.*, 1987; Wold *et al.*, 1988) and in its absence, leading strand DNA synthesis is abolished (Prelich and Stillman, 1988). By implication, DNA polymerase δ is required for SV40 DNA replication and possibly also for carrying out leading strand DNA synthesis from chromosomal origins.

Three nuclear DNA polymerases have been identified in the yeast, *Saccharomyces cerevisiae*, by biochemical methods (Bauer *et al.*, 1988; Burgers and Bauer, 1988). Of these, DNA polymerase III is the analog of mammalian DNA polymerase δ because of its interaction with PCNA (Bauer and Burgers, 1988a). DNA polymerase III consists of two subunits of 125 and 55 kd and contains a proofreading 3′–5′ exonuclease activity but no primase activity (Bauer *et al.*, 1988; Bauer and Burgers, 1988a). DNA polymerase I in a complex with DNA primase is highly homologous to DNA polymerase α (Campbell, 1986; Pizzagalli *et al.*, 1988), whereas DNA polymerase II may be analogous to a PCNA-independent form of DNA polymerase δ (reviewed by Burgers, 1989).

Numerous temperature-sensitive mutants impeding the cell division cycle of *S.cerevisiae* (*cdc* mutants) have been isolated (Pringle and Hartwell, 1981). When grown at the non-permissive temperature, these mutants are blocked at characteristic points in the cell cycle. A large number of *cdc* mutants involved in the DNA synthesis phase of the cell cycle have been cloned and characterized. They contain the initiation genes *CDC4* and *CDC7*, genes such as *CDC8* and *CDC21* involved in nucleotide biosynthesis and DNA biosynthetic genes *CDC9* and *CDC17* (*POL1*) (Birkenmeyer *et al.*, 1984; Sclafani and Fangman, 1984; Barker *et al.*, 1985; Patterson *et al.*, 1986; Carson, 1987; Taylor *et al.*, 1987; Yochem and Byers, 1987). The *CDC2* gene has so far not been cloned and characterized. Johnston and Williamson (1978) have shown that at the non-permissive temperature, DNA synthesis of *cdc2* mutants is closely similar to that of the wild type. Nevertheless, some step of DNA replication must be defective, since *cdc2* arrested cells are unable to undergo a single round of division in the presence of the DNA synthesis inhibitor hydroxyurea after release to permissive temperature (Hartwell, 1976). More recently, Conrad and Newlon (1983), in a detailed study, have shown that different alleles of *cdc2* failed to replicate approximately one-third of the nuclear genome at 37°C; the size and the structure of the replicated DNA molecules appeared normal. *CDC2* is also required for meiosis (Schild and Byers, 1978). Finally, *cdc2* mutants are deficient for DNA replication in a permeable cell system (Kuo *et al.*, 1983).

During a recent mapping and sequencing study of the *KIN28* gene, homologous to the *CDC28* gene, and of its surrounding chromosomal locations, we have cloned and sequenced several other genes adjacent to *KIN28* (Simon *et al.*, 1986). Here we present the nucleotide sequence of the *CDC2* gene and show biochemically that this gene codes for the large subunit of DNA polymerase III. Part of this work has been presented elsewhere (Boulet *et al.*, 1988).

Results

Cloning and sequence analysis of the CDC2 gene

As we mapped gene *KIN28* (Simon *et al.*, 1986) very close to *pho2* and *cdc2* on chromosome IV (Sengstag and Hinnen, 1987), we also undertook the cloning of the *CDC2* gene. Strain YAB2 with the temperature-sensitive *cdc2-1* allele grows at 24°C, but cells cease to divide and acquire a characteristic double-cell phenotype when the temperature is shifted to 36°C (Culotti and Hartwell, 1971). The wild-type *CDC2* gene was isolated from a genomic library in vector YEp13 by complementation of the *cdc2-1* mutant. A transformant plasmid, pAB15, allowed the growth of

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Fig. 1. Gene *CDC2*. (**A**) *Hin*dIII restriction map of the regions surrounding gene *CDC2*. pCS19/3.1 is a recombinant plasmid isolated by Sengstag and Hinnen (1987) as complementing a *pho2* mutation; *KIN28* codes for a protein kinase (Simon *et al.*, 1986); *MSS2* is a gene required for the synthesis of cytochrome *c* oxidase subunit II (M.Simon and G.Faye, unpublished result). (**B**) Nucleotide sequence and deduced amino acid sequence of *CDC2*. The amino acid sequence is numbered from the first ATG codon of the open reading frame. The ACGCGT sequence found in the 5′ non-coding region of several periodically expressed genes is underlined. Two putative zinc fingers present near the C-terminal end of *CDC2* are indicated. Note that the zing finger sequence starting from residues 1005 shares more residues with the zinc finger consensus sequence described by Johnston (1987) than the zinc finger starting from residue 1052.

