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Molecular Characterization of Cell Cycle Gene *CDC7* from *Saccharomyces cerevisiae*

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The product of the *CDC7* gene of *Saccharomyces cerevisiae* appears to have multiple roles in cellular physiology. It is required for the initiation of mitotic DNA synthesis. While it is not required for the initiation of meiotic DNA replication, it is necessary for genetic recombination during meiosis and for the formation of ascospores. It has also been implicated in an error-prone DNA repair pathway. Plasmids capable of complementing temperature-sensitive *cdc7* mutations were isolated from libraries of yeast genomic DNA in the multicopy plasmid vectors YRp7 and YEp24. The complementing activity was localized within a 3.0-kilobase genomic DNA fragment. Genetic studies that included integration of the genomic insert at or near the *CDC7* locus and marker rescue of four *cdc7* alleles proved that the cloned fragment contains the yeast chromosomal *CDC7* gene. The RNA transcript of *CDC7* is about 1,700 nucleotides. Analysis of the nucleotide sequence of a 2.1-kilobase region of the cloned fragment revealed the presence of an open reading frame of 1,521 nucleotides that is presumed to encode the *CDC7* protein. Depending on which of two possible ATG codons initiates translation, the calculated size of the *CDC7* protein is 58.2 or 56 kilodaltons. Comparison of the predicted amino acid sequence of the *CDC7* gene product with other known protein sequences suggests that *CDC7* encodes a protein kinase.

Use of the budding yeast *Saccharomyces cerevisiae* as a model for studies on the eucaryotic cell cycle relies heavily on temperature-sensitive mutations in cell division cycle (*CDC*) genes (32, 35). Characterization of these mutants has led to the formulation of a model in which progression through the cell cycle is determined by a set of interrelated pathways, each organized as a dependent sequence of events requiring the action of specific gene products. One such pathway, operating late in the G1 phase of the cell cycle, requires the function of the *CDC7* gene product (12). Cells carrying a thermosensitive lesion in the *CDC7* gene arrest at the restrictive temperature as budded cells with separated spindle-pole bodies but without an elongated spindle apparatus and without initiating DNA synthesis (5, 12). Upon return to permissive conditions *cdc7* cells are able to enter the S phase and subsequently complete a round of DNA synthesis without further protein synthesis (14).

In contrast to the requirement for *CDC7* function to initiate mitotic DNA synthesis, premeiotic DNA replication occurs normally in *cdc7* homozygous diploids under the restrictive condition (43). However, these diploids fail to form a synaptonemal complex, to show commitment to genetic recombination, or to form ascospores (40). Thus, although *cdc7* strains are defective in both mitotic and meiotic cell cycles, the lesion appears to affect each pathway in a quite distinct manner. In addition to having roles in the mitotic and meiotic pathways, the *CDC7* gene product has been implicated in DNA repair as a member of the *RAD6* epistasis group, since strains carrying a *cdc7* mutation show almost no mutagenic repair in response to a variety of damaging agents (31).

To elucidate the role of the *CDC7* gene product in the various cellular functions in which it is implicated and to determine whether differential expression of the *CDC7* gene is involved with its cell cycle functions, we and others (24) have begun a molecular analysis of the *CDC7* gene. In this paper we describe the cloning of the *CDC7* gene, the characterization of its transcriptional product, the nucleotide sequence of the gene, and the regions of homology between the predicted protein products of the *CDC7* and *CDC28* genes.

MATERIALS AND METHODS

Strains and media. *Escherichia coli* HB101 (F^- *thi leu pro hsdR hsdM recA endI*) and HW87 [F^- Δ(*araD139-leu*) *lacX74 galK hsdR rpsL srb recA*] were used as hosts for the routine maintenance and propagation of plasmids. Bacterial cultures were grown in L broth or supplemented M9 medium (26); when necessary, ampicillin was added to media to a final concentration of 50 μg/ml.

Yeast cells were grown in either yeast extract-peptone-glucose (YPD) or supplemented synthetic minimal medium (42). *S. cerevisiae* strains used in this work were SB155 (*MAT*α *trp1 cdc7-1*), SB158 (*MAT*α *trp1 ura3-52 cdc7-1*), S288C (*MAT*α), 136 (*MAT*α *trp1 ura3-52 leu2-3,112 cdc7-2*), 142 (*MAT***a** *trp1 ura3-52 leu2-3,112 cdc7-4*), 208 (*MAT*α *ura3-52 leu2-3,112 cdc7-1*), and 209 (*MAT***a** *ura3-52 leu2-3,112 cdc7-3*). Strains containing the *cdc7* alleles were constructed by standard genetic procedures (42) from the original *cdc7* isolates obtained from L. H. Hartwell (13).

Preparation of DNA. Plasmid DNA was extracted from cultures of *E. coli* on an analytical scale by alkaline lysis (2) and purified from larger cultures by CsCl-ethidium bromide

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TABLE 1. Marker rescue analysis of four *cdc7* mutant alleles

cdc7 allele	Control reversion[b] ($\times 10^{-5}$)	pRS5 reversion ($\times 10^{-5}$)	Relative increase[c]
7-1	0.23	1.6	7.0
7-2	0.58	2.8	4.8
7-3	1.50	180	120
7-4	1.50	150	100

[a] Strains were transformed with control plasmid pRS3 ($CDC7^+$) or with plasmid pRS5 which contains the 5.2-kb DNA fragment located at the left of the *Bam*HI site in the *CDC7*-complementing region of pRS3 (Fig. 1; Materials and Methods).

[b] Reversion frequencies were not significantly different for strains without plasmid and the same strains harboring the vector plasmid or pRS4.

[c] The increase in the frequency of reversion produced by strains harboring plasmid pRS5.

density gradient centrifugation after detergent lysis (16). DNA was prepared from rapid lysates of yeast transformants essentially as described by Naumovski and Friedberg (30), while genomic DNA was prepared from *S. cerevisiae* S288C by the method of Cryer et al. (8). Single-stranded viral DNA was prepared from recombinant M13mp8 and M13mp9 bacteriophage by phenol extraction of polyethylene glycol-precipitated phage particles (25).

Characterization of DNA. Restriction enzymes, T4 DNA ligase, DNA polymerase, and Klenow fragment were purchased from New England BioLabs, Inc. (Keene, N.H.) or Bethesda Research Laboratories, Inc. (Gaithersburg, Md.) and were used according to the recommendations of the manufacturers. DNA was nick translated as described by Rigby et al. (37) with [α-^{32}P]dATP supplied by Amersham International and New England Nuclear Corp. (Boston, Mass.).

Yeast genomic libraries. Pools of recombinant plasmids containing quasi-random fragments of yeast genomic DNA (strain S288C) were prepared essentially as described previously, using the vector YRp7 (29, 32, 45). The Carlson YEp24 genomic library (6) was obtained from D. Koshland and D. Botstein.

DNA sequencing. Nucleotide sequences were determined by the dideoxy chain-termination method (38), for which DNA fragments to be sequenced were cloned into M13mp8 or M13mp9 (25). Reaction products were resolved by electrophoresis through 6% acrylamide gels under denaturing conditions and detected by autoradiography overnight at room temperature.