Fig. 2. Protein *CDC2* is a DNA polymerase. Amino acid sequence homology between yeast PolI (Pizzagalli *et al.*, 1988), human Polα (Wong *et al.*, 1988), *CDC2* and EBV DNA polymerase (Baer *et al.*, 1984). Identical residues are boxed. Related residues are indicated with points. The six conserved regions of DNA polymerases, disclosed by Wong *et al.* (1988) are underlined by wavy lines.

Fig. 3. *CDC2* belongs to the viral sub-family of DNA polymerases. Comparison of the N- and C-terminal regions of *CDC2* with the corresponding regions of the EBV and the related HCMV and HSV DNA polymerases (Kouzarides *et al.*, 1987). Identical residues are boxed. The amino acid substitution in herpes simplex mutant TP2.5 (Larder *et al.*, 1987) (residue 355) is indicated.

strain YAB2 at 36°C. pAB15 contains a 10 kb yeast DNA insert (Figure 1A). This fragment overlaps with the insert of a plasmid isolated by Sengstag and Hinnen (1987) as complementing a mutation in gene *PHO2* (Figure 1A). This demonstrates that pAB15 bears the structural gene for *CDC2* rather than a suppressor, since *cdc2* maps very close to gene *PHO2* (Arndt *et al.*, 1987). A *Hin*dIII restriction map is presented in Figure 1A. The *Hin*dIII fragments of the insert of pAB15 were subcloned in vector pEMBL31; a 3.9 kb *Hin*dIII fragment was able to complement the *cdc2-1* mutation.

The DNA sequence of the *Hin*dIII fragment was determined. An open reding frame coding for a 1093 amino acid long putative protein is present (Figure 1B). Its calculated mol. wt is 124 518 daltons. A codon bias index

of 14% indicates that *CDC2* is moderately expressed (Bennetzen and Hall, 1982; Kammerer *et al.*, 1984).

Protein CDC2 is a DNA polymerase

When we searched the protein databases for sequences homologous to the *CDC2* protein, we found several regions of homology with eukaryotic DNA polymerases. The six conserved regions identified by Wong *et al.* (1988) in human DNA polymerase α, yeast DNA polymerase I, DNA polymerases of herpes, Epstein–Barr, cytomegalo, vaccinia and adeno-2 viruses and of *Escherichia coli* bacteriophage T4 are present in the same linear spatial arrangement on protein *CDC2* (Figure 2). This strongly suggests that *CDC2* has a DNA polymerase activity. Figure 2 shows a comparison of the portions of the primary amino acid sequences of *S.cerevisiae* DNA polymerase I, human DNA polymerase α, the DNA polymerase of Epstein–Barr virus (EBV), and of the *CDC2* protein which present significant homologies (Baer *et al.*, 1984; Pizzagalli *et al.*, 1988; Wong *et al.*, 1988). The EBV DNA polymerase gives the highest homology score with *CDC2*. In fact, homology between these two proteins continues outside of the segment delimited by the six conserved regions. A comparison of *CDC2* with the DNA polymerases of three distinctly related herpes virus [human cytomegalovirus (HCMV), Epstein–Barr virus (EBV) and herpes simplex virus (HSV)] disclose four stretches of homology recognized by Kouzarides *et al.* (1987) (Figure 3). These sequences are absent in PolI and Polα. This indicates that *CDC2*, EBV, HCMV and HSV DNA polymerases belong to the same sub-family. One of the mutations of herpes simplex virus conferring anti-viral drug resistance (Larder *et al.*, 1987) was found in the second domain displayed in Figure 3: a glycine is changed to an aspartic acid; interestingly, an aspartic residue is present in the *CDC2* homologous sequence.

Proteins interacting with nucleic acids possess sequences with cysteine or histidine residues able to complex metal ions by forming tetrahedral box structures named zinc fingers. Two such putative nucleic acid binding domains are present near the C-terminal end of *CDC2* (Figure 1B). They belong to the C4 class (Johnston, 1987; Evans and Hollenberg, 1988). These two finger motifs are separated by 28 amino acids. Their putative loops are 11 and nine amino acids long. These zinc fingers most likely promote non-specific interactions with the DNA.

We have not yet determined whether gene *CDC2* is transitorily expressed during the cell cycle as are other genes involved in DNA metabolism (Breeden, 1988). However, Pizzagalli *et al.* (1988) have recently identified a sequence, ACGCGT, which is present in the 5′ non-coding region of genes *POLI(CDC17), CDC8, CDC9, CDC21* and *TOP2.* This sequence is also present 165 bases upstream of the putative start codon of *CDC2* and near a TATA box sequence (Figure 1B), indicating that gene *CDC2* may be periodically expressed during the cell cycle and possibly at G_1/S.

DNA polymerase activity in cdc2 mutants

Crude enzyme preparations were made from exponentially growing yeast cells which had been incubated at 36°C for 2 h, and fractionated by DEAE Silica gel HPLC. Figure 4A shows the chromatogram obtained with the parental wild-type strain A364a. Elution positions of DNA polymerases I, II and III are indicated. The purification procedure has

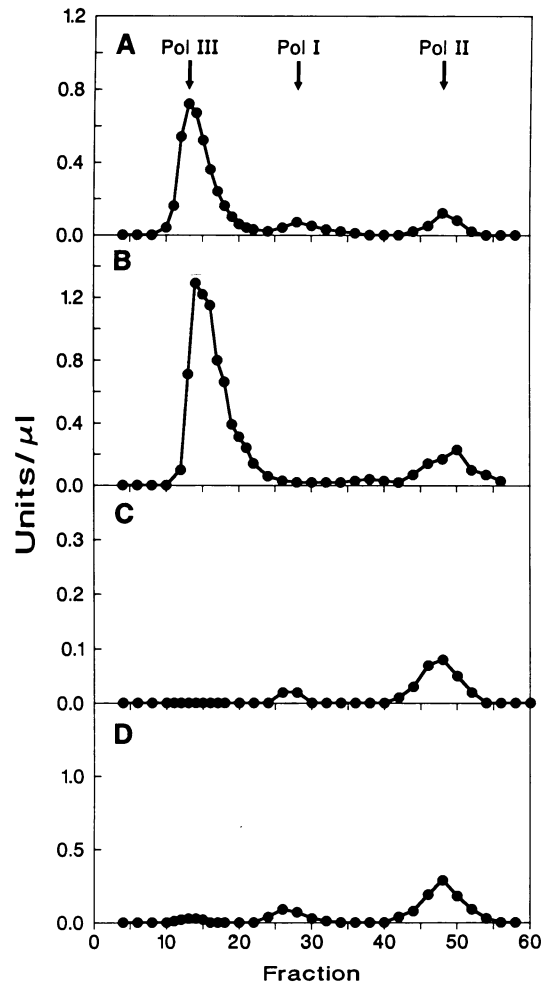

Fig. 4. DEAE-Silica gel HPLC of various extracts. The scale of the *y*-axis (DNA polymerase activity in units/µl) has been adjusted to compensate for the different amounts of protein injected onto the column: (**A**), A364a, 16 mg; (**B**), *cdc17*, 20 mg; (**C**), 370 (*cdc2-1*), 5.1 mg; (**D**), H2C2A (*cdc2-2*), 23 mg. The exact fraction numbers of elution of the DNA polymerases vary slightly between separations. Elution positions, however, coincide exactly with certain positions in the A_{280} profiles, which are identical for all separations (not shown).