Genetic techniques. Transformation of *E. coli* by plasmid DNA was by the method of Warren and Sherratt (48). *S. cerevisiae* strains were transformed after spheroplasting (42). Construction of diploids, sporulation, and dissection of yeast spore tetrads were performed by standard genetic techniques (42).

Marker rescue analysis. Plasmid pRS4 was produced from pRS3 by digestion with *Bam*HI and subsequent recircularization and self-ligation. Plasmid pRS5 was produced by subcloning the 5.2-kilobase (kb) *Bam*HI fragment from pRS3 into the *Bam*HI site of vector YEp13 (4). All *cdc7* mutant strains were complemented by plasmid pRS3 (7.4 kb) but not by plasmids pRS4 (2.2 kb) and pRS5 (5.2 kb). For the marker rescue analysis, Ura^+ (or Leu^+) transformant colonies selected at 23°C were picked, and the cells were plated at 23 and 36°C on fully supplemented medium (YPD). The frequency of reversion was calculated as the number of colonies produced at the restrictive temperature (36°C) divided by the total number of colonies which harbored the plasmid at the permissive temperature (23°C). Usually about 80% of the cells contained the plasmid under these conditions. Values in Table 1 are averages of five trials on each of two independent transformants.

Preparation of RNA from *S. cerevisiae*. Total RNA was prepared from 50-ml cultures of exponentially growing yeast cells at a density of 1×10^7 to 2×10^7 cells per ml as follows. Cells were harvested from YPD, washed once in ice-cold water, and suspended in 3 ml of ice-cold breaking buffer (0.1 M Tris [pH 7.5], 0.1 M LiCl, 0.1 mM EDTA, 0.5 mg of heparin per ml). An equal volume of glass beads was added, and the cells were broken by vortexing for four periods of 30 s with cooling on ice in between. Sodium dodecyl sulfate was then added to a final concentration of 0.5%, and the aqueous phase was extracted with an equal volume of phenol-chloroform (1:1) by vortexing for 10 s. After centrifugation at $15,000 \times g$ for 5 min at 4°C the aqueous phase was removed, and the phenol-chloroform phase was reextracted with 3 ml of breaking buffer containing 0.5% sodium dodecyl sulfate and 0.1 M Tris (pH 9.0). The aqueous phases were then pooled, and RNA was precipitated with 2 volumes of ethanol at −20°C for 1 h. The RNA was collected by centrifugation, washed in 95% ethanol, dried, and suspended in 2 ml of 1% potassium acetate. Two volumes of ethanol were added, and the RNA was stored in this state at −20°C.

RNA transcript identification. RNA was denatured by treatment with glyoxal, fractionated on 1.4% agarose gels, and transferred to nitrocellulose filter paper by methods described previously (46). The filters were prehybridized for 6 to 16 h at 42°C, hybridized to the appropriate ^{32}P-labeled probes (5×10^7 to 2×10^8 cpm/μg) for 16 to 24 h, and then washed as described previously (46). After drying, the filters were exposed to X-ray film for 1 to 4 days at −70°C with Cronex Lightning-Plus intensifying screens.

RESULTS

Isolation of DNA fragments able to complement the *cdc7* mutation. Genomic DNA fragments capable of complementing the temperature-sensitive *cdc7* mutation were isolated from a library of random *Sau*3A fragments of *S. cerevisiae* DNA in the vector YEp24. Plasmid YEp24 consists of pBR322 carrying the yeast *URA3* gene and the 2μm plasmid replication origin (3); the recombinant plasmids of the library replicate autonomously in yeasts and express the *URA3* gene. A *ura3 cdc7* strain of *S. cerevisiae* (strain 136) was transformed with the library, and Ura^+ transformants were selected by allowing spheroplasts to regenerate in agar medium lacking uracil at 23°C for 5 days. The required URA^+ TSM^+ transformants were identified by replica plating URA^+ transformants to fresh agar medium lacking uracil and incubating at 36°C. Two URA^+ TSM^+ transformants of *S. cerevisiae* were obtained in this way, and total nucleic acid extracted from each of these was used to transform *E. coli* HB101 to ampicillin resistance. Isolation and characterization of the transforming plasmids indicated that they carried nonidentical but overlapping genomic fragments. These plasmids were designated pRS3 and pRS7 (Fig. 1).

Genomic fragments capable of complementing *cdc7* were also isolated independently from a *Sau*3A genomic library in the vector YRp7. This vector carries the yeast *TRP1* gene and a putative chromosomal replication origin that permits autonomous replication in yeasts (45). In this case, a *trp1 cdc7* strain (strain SB155) was transformed with the library, and transformants that grew at 36°C on agar medium lacking tryptophan were selected directly. Total DNA was extracted from two such yeast transformants and was used to trans-

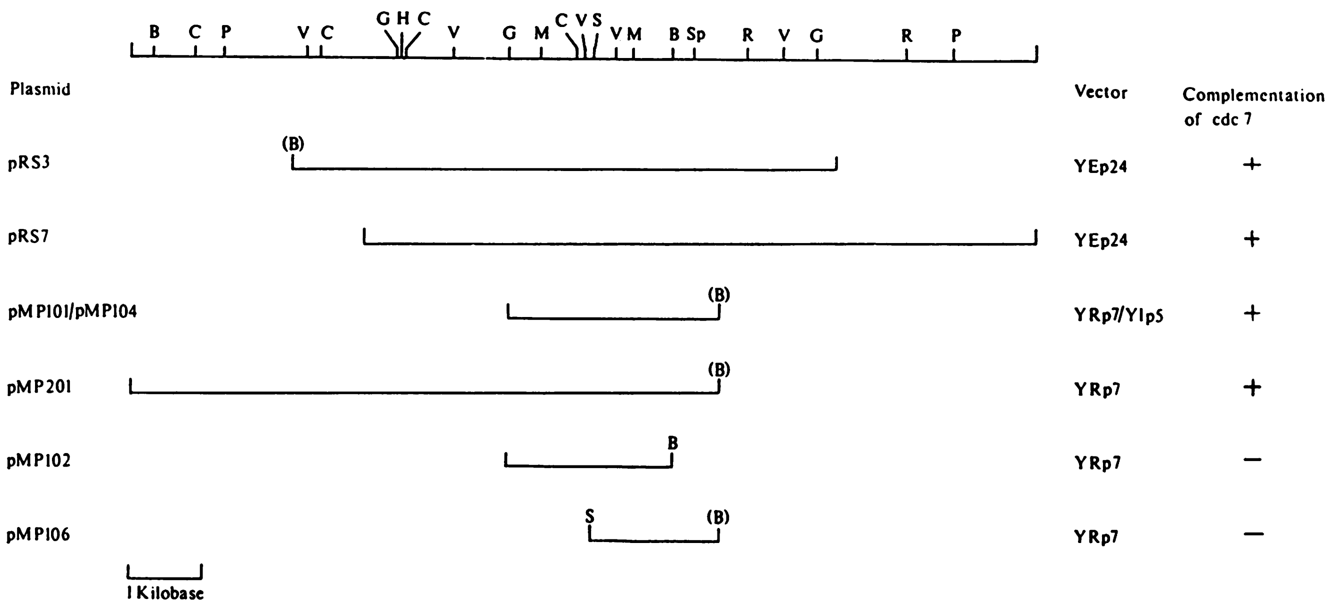

FIG. 1. Restriction map of *cdc7*-complementing clones and subcloned DNA fragments. The upper line represents a composite of the data for individual clones. Abbreviations: +, able to complement *cdc7*; −, unable to complement *cdc7*; (B), new *Bam*HI sites created at the vector-insert junctions; B, *Bam*HI site; C, *Cla*I site; G, *Bgl*II site; H, *Hin*dIII site; M, *Mlu*I site; P, *Pst*I site; R, *Eco*RI site; S, *Sac*I site; Sp, *Sph*I site; V, *Eco*RV site.

form *E. coli* HB101 to ampicillin resistance. The plasmids recovered in this manner were designated pMP101 and pMP201 (Fig. 1).