been optimized to obtain a high yield of relatively unproteolyzed DNA polymerase III after cell extraction and DEAE Silica gel chromatography (see Materials and methods). In addition, however, this procedure also leads to very low yields of DNA polymerase I after DEAE Silica gel HPLC (Figure 4A) (Bauer *et al.*, 1988; Burgers and Bauer, 1988). This low DNA polymerase I signal was completely eliminated when an extract from a *cdc17* strain, mutant in the catalytic subunit of DNA polymerase I (Carson, 1987), was fractionated by HPLC (Figure 4B). However, DNA polymerase III was still present at wild-type levels, indicating that the imposition of a cell cycle block by a temperature shift did not hamper the subsequent recovery of this labile enzyme. Similarly, DNA polymerase III was fully recovered from cells which had been blocked in the S-phase by hydroxyurea treatment (data not shown; Hartwell, 1976). In contrast, no DNA polymerase III activity was recovered from a *cdc2-1* mutant (strain 370), or from a *cdc2-2* mutant (strain H2C2A) (Figure 4C and D). This was also the case when the temperature of the assay was lowered from 37 to

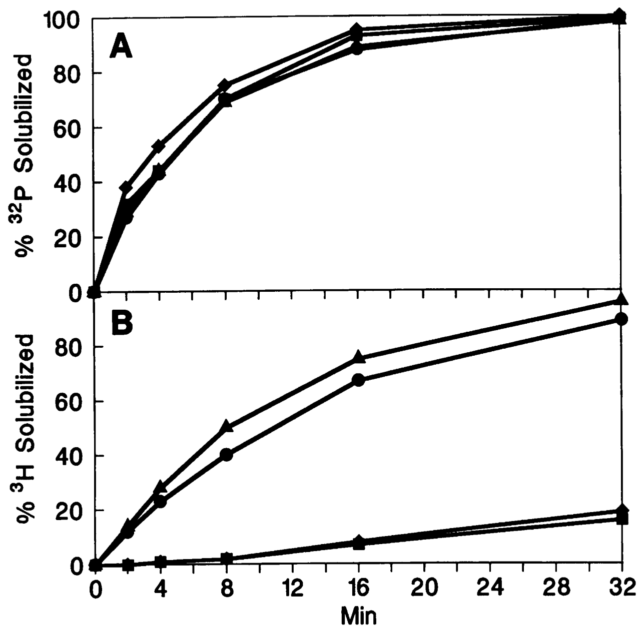

Fig. 5. *cdc2* mutants are likely deficient for the proofreading activity of DNA polymerase III. The assay measures the release of ^{32}P-label (**A**) and ^{3}H-label (**B**) from 5'[^{32}P]−[^{3}H] doubly labeled single-stranded calf thymus DNA. Each 300 μl assay contained 1.6 μg of pooled DEAE fractions (fraction III). Aliquots of 50 μl were processed at the indicated times. A364a, ●; *cdc17*, ▲; 370 (*cdc2-1*), ◆; H2C2A (*cdc2-2*), ■.

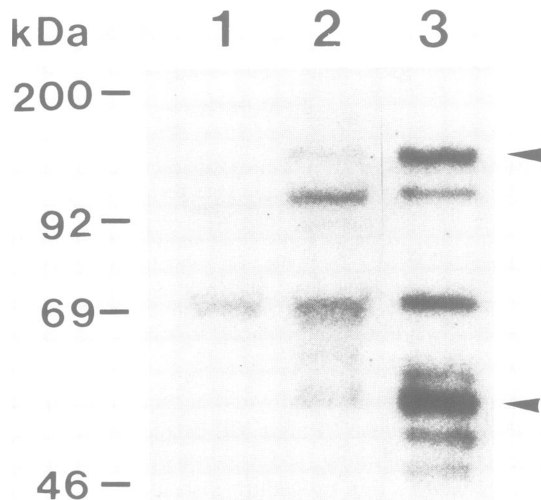

Fig. 6. Western blot using antibodies against partially purified yeast DNA polymerase III. **Lane 1**, 5 μg of *cdc2-2* fraction III; **lane 2**, 5 μg of *cdc17* fraction III: **lane 3**, 0.5 μg of yeast DNA polymerase III fraction VII (Bauer *et al.*, 1988) (see Materials and methods for details). Arrows indicate the positions of the unproteolyzed subunits of DNA polymerase III.

22°C (data not shown). For each of these four separations, a similar yield of DNA polymerase II was obtained. These results show that DNA polymersae I is deficient in a *cdc17* mutant, DNA polymerase III in *cdc2* mutants and DNA polymerase II in neither.

Exonuclease III activity in cdc2 mutants

Because of the presence of a multitude of nucleases in yeast, it is impossible to assay reliably all fractions of an HPLC separation for the absence of the proofreading 3′−5′ exonuclease activity (exonuclease III) of DNA polymerase III. Thus, we focussed only on that part of the chromatogram where DNA polymerase III elutes. A second exonuclease, exonuclease IV, coelutes with DNA polymerase III on DEAE Silica gel chromatography (Bauer *et al.*, 1988). Exonuclease IV, however, is a 5′−3′ exonuclease which is non-processive when assayed in the presence of 100 mM NaCl (Bauer *et al.*, 1988). To distinguish between exonucleases III and IV, single-stranded calf thymus DNA (500−2000 nucleotides long) was doubly labeled with ^{32}P at the 5′ end and ^{3}H at the 3′ end. DEAE-peak fractions of DNA polymerase III activity from the wild type or from the *cdc17* strain were pooled. Analogous fractions from the *cdc2* mutants were also pooled. These pools were assayed for 5′−3′ and 3′−5′ exonuclease activity by simultaneously monitoring the release of ^{32}P and ^{3}H label respectively, as acid-soluble radioactivity (Figure 5A and B). Whereas virtually identical levels of 5′−3′ exonuclease activity were present in the four DEAE pools (Figure 5A), the *cdc2-1* and *cdc2-2* DEAE pools contained low or no 3′−5′ exonuclease activity (Figure 5B). The experiments suggest that *cdc2* mutants are also deficient for exonuclease III, although the possibility that exonuclease III, when isolated from these mutant strains has altered elution position cannot be excluded.

Analysis of DNA polymerase III subunits with antisera

A rabbit antiserum has been developed against partially purified DNA polymerase III (Bauer *et al.*, 1988). In addition to the 125 and 55 kd subunits, other polymerase unrelated polypeptides, including members of the 70 kd heat-shock protein family, are also detected by this serum (Burgers and Bauer, 1988). Protein aliquots from the DEAE pools obtained from the *cdc17* strain or the *cdc2-2* strain (H2C2A) were separated by SDS−PAGE transferred to nitrocellulose membranes and probed with the antiserum (Towbin *et al.*, 1979). Both the 125 and 55 kd polypeptides present in the DEAE pool from the *cdc17* strain and in partially purified DNA polymerase III are absent from the DEAE pool from the *cdc2-2* mutant (Figure 6). As a control, the 70 kd heat-shock protein is present in all lanes.

Discussion

Several conclusions can be drawn from the experiments. First, the obvious homology of the *CDC2* protein sequence with those of a large number of eukaryotic DNA polymerases together with the absence of DNA polymerase III activity, as well as its subunit polypeptides from *cdc2* mutant extracts, show that the *CDC2* gene codes for the large subunit of this enzyme. The close agreement between the calculated mol. wt of the *CDC2* protein deduced from the sequence (124 518) and the measured size of the large subunit (125 kd; Bauer and Burgers, 1988a) is also an indication that our biochemical studies of the enzyme have been performed on the intact enzyme. Second, from the nature of the *cdc2* mutation, i.e. temperature sensitive for growth with its execution point in the S phase, we can conclude that DNA polymerase III is essential for yeast DNA replication. Third,

the presence of wild-type levels of DNA polymerase II in *cdc2* mutant extracts confirms our previous contention that DNA polymerases II and III are structurally unrelated (Burgers, 1988; Burgers and Bauer, 1988). Fourth, the absence of the 55 kd subnit in DEAE silica gel fractions of the *cdc2-2* mutant, as compared to analogous fractions of the control strain, strongly suggests that the 55 kd polypeptide is a true subunit of DNA polymerase III and not a contaminant with identical chromatographic properties. In contrast, non-polymerase polypeptides that are recognized by the impure antiserum are present at comparable levels in fractions from the *cdc17* or *cdc2-2* strain (Figure 6). Fifth, because both subunits are absent in fractions from the *cdc2-2* mutant, we cannot draw a definite conclusion about the proofreading exonuclease activity of DNA polymerase III. Although the more extensive homology with the viral DNA polymerases (EBV, HCMV and HSV), all of which carry out exonucleolytic proofreading (Kouzarides *et al.*, 1987), than with human DNA polymerase α or yeast DNA polymerase I suggests that the *CDC2* gene product has an exonuclease domain, it is still formally possible that the proofreading function is located in the 55 kd subunit. Such is the case with *E.coli* DNA polymerase III, with the polymerase activity residing in the α subunit and the exonuclease activity in the ϵ subunit (Scheuerman *et al.*, 1984; Maki and Kornberg, 1987).