Restriction enzyme mapping showed that the genomic fragments in each of the complementing plasmids were different. pRS3 and pRS7 contained genomic fragments of 8.2 and 10.9 kb, respectively, while pMP101 and pMP201 carried inserts of 3.0 and 8.1 kb, respectively. There was significant overlap between the four cloned genomic fragments, and in particular, the insert of pMP101 was found to be contained within the three other plasmids which complemented *cdc7* (Fig. 1). Southern analysis of chromosomal DNA showed that the distribution of sites in the cloned DNA fragments of pRS3 and pRS7 was the same as at the homologous region of the genome of *S. cerevisiae* (data not shown). Figure 1 shows a composite map of restriction sites within this region of the genome.

Cloned fragments contain the authentic *CDC7* gene. Although the isolation of homologous genomic fragments capable of complementing *cdc7* from two independently constructed genomic libraries suggests that the cloned fragments carry the *CDC7* gene itself, the observation that the effects of many conditional lethal mutations in yeasts can be alleviated by extragenic suppression (20) made it necessary to demonstrate further that the plasmids contained the authentic *CDC7* gene within the cloned genomic fragment. The genomic fragment from pMP101 was first subcloned into the vector YIp5, which carries the yeast *URA3* gene in PBR322. Since this recombinant plasmid, designated pMP104 (Fig. 1), is unable to replicate autonomously in yeasts (39), stable yeast transformants arise only if the plasmid integrates into a chromosome by homologous recombination (15). Thus, plasmid pMP104 was used to transform a *ura3 cdc7* strain (strain SB158), and URA^+ TSM^+ transformants were selected directly. Since the *ura3-52* mutation appears to preclude recombination between this locus and the vector $URA3^+$ sequences (39), integration would be likely to occur at the chromosomal site homologous to the genomic fragment of pMP104.

A standard genetic cross was then performed to determine whether pMP104 had indeed integrated at the *CDC7* locus, which is tightly linked to both the *TRP1* locus (3.8 centimorgans) and the centromere of chromosome IV (27, 28). One integrant (*MAT*α *his ade trp1 ura3-52 cdc7-1::URA3*$^+$ TSM^+) was crossed with SB107 (*MAT***a** *leu2-3 ura3-1*); tetrads were dissected and scored for TSM, URA, and TRP phenotypes. In 25 of 25 tetrads, TSM^+ segregated 4+:0−. Twenty-four tetrads showed parental ditype, and one showed tetratype segregation for URA and TRP, indicating that the integrated sequences are about 2 centimorgans from *TRP1* (33). We conclude that plasmid pMP104 had integrated at or near the *CDC7* locus. Therefore, plasmids pMP101 and pMP104 carry a genomic insert which is most likely the *CDC7* gene.

Location of *CDC7* gene within cloned DNA fragments. The observation that the genomic fragment of pMP101 is common to the cloned fragments of pRS3, pRS7, and pMP201 suggests that the complete *CDC7* gene is contained entirely within this 3.0-kb segment of DNA. To localize the functional *CDC7* gene more precisely, we prepared two subclones of the genomic DNA insert of pMP101.

In the first of these, the 650-base-pair (bp) fragment between the new *Bam*HI site created at the vector-insert junction and the natural *Bam*HI site was removed by digesting pMP101 to completion with *Bam*HI and religating after dilution. The resulting plasmid, pMP102 (Fig. 1), transformed *S. cerevisiae* to TRP^+ at high frequency at 23°C but was unable to complement the *cdc7-1* mutation, suggesting that the *CDC7* gene extends to the right of the authentic *Bam*HI site of pMP101 as drawn in Fig. 1.

Plasmid pMP106 was constructed by digesting pMP101 to completion with *Cla*I and *Sac*I, removing single strands with

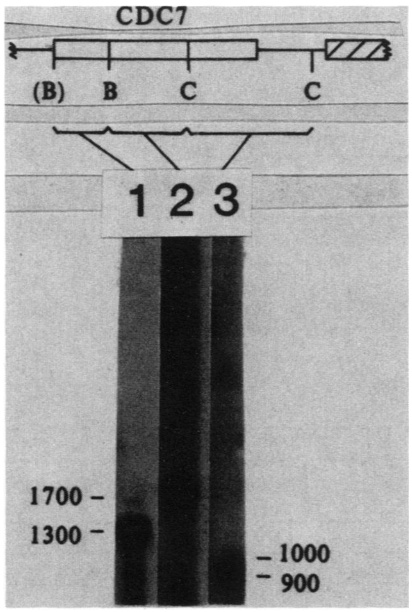

FIG. 2. Identification of *CDC7* RNA transcript. Three 15-μg samples of RNA were denatured with glyoxal, resolved by agarose gel electrophoresis, and transferred to nitrocellulose as described by Thomas (46). The nitrocellulose membrane was cut into strips, each of which was hybridized to a unique ^{32}P-labeled DNA probe derived from pMP101. The location of each of these DNA probes within pMP101 is shown schematically, together with the approximate location of the *CDC7* gene. The open box represents the cloned yeast genomic fragment carrying *CDC7*; the thin line represents pBR322 vector sequences; and the hatched box represents *TRP1 ARS1* vector sequences. Restriction site abbreviations are as described in the legend to Fig. 1. The map orientation in this figure is reversed compared with that in Fig. 1. Lanes: 1, 0.65-kb new *Bam*HI-*Bam*HI DNA probe; 2, 1.3-kb *Bam*HI-*Cla*I DNA probe; 3, 1.5-kb *Cla*I-*Cla*I DNA probe. Chain lengths of the RNA transcripts homologous to each probe were determined by comparison with the migration of nucleic acids of known size (not shown). Numbers show number of nucleotides.

S1 nuclease, and religating. This removed about 1.6 kb comprising about 1.3 kb of the pMP101 genomic fragment and 350 bp of vector sequences. Like pMP102, pMP106 also transformed *S. cerevisiae* to *TRP1*$^+$ at high frequency at 23°C but failed to complement *cdc7*, suggesting that *CDC7* extends to the left of the unique *Sac*I site of pMP101 (Fig. 1).