With the identity of the yeast DNA polymerases clearly established, we can now draw comparisons with the mammalian enzymes in order to gain better insight into their identity, structure and function. Clearly, the original definition of DNA polymerase δ, i.e. a mammalian DNA polymerase with a proofreading $3'-5'$ exonuclease activity (Byrnes *et al.*, 1976) may need to be modified. Yeast has two analogs to DNA polymerase δ: DNA polymerase III (form δ_1) with two subunits, a non-processive enzyme which becomes very processive by protein–protein interaction with PCNA (Bauer and Burgers, 1988a,b); and DNA polymerase II (form δ_2), a very processive enzyme in the absence of PCNA (Bauer and Burgers, 1988a; Burgers, 1988). One form of DNA polymerase δ from calf thymus (form δ_1) studied extensively by So and Downey (1988) and coworkers is clearly the mammalian analog of DNA polymerase III. It consists of two subunits of 125 and 48 kd and is non-processive in the absence of PCNA but very processive in its presence. Other forms of DNA polymerase δ from calf thymus (forms δ_2) studied by Hübscher and coworkers (Focher *et al.*, 1988) and Bambara and coworkers (Sabatino *et al.*, 1988) are analogous to DNA polymerase II because of their high processivity in the absence of PCNA and lack of interaction with PCNA. Similarly, two different forms of DNA polymerase δ have been isolated from human cells (Lee and Toomey, 1987; Syvaoja and Linn, 1989) with the additional knowledge that the PCNA-independent form δ_2 from HeLa cells functions as a repair enzyme in permeable cell assays (Nishida *et al.*, 1988).

The combined evidence from mammalian cells of the proliferation dependence of PCNA synthesis, the localization of PCNA to sites of ongoing DNA replication (reviewed by Celis *et al.*, 1987) and the requirement for PCNA for leading strand DNA synthesis from the SV40 origin (Prelich and Stillman, 1988), with the evidence from yeast of the essential nature of the *CDC2* gene for DNA replication during vegetative growth and meiosis, overwhelmingly points at the importance of DNA polymerase III (mammalian DNA polymerase δ, form δ_1) as a replicative enzyme in the eukaryotic cell. DNA polymerase II (mammalian DNA polymerase δ, form δ_2), on the other hand, may function as a repair enzyme.

Materials and methods

Strains

The yeast strains were: A364a (*MATa, ade1, ade2, gal1, his7, lys2, tyr1* and *ura1*), *cdc17* (as A364a, but *cdc17-1*) and 370 (as 364a, but *cdc2-1*) from L.Hartwell (University of Washington; H2C2A (*MATa, his7, ura1* and *cdc2-2*) from J.Rosamond (University of Manchester); YAB2 (MATα, *cdc2-1 leu2, trp1, ade, ura* and *his7*), this work.

The *E.coli* strains used in cloning procedures were MC1066, JM83 and JM101 (Casadaban *et al.*, 1983; Yanisch-Perron *et al.*, 1985).

Vectors

The *E.coli*/yeast shuttle vectors used were YEp13 (Broach *et al.*, 1979) and pEMBL31 (Baldari and Cesarini, 1985). DNA fragments to be sequenced were cloned into pTZ18 and 19 (Pharmacia).

Cloning and sequencing

Restriction analysis and cloning in *E.coli* were carried out as described in Maniatis *et al.* (1982). Yeast transformation was according to Beggs (1978). A yeast genomic library in vector YEp13 (Nasmyth and Tatchell, 1980) was used to transform strain YAB2. Selection for $CDC2^+$ transformants was accomplished by culturing transformed spheroplasts on media lacking leucine, overnight at 24°C before their transfer to 36°C. One transformant, pAB15, was obtained. The *Hin*dIII fragments of the inset of pAB15 were subcloned in the vector pEMBL31. The resulting plasmids were used to transform strain YAB2. A 3.9 kb *Hin*dIII fragment was able to complement the *cdc2-1* mutation. The *Hin*dIII fragment was cleaved into two fragments by *Hpa*I. These *Hin*dIII–*Hpa*I fragments were cloned in pTZ18 and -19. Subclones obtained according to the strategy of Lin *et al.* (1983) were submitted to sequence analysis by the Sanger dideoxy sequencing technique (Sanger *et al.*, 1977).

Preparation of extracts

Yeast cells were grown aerobically in 3 l of YPD medium at 24°C. At OD_{660} = 1 (0.4 for strain 370), the temperature of the medium was increased to 36°C and the flasks were further shaken at 36°C for 2 h. Phenylmethylsulfonyl fluoride (PMSF) and leupeptin were then added to 1 mM and 10 μM respectively. The cells were then chilled and harvested for 5 min at 4000 r.p.m. in a Sorvall GS-3 rotor, and resuspended in an equal volume of ice-cold lysis buffer (0.2 M Tris–HCl, pH 8.1, 10% glycerol, 8 mM EDTA, 0.1% Brij 58, 6 mM DTT, 10 mM $NaHSO_3$, 1 mM PMSF, 10 μM pepstatin A, 20 μM leupeptin, 10 mM benzamidine). Cells were broken in a bead beater (Bio-Spec), with ice-water cooling, by blending with glass beads (0.4–0.5 mM, 15 ml) for 3 × 1 min interspersed with 1-min cooling periods. All further steps were carried out at 0–4°C. The lysate was poured off and the beads washed with 2 × 5 ml of lysis buffer diluted with an equal volume of water. To each milliliter of lysate were added 47 μl of saturated ammonium sulfate (at 0°C) followed by 35 μl of 10% v/v polymin P. After stirring for 15 min, the lysate was centrifuged for 30 min at 18 000 r.p.m. in an SS34 rotor. To the supernatant was added 0.28 g/ml of solid ammonium sulfate, and the suspension was stirred for 1 h. The precipitate was collected at 18 000 r.p.m. for 30 min and dissolved in 3 ml of DE buffer (25 mM potassium phosphate, pH 7.0, 10% v/v glycerol, 2 mM EDTA, 5 mM DTT, 10 mM $NaHSO_3$, 5 μM leupeptin, 2 mM benzamidine, 2 μM pepstatin A, 1 mM PMSF) and dialyzed against 400 ml of the same buffer for 4 h. After two clearing spins at 18 000 r.p.m. for 20 min each, to remove particulate matter, the enzyme fraction was analyzed by HPLC.

DEAE Silica gel HPLC on a 25 × 4.6 cm Synchrom AX1000 column was exactly as described (Bauer *et al.*, 1988), except that benzamidine at 0.5 mM and pepstatin at 2 μM were the only protease inhibitors added to the buffers, and fractions of 0.8 ml were collected. The three peak fractions containing DNA polymerase III activity from the A364a and *cdc17* HPLC separations and analogous fractions from 370 (*cdc2-1*) and H2C2A (*cdc2-2*) separations were pooled and used for exonuclease assays and Western blotting (Fraction III).

Enzyme assays

DNA polymerase assays using activated calf thymus DNA as a substrate and exonuclease assays using doubly labeled single-stranded calf thymus

DNA as a substrate were as described (Bauer *et al.*, 1988). The exonuclease assays contained, in addition, 100 mM NaCl.

Other methods

All genetic procedures and growth media were as described by Sherman *et al.* (1983). Protein-immuno blots were performed as described (Towbin *et al.*, 1979). ^{125}I-labeled protein A was used to detect bound antibodies. Protein concentrations were determined according to Bradford (1976).

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While this work was in press, a report was published [Sitney,K.C., Budd,M.E. and Campbell,J.L. (1989) *Cell*, **56**, 599–605], in which it was shown that *cdc2* mutants are deficient for the polymerase activity of DNA polymerase III. The results of these authors are in complete agreement with those published here.