Since plasmid-chromosome recombination in yeasts is an extremely sensitive method for fine-structure genetic analysis (9), we determined whether the putative *CDC7*$^+$ DNA sequences could recombine with the *CDC7* locus in marker rescue experiments. Noncomplementing fragments of *CDC7* DNA were subcloned onto high-copy-number yeast 2μm plasmids and transformed into strains with different *cdc7* mutant alleles. Only a plasmid containing the correct wild-type DNA sequences will be able to rescue, by homologous recombination, a given mutant allele. Table 1 shows that plasmid pRS5, containing the leftward 5.2-kb *Bam*HI fragment of pRS3 (Fig. 1; Materials and Methods), could rescue the four mutant alleles *cdc7-1* through *cdc7-4*. On the other hand, neither plasmid pRS4 which contains the 2.2-kb DNA fragment to the right of the *Bam*HI site nor the vector alone produced any significant increase in the reversion rate. Therefore, by plasmid-chromosome deletion mapping we showed that the cloned DNA sequences must contain the *CDC7*$^+$ gene and that all four *cdc7* alleles map to the left of the *Bam*HI site (Fig. 1). Although the four *cdc7* alleles were isolated as independent clones (12), it is possible that they represent the same mutation. However, it is evident from the data presented in Table 1 that at least *cdc7-1* and *cdc7-2* must be different from *cdc7-3* and *cdc7-4* because the frequency of recombination with pRS5 was very different (about 20-fold). Further deletion mapping revealed that at least three of the alleles, *cdc7-1*, *-2*, and *-3*, are located between the *Bam*HI and *Eco*RV sites on a 0.77-kb DNA fragment (data not shown).

Identification of *CDC7* RNA transcript. RNA was prepared from wild-type *S. cerevisiae* and was denatured by treatment with glyoxal (46). Three equal samples of RNA were fractionated by electrophoresis through 1.5% agarose gels, and the RNA was transferred to nitrocellulose as described previously (46). The nitrocellulose filter was divided into three separate strips, and each was hybridized with a nick-translated DNA probe as indicated in Fig. 2.

Four different RNA species were identified as being homologous to regions of the cloned DNA around the *CDC7* gene. The 1.3-kb *Bam*HI-*Cla*I fragment hybridized to a single RNA species of 1,700 nucleotides (Fig. 2, lane 2). In other experiments, the 0.77-kb *Bam*HI-*Eco*RV fragment which could rescue three *cdc7* alleles (see above) also hybridized to a 1,700-nucleotide RNA which is poly(A)$^+$ (data not shown). From these data we conclude that the 1,700-nucleotide RNA is the transcript of the *CDC7* gene, which is consistent with a previous report (24). The 650-bp DNA fragment between the *Bam*HI site and the new *Bam*HI site at the vector-insert boundary also hybridized to a 1,700-nucleotide RNA species (Fig. 2, lane 1), which is consistent with the results of a subcloning experiment that showed that at least part of this region was necessary for *CDC7* expression. The 650-bp DNA probe also hybridized to a 1,300-nucleotide RNA that we presume to be the transcript of a gene adjacent to *CDC7*. The 1.5-kb *Cla*I-*Cla*I fragment hybridized to two RNA transcripts of about 1,000 and 900 nucleotides (Fig. 2, lane 3). We did not detect hybridization between this fragment and the 1,700-nucleotide RNA, which supports the view that regions to the left of the *Cla*I site in pMP101 are not essential for complementation of the *cdc7* mutation. However, we cannot exclude the presence of a short region of homology between the 1.5-kb *Cla*I-*Cla*I fragment and the putative *CDC7* transcript.

Nucleotide sequence of the *CDC7* gene. The nucleotide sequence of the genomic fragment in the region of the *CDC7* gene was determined initially from the new *Bam*HI site at one vector-insert junction in pMP101 to the *Cla*I site and subsequently to a point 110 nucleotides beyond *Cla*I, using the strategy shown in Fig. 3A. When possible, restriction subfragments of the genomic clone were sequenced directly. Complete sequencing, however, required the analysis of randomly isolated subclones of *Rsa*I and *Sau*3A fragments.

The location of all potential termination codons in this region is shown in Fig. 3B, from which it can be seen that only one of the six possible reading frames contains a long stretch (507 triplets) uninterrupted by stop codons. The size of this long reading frame (1,521 bases) is compatible with the size of the *CDC7* mRNA transcript (~1,700 bases; Fig. 2). Moreover, the reading frame contains both the *Bam*HI and *Sac*I sites, consistent with the inability of clones terminating at these sites to complement the *cdc7* mutation (Fig. 1). This reading frame also contains the 0.77-kb *Bam*HI-*Eco*RV fragment which can rescue three *cdc7* alleles (see above). From these data we conclude that this open reading frame encodes the *CDC7* gene product.

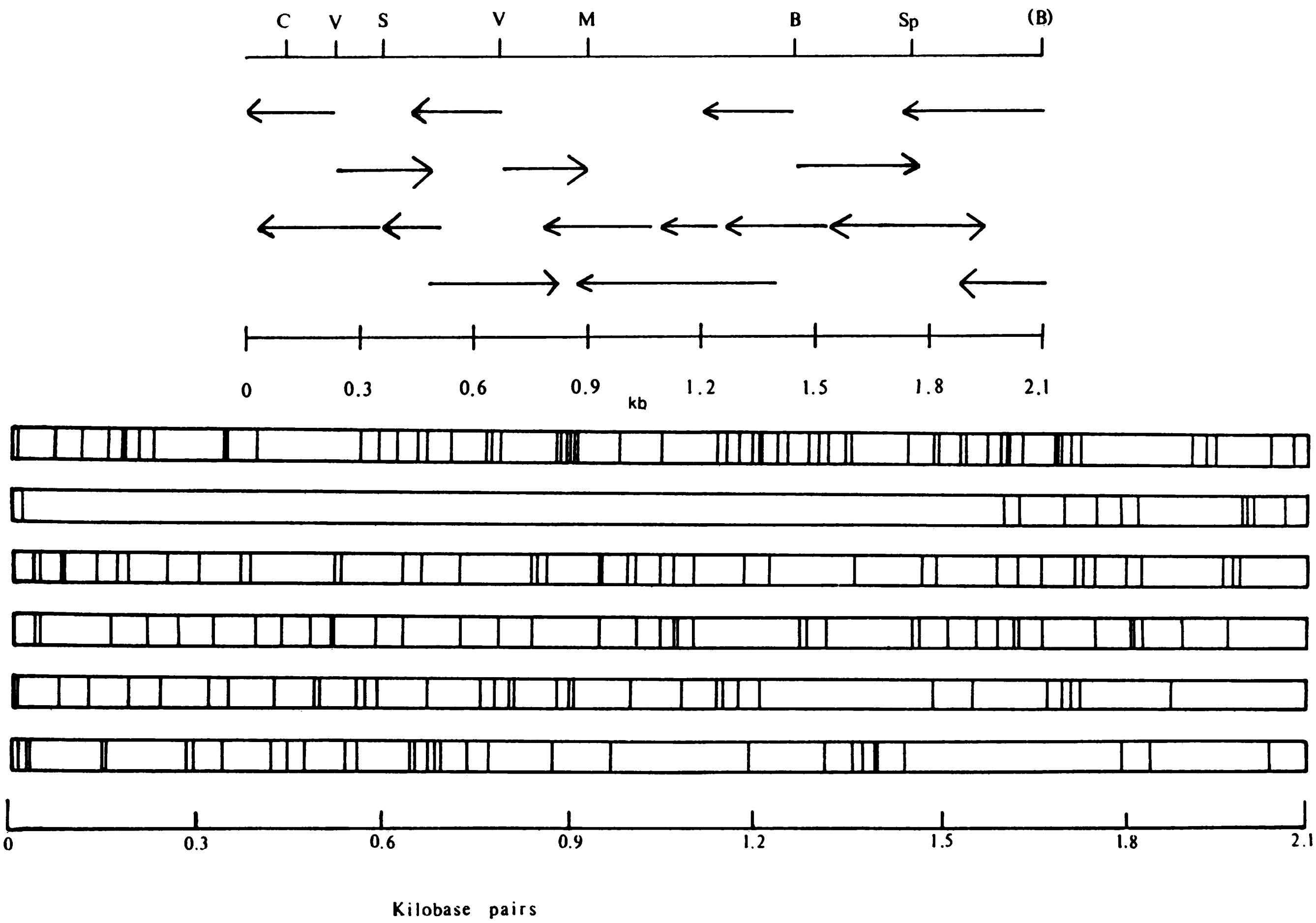

FIG. 3. Strategy for nucleotide sequencing and the distribution of translation termination codons within the DNA sequence of the *CDC7* gene. (Top) The upper line shows the restriction enzyme sites in the region of the *CDC7* gene. The upper two rows of arrows represent the direction and extent of sequence information derived from fragments obtained by using these sites; the lower two rows of arrows represent sequence information obtained by shotgun cloning *Sau*3A and *Rsa*I subfragments. (Bottom) The locations of translation termination codons in each of the six possible reading frames are shown by vertical lines.

Figure 4 shows the nucleotide sequence of a 2.1-kb region around the long open reading frame, together with a predicted amino acid sequence for the translational product. The nucleotide A of the first in-phase ATG codon within the open reading frame is numbered 1. However, it is not clear that this ATG codon is the translational initiation site, since there is a second in-frame ATG codon at nucleotides 55 to 57 that could act as the initiator (Fig. 4). Depending on which of these codons is used for initiation, the calculated molecular weight of the *CDC7* protein is either 58,250 or 56,000.

The nucleotide sequence downstream from the TAG termination codon (1522 to 1524) contains several short nucleotide sequences found in the 3′-untranslated regions of other *S. cerevisiae* genes, notably the sequences TAGT and TTT, which occur repetitively between nucleotides 1544 and 1678. The TAGT at nucleotides 1544 to 1547 is part of the sequence TAGTCT that occurs repetitively downstream of the *CDC8* gene (1) and may be associated with transcription termination and polyadenylation.

Homology between *CDC7* gene product and protein kinases. The predicted amino acid sequence of the *CDC7* gene product was compared with other known and predicted protein sequences. This search revealed a statistically significant homology between the *CDC7* protein and the protein product of the *CDC28* gene, which has been shown to be a protein kinase (22, 36). The homology is not randomly distributed throughout the proteins though, but is confined largely to four domains comprising residues 40 to 52, 73 to 78, 155 to 186, and 275 to 308 of the *CDC7* sequence (Fig. 5). These domains correspond to two functionally important regions within the *CDC28* and other protein kinases, one around an ATP-binding site and the other surrounding a putative phosphorylation receptor site (11, 17, 22). Both these sites are thought to be essential for protein kinase activity, and their amino acid sequences are highly conserved in a number of known and putative kinases including bovine cyclic AMP-dependent protein kinase and the *src* family of oncogene kinases (11, 17, 22).

The majority of the consensus sequence information for both sites found in protein kinases was present in the *CDC7* gene product (Fig. 6) and constituted the region of maximum homology with *CDC28*. Thus, codons 40 to 52 and 73 to 78 approximate the ATP-binding site, while codons 155 to 186 and 275 to 308 encompass the phosphorylation receptor site. These homologies suggest that *CDC7* is a protein kinase. However, while the *CDC7* protein contains the consensus

-87 GTAACAGACT ACCTTAAATT TCAATAACAA TTGTGCTATT ATCTAATTTT CTTAGGAAAG AGGCAGTTTC GAAGTAGAAC AATCATA ATG ACA AGC 9
Met Thr Ser

AAA ACG AAG AAT ATC GAT GAT ATA CCT CCA GAA ATC AAA GAA GAG ATG ATA CAG CTC TAT CAT GAT CTA CCG GGT ATA GAA 90
Lys Thr Lys Asn Ile Asp Asp Ile Pro Pro Glu Ile Lys Glu Glu Met Ile Gln Leu Tyr His Asp Leu Pro Gly Ile Glu

AAT GAA TAT AAA CTC ATA GAC AAG ATC GGT GAG GGA ACA TTT TCG TCA GTG TAT AAA GCC AAA GAT ATC ACT GGG AAA ATA 171
Asn Glu Tyr Lys Leu Ile Asp Lys Ile Gly Glu Gly Thr Phe Ser Ser Val Tyr Lys Ala Lys Asp Ile Thr Gly Lys Ile

ACA AAA AAA TTT GCA TCA CAT TTT TGG AAT TAT GGT TCG AAC TAT GTT GCT TTG AAG AAA ATA TAC GTT ACC TCG TCA CCG 252
Thr Lys Lys Phe Ala Ser His Phe Trp Asn Tyr Gly Ser Asn Tyr Val Ala Leu Lys Lys Ile Tyr Val Thr Ser Ser Pro

CAA AGA ATT TAT AAT GAG CTC AAC CTG CTG TAC ATA ATG ACG GGA TCT TCG AGA GTA GCC CCT CTA TGT GAT GCA AAA AGG 333
Gln Arg Ile Tyr Asn Glu Leu Asn Leu Leu Tyr Ile Met Thr Gly Ser Ser Arg Val Ala Pro Leu Cys Asp Ala Lys Arg

GTG CGA GAT CAA GTC ATT GCT GTT TTA CCG TAC TAT CCC CAC GAG GAG TTC CGA ACT TTC TAC AGG GAT CTA CCA ATC AAG 414
Val Arg Asp Gln Val Ile Ala Val Leu Pro Tyr Tyr Pro His Glu Glu Phe Arg Thr Phe Tyr Arg Asp Leu Pro Ile Lys

GGA ATC AAG AAG TAC ATT TGG GAG CTA CTA AGA GCA TTG AAG TTT GTT CAT TCG AAG GGA ATT ATT CAT AGA GAC ATC AAA 495
Gly Ile Lys Lys Tyr Ile Trp Glu Leu Leu Arg Ala Leu Lys Phe Val His Ser Lys Gly Ile Ile His Arg Asp Ile Lys

CCG ACA AAT TTT TTA TTT AAT TTG GAA TTG GGG CGT GGA GTG CTT GTT GAT TTT GGT CTA GCC GAG GCT CAA ATG GAT TAT 576
Pro Thr Asn Phe Leu Phe Asn Leu Glu Leu Gly Arg Gly Val Leu Val Asp Phe Gly Leu Ala Glu Ala Gln Met Asp Tyr

AAA AGC ATG ATA TCT AGT CAA AAC GAT TAC GAC AAT TAT GCA AAT ACA AAC CAT GAT GGT GGA TAT TCA ATG AGG AAT CAC 657
Lys Ser Met Ile Ser Ser Gln Asn Asp Tyr Asp Asn Tyr Ala Asn Thr Asn His Asp Gly Gly Tyr Ser Met Arg Asn His

GAA CAA TTT TGT CCA TGC ATT ATG CGT AAT CAA TAT TCT CCT AAC TCA CAT AAC CAA ACA CCT CCT ATG GTC ACC ATA CAA 738
Glu Gln Phe Cys Pro Cys Ile Met Arg Asn Gln Tyr Ser Pro Asn Ser His Asn Gln Thr Pro Pro Met Val Thr Ile Gln

AAT GGC AAG GTC GTC CAC TTA AAC AAT GTA AAT GGG GTG GAT CTG ACA AAG GGT TAT CCT AAA AAT GAA ACG CGT AGA ATT 819
Asn Gly Lys Val Val His Leu Asn Asn Val Asn Gly Val Asp Leu Thr Lys Gly Tyr Pro Lys Asn Glu Thr Arg Arg Ile

AAA AGG GCT AAT AGA GCA GGG ACT CGT GGA TTT CGG GCA CCA GAA GTG TTA ATG AAG TGT GGG GCT CAA AGC ACA AAG ATT 900
Lys Arg Ala Asn Arg Ala Gly Thr Arg Gly Phe Arg Ala Pro Glu Val Leu Met Lys Cys Gly Ala Gln Ser Thr Lys Ile

GAT ATA TGG TCC GTA GGT GTT ATT CTT TTA AGT CTT TTG GGC AGA AGA TTT CCA ATG TTC CAA AGT TTA GAT GAT GCG GAT 981
Asp Ile Trp Ser Val Gly Val Ile Leu Leu Ser Leu Leu Gly Arg Arg Phe Pro Met Phe Gln Ser Leu Asp Asp Ala Asp

TCT TTG CTA GAG TTA TGT ACT ATT TTT GGT TGG AAA GAA TTA AGA AAA TGC GCA GCG TTG CAT GGA TTG GGT TTC GAA GCT 1062
Ser Leu Leu Glu Leu Cys Thr Ile Phe Gly Trp Lys Glu Leu Arg Lys Cys Ala Ala Leu His Gly Leu Gly Phe Glu Ala

AGT GGG CTC ATT TGG GAT AAA CCA AAC GGA TAT TCT AAT GGA TTG AAG GAA TTT GTT TAT GAT TTG CTT AAT AAA GAA TGT 1143
Ser Gly Leu Ile Trp Asp Lys Pro Asn Gly Tyr Ser Asn Gly Leu Lys Glu Phe Val Tyr Asp Leu Leu Asn Lys Glu Cys

ACC ATA GGT ACG TTC CCT GAG TAC AGT GTT GCT TTT GAA ACA TTC GGA TTT CTA CAA CAA GAA TTA CAT GAC AGG ATG TCC 1224
Thr Ile Gly Thr Phe Pro Glu Tyr Ser Val Ala Phe Glu Thr Phe Gly Phe Leu Gln Gln Glu Leu His Asp Arg Met Ser

ATT GAA CCT CAA TTA CCT GAC CCC AAG ACA AAT ATG GAT GCT GTT GAT GCC TAT GAG TTG AAA AAG TAT CAA GAA GAA ATT 1305
Ile Glu Pro Gln Leu Pro Asp Pro Lys Thr Asn Met Asp Ala Val Asp Ala Tyr Glu Leu Lys Lys Tyr Gln Glu Glu Ile

TGG TCC GAT CAT TAT TGG TGC TTC CAG GTT TTG GAA CAA TGC TTC GAA ATG GAT CCT CAA AAG CGT AGT TCA GCA GAA GAT 1386
Trp Ser Asp His Tyr Trp Cys Phe Gln Val Leu Glu Gln Cys Phe Glu Met Asp Pro Gln Lys Arg Ser Ser Ala Glu Asp

TTA CTG AAA ACC CCG TTT TTC AAT GAA TTG AAT GAA AAC ACA TAT TTA CTG GAT GGC GAG AGT ACT GAC GAA GAT GAC GTT 1467
Leu Leu Lys Thr Pro Phe Phe Asn Glu Leu Asn Glu Asn Thr Tyr Leu Leu Asp Gly Glu Ser Thr Asp Glu Asp Asp Val

GTC AGC TCA AGC GAG GCA GAT TTG CTC GAT AAG GAT GTT CTC CTA ATA TCT GAA TAG CAAAGTGAT AAATTACTGC TAGTCTGAAA 1553
Val Ser Ser Ser Glu Ala Asp Leu Leu Asp Lys Asp Val Leu Leu Ile Ser Glu Ter

TAATTTCCTT TTCTTTGGAA AGAGAATTTT AAAAGTACTT ACATATTTGC ATAGTGAAAG ATTTAAATAA AAATTTCTTA AAGTGAAACG GTTTAGCA 1651

TAATTGGTGG CATGCGCTTA AGATAGTGCC AAATACCAAA AGGCACAGGT GCAGACGATA CTTAAACAAT AGTGCTACTA CGCACTTCGT GAAAGCTA 1749

ATATCTCTTT ACCTTGCATT TGGGCATGTT GCAAACAGGA GGATCAAAAT ACAAATGGAA TCAAGAATGC TCTTGTGGTA TGATACTTTT TGTTTTTC 1847

TTTTGAGCCC ATGCGTACAT TTGAGCTGTT GAAACAGTCA AAAATAAAAC GGCAAATAAA TTGAACTTGA ACACAAAAGT AAACCAAATC CAAGACCA 1945

AACTTCAAAA GTATAGTTGG GAGCAACAAA AAGATTGAAA ATACCTTGAT TCAATGGGAC ACGGATCC 2013

FIG. 4. Nucleotide sequence and predicted amino acid sequence of *S. cerevisiae CDC7* gene and its protein product.

sequence information of the protein kinase functional domains, the organization of that sequence differs significantly from the consensus. In particular, the regions within each site that exhibit length heterogeneity are larger in *CDC7* by some 10 amino acids in the ATP-binding site and by about 80 amino acids at the phosphorylation receptor site.

DISCUSSION

Four plasmids capable of complementing the *cdc7* mutation were isolated from libraries of *S. cerevisiae* genomic DNA. One of these plasmids (pMP101) carried an insert of 3.0 kb that was present within the cloned fragments of the

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             10        20        30        40        50        60        70        80
CDC7  MTSKTKNIDDIPPEIKEEMIQLYHDLPGIENEYKLIDKIGEGTFSSVYKAKDITGKITKKFASHFWNYGSNYVALKKIYVTSSPQRIYN
                                     :  ::   : ::::  :::: :                   :::::    :
CDC28                                MSGELANYKRLEKVGEGTYGVVYKALDLRPGQGQRV     VALKKIRLESEDEGVPST
                                              10        20        30            40        50

             90        100       110       120       130       140                 150
CDC7         ELNL      LYIMTGSSRVAPLCDAKRVRDQVIAVLPYYPHEEFRTFYRDLPIKGI            KKYIWELLRALKFV
             ::        ::    :          ::                :   ::       ::               ::   :
CDC28 AIREISLLKELKDDNIVRLYDIVHS          DAHKL              YLVFEFLDLDLKRYMEGIPKDQPLGADIVKKFMMQLCKGIAYC
        60        70                   80                   90        100       110       120

        160       170         180       190       200       210       220       230       240
CDC7  HSKGIIHRDIKPTNFLF     NLELGRGVLVDFGLAEAQMDYKSMISSQNDYDNYANTNHDGGYSMRNHEQFCPCIMRNQYSPNSHNQTP
      ::  : ::: :: :  :     :: ::       :::::
CDC28 HSHRILHRDLKPQNLLINKDGNLKLG       DFGLA
      130       140       150

             250       260       270       280         290       300       310
CDC7  PMVTIQNGKVVHLNNVNGVDLTKGYPKNETRRIKRAN    RAGTRGF      RAPEVLMKCGAQSTKIDIWSVGVILLSLLGR  RFPM
                                        ::     :: :         ::::::     ::  : :: : :        :   :
CDC28                                   RAFGVPLRAYTHEIVTLWYRAPEVLLGGKQYSTGVDTWSIGCIFAEMCNRKPIFSG
                                        160       170       180       190       200       210

             330       340       350       360       370       380       390       400
CDC7  FQSLDDADSLLELCTIFGWKELRKCAALHGLGFEASGLIWDKPNGYSNGLKEFVYDLLNKECTIGTFPEYSVAFVTFGFLQQEL
           :                     :                     :     ::: :         :          ::
CDC28 DSEIDQIFKIFRVLGTPNEAIWPDIVYLPDFKPSFPQWRRKDLSQVVPSLDPRGIDLLDKLLAYDPINRISARRAAIHPYFQES
        220       230       240       250       260       270       280       290
```

FIG. 5. Comparison of the amino acid sequence of *CDC7* protein (upper) predicted from the DNA sequence shown in Fig. 4 with the predicted *CDC28* protein sequence (lower) from reference 22. Amino acids are designated by the standard IUPAC single-letter code, and homologies between the two sequences are indicated by colons. The boxed regions represent functionally significant homologies (see text). The amino acid sequence of the entire *CDC28* protein is shown, whereas, for clarity, the *CDC7* sequence is truncated to show only residues 1 to 403.

other three plasmids. The *cdc7*-complementing activity was localized within this fragment by subcloning and shown to correspond to an open reading frame of 1,521 bp that is transcribed to produce an mRNA of 1,700 nucleotides.

The genomic fragment carrying this reading frame was subcloned into the integrative vector YIp5, and the resulting plasmid (pMP104) was used to transform an *S. cerevisiae cdc7* strain such that the plasmid integrated into the yeast genome at the site homologous to the cloned insert. Tetrad analysis showed that this site maps at or near the known locus of the *CDC7* gene, near the *TRP1* gene on chromosome IV. Furthermore, by employing plasmid-chromosome recombination, the mutant sites in all of four *cdc7* alleles (*cdc7-1*, *-2*, *-3*, and *-4*) were shown to be located in the regions corresponding to specific cloned DNA fragments (Fig. 1; Table 1). Therefore, the cloned fragments contain the authentic *CDC7* gene and not an extragenic suppressor.

```
ATP-BINDING DOMAIN
CDC7       G-G-FS-V (25)  VA-K
Consensus  G-G-FG-V (8-16) VA-K
CDC28      G-G-YG-V (14)  VA-K

PHOSPHORECEPTOR DOMAIN
CDC7       RDI(3)N-L(11)DFG--   (99)         APE(12)D-W--G-I--S
Consensus  RDL(3)N-L(10)DFG--R/K(16-21)APE(11)D-W--G-L--E
CDC28      RDL(3)N-L(10)DFG--R  (19)         APE(12)D-W--G-I--E
```

FIG. 6. Comparison of *CDC7* and *CDC28* proteins with consensus sequences for the conserved functional domains of protein kinases. The consensus sequence is derived from cyclic AMP-dependent protein kinase and eight oncogene kinases (17, 32) for the ATP-binding region and from cyclic AMP-dependent protein kinase and five oncogene kinases (11, 32) for the phosphoreceptor region.

It is interesting to note that the recombinant plasmid pMP104 transformed *S. cerevisiae* at the very low frequency (1 to 5 transformants per μg of DNA) characteristic of yeast vectors lacking the sequences necessary for autonomous replication (*ARS* elements [7, 45]), implying that neither YIp5 nor the cloned fragment carrying *CDC7* contain a functional *ARS* element. However, the nucleotide sequence of the cloned fragment reveals a sequence that is closely related to the consensus core sequence, —$^{A}_{T}$TTTATPuTT T$^{A}_{T}$—, found within identified *ARS* elements (4, 18, 44, 47). The sequence —ATTTGTATTTT—, complementary to nucleotides 1795 to 1805 in the 3′-flanking region of the *CDC7* gene, differs by only one nucleotide from the *ARS* core consensus. We presume that either the change from A to G at nucleotide 5 of this sequence is sufficient to prevent *ARS* activity or that adjacent sequences necessary for autonomous replication are lacking (7, 18).

In addition to the approximate consensus *ARS* element, the 3′-flanking region of the *CDC7* gene contains sequences found in similar regions of other yeast genes, notably the consensus polyadenylation sequence AATAAA (10) at nucleotides 1619 to 1624 (Fig. 4), 95 nucleotides downstream from the TAG termination codon. It is part of a larger sequence, TAG—(A-T rich)$_{19}$—TTT, occurring between nucleotides 1605 and 1629 that strongly resembles a sequence Zaret and Sherman (50) have proposed to be required for efficient transcription termination and polyadenylation.

In contrast to the 3′-flanking region, there is no evidence for any of the consensus signal sequences (TATAAA and PuCACACA) that occur in the 5′-flanking regions of many yeast genes (49). This failure may be a consequence of the short amount of DNA sequenced 5′ to the open reading frame; while the consensus TATA box signal is located about 35 bp upstream of the transcription start site in most eucaryotes, it may be far as 220 bp upstream in yeasts (41).

The open reading frame itself could encode two proteins of molecular weight 58,250 or 56,000 depending on which of two ATG codons is used for the initiation of translation. Both ATG codons, at nucleotides 1 to 3 and 55 to 57, are located within sequences that approximate the PuXXATGPuXT that is found in the translational start site of many *S. cerevisiae* genes (19). It may be significant that a plasmid construct derived from pMP101 in which the genomic insert terminates at the *Cla*I site (nucleotides 22 to 27) retains the ability to transform *S. cerevisiae* to TRP$^+$ and TSM$^+$ at high frequency (M. N. Patterson, unpublished data). Since this plasmid lacks genomic sequences upstream of nucleotide 22 (Fig. 4), it seems likely that in this case the ATG codon at nucleotides 55 to 57 is used for translational initiation. This observation would be consistent with the Northern hybridization studies (Fig. 2) that suggested that the *CDC7* mRNA did not extend beyond the *Cla*I site. However, this interpretation would require that all the regulatory and promoter sequences for *CDC7* expression be located between nucleotides 22 and 54 of the sequence shown in Fig. 4, which seems unlikely. Another possibility is that in this construct *CDC7* expression is dependent on adjacent vector sequences, such that a truncated but functional protein is being produced. Definitive conclusions on this point require accurate mapping of the 5′ terminus of the *CDC7* transcript.

The *CDC7* protein sequence has regions of homology with the *CDC28* and oncogene protein kinases (Fig. 6) (11, 17, 22). However, *CDC7* differs from all known protein kinases by virtue of a large region of heterology within the phosphorylation receptor domain. One explanation for this could be the presence of introns within the *CDC7* gene. However, only one RNA species has been observed in Northern hybridization studies, and *CDC7* lacks any of the consensus exon-intron junction and 3′ splice signal sequences (21, 34), implying that the extra amino acid sequences predicted within the phosphorylation receptor domain are indeed present in the *CDC7* gene product. This being the case, it is less obvious that these domains are associated within a protein kinase activity of the *CDC7* protein. The additional heterogeneity might modify the phosphorylation receptor domain such that the protein performs functions unrelated to protein phosphorylation. Alternatively, the *CDC7* protein may have protein kinase activity that is modified or regulated in a specific manner as a result of the heterogeneity.

The demonstration that *CDC28* encodes a protein kinase (36) suggests that commitment to the mitotic cell cycle is accomplished in part by the activation of target proteins by phosphorylation. The finding that the *CDC7* protein may also be a protein kinase suggests that the initiation of mitotic DNA synthesis also requires the phorphorylation of certain specific proteins. Moreover, it implies that the nuclear division pathway operating in the late G1 phase is associated with events that resemble a cascade of protein phosphorylation that result ultimately in the transition from G1 to the S phase. The identification of other components of the cascade and the characterization of the targets for phosphorylation by *CDC7* and *CDC28* protein kinases would help test this idea.

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LITERATURE CITED

1. **Birkenmeyer, L. G., J. C. Hill, and L. B. Dumas.** 1984. *Saccharomyces cerevisiae CDC8* gene and its product. Mol. Cell. Biol. **4**:583–590.
2. **Birnboim, H. C., and J. Doty.** 1979. A rapid alkaline extraction procedure for screening recombinant plasmid DNA. Nucleic Acids Res. **7**:1513–1523.
3. **Botstein, D., S. C. Falco, S. E. Stewart, M. Brennan, S. Scherer, D. T. Stinchcomb, K. Struhl, and R. W. Davis.** 1979. Sterile host yeasts (SHY): a eukaryotic system of biological containment for recombinant DNA experiments. Gene **8**:17–24.
4. **Broach, J., Y. Li, J. Feldman, M. Jayaram, J. Abraham, K. Nasmyth, and J. Hicks.** 1983. Localisation and sequence analysis of yeast origins of DNA replication. Cold Spring Harbor Symp. Quant. Biol. **47**:1165–1173.
5. **Byers, B., and L. Goetsch.** 1974. Duplication of spindle plaques and integration of the yeast cell cycle. Cold Spring Harbor Symp. Quant. Biol. **38**:123–131.
6. **Carlson, M., and D. Botstein.** 1982. Two differently regulated mRNAs with different 5′ ends encode secreted and intracellular forms of yeast invertase. Cell **28**:145–154.
7. **Celniker, S. E., K. Sweder, F. Srienc, J. E. Bailey, and J. L. Campbell.** 1984. Deletion mutations affecting autonomously replicating sequence *ARS1* of *Saccharomyces cerevisiae*. Mol. Cell. Biol. **4**:2455–2466.
8. **Cryer, D. R., R. Eccleshall, and J. Marmur.** 1975. Isolation of yeast DNA. Methods Cell Biol. **12**:39–44.
9. **Falco, C. S., M. Rose, and D. Botstein.** 1983. Homologous recombination between episomal plasmids and chromosomes in yeast. Genetics **105**:843–856.
10. **Fitzgerald, M., and T. Shenk.** 1981. The sequence 5′-AAUAAA-3′ forms part of the recognition site for polyadenylation of late SV40 mRNAs. Cell **24**:251–260.
11. **Groffen, J., N. Heisterkamp, F. H. Reynolds, Jr., and J. R. Stephenson.** 1983. Homology between phosphotyrosine acceptor site of human c-*abl* and viral oncogene products. Nature (London) **304**:167–169.
12. **Hartwell, L. H.** 1973. Three additional genes required for deoxyribonucleic acid synthesis in *Saccharomyces cerevisiae*. J. Bacteriol. **115**:966–974.
13. **Hartwell, L. H., R. K. Mortimer, J. Culotti, and M. Culotti.** 1973. Genetic control of the cell division cycle in yeast. V. Genetic analysis of *cdc* mutants. Genetics **74**:267–286.
14. **Hereford, L. M., and L. H. Hartwell.** 1974. Sequential gene function in the initiation of *S. cerevisiae* DNA synthesis. J. Mol. Biol. **84**:445–461.
15. **Hicks, J. B., A. Hinnen, and G. R. Fink.** 1979. Properties of yeast transformation. Cold Spring Harbor Symp. Quant. Biol. **43**:1305–1314.
16. **Humphreys, G. O., G. A. Willshaw, and E. S. Anderson.** 1975. A simple method for the preparation of large quantities of pure plasmid DNA. Biochim. Biophys. Acta **383**:457–463.

17. **Kamps, M. P., S. S. Taylor, and B. M. Sefton.** 1984. Direct evidence that oncogenic tyrosine kinases and cyclic AMP-dependent protein kinase have homologous ATP-binding sites. Nature (London) **310**:589–592.
18. **Kearsey, S.** 1984. Structural requirements for the function of a yeast chromosomal replicator. Cell **37**:299–307.
19. **Kozak, M.** 1983. Comparison of initiation of protein synthesis in procaryotes, eucaryotes, and organelles. Microbiol. Rev. **47**:1–45.
20. **Kuo, C.-L., and J. L. Campbell.** 1983. Cloning of *Saccharomyces cerevisiae* DNA replication genes. Isolation of the *CDC8* gene and two genes that compensate for the *cdc8-1* mutation. Mol. Cell. Biol. **3**:1730–1737.
21. **Langford, C., and D. Gallwitz.** 1983. Evidence for an intron-contained sequence required for the splicing of yeast RNA polymerase II transcripts. Cell **33**:519–527.
22. **Lorincz, A. T., and S. I. Reed.** 1984. Primary structure homology between the product of yeast cell division control gene *CDC28* and vertebrate oncogenes. Nature (London) **307**:183–185.
23. **Maniatis, T., E. F. Fritsch, and J. Sambrook.** 1982. Molecular cloning, a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
24. **Meddle, C. C., P. Kumar, J. Ham, D. A. Hughes, and I. R. Johnston.** 1985. Cloning of the *CDC7* gene of *Saccharomyces cerevisiae* in association with centromeric DNA. Gene **34**:179–186.
25. **Messing, J., and J. Vieira.** 1982. A new pair of M13 vectors for selecting either DNA strand of double-digest restriction fragments. Gene **19**:269–276.
26. **Miller, J. H.** 1972. Experiments in molecular genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
27. **Mortimer, R. K., and D. C. Hawthorne.** 1973. Genetic mapping in *Saccharomyces*. Mapping of temperature-sensitive genes and use of disomic strains in localising genes. Genetics **74**:33–54.
28. **Mortimer, R. K., and D. Schild.** 1980. Genetic map of *Saccharomyces cerevisiae*. Microbiol. Rev. **44**:519–571.
29. **Nasmyth, K. A., and S. I. Reed.** 1980. The isolation of genes by complementation in yeast: the molecular cloning of a cell cycle gene. Proc. Natl. Acad. Sci. USA **77**:2119–2123.
30. **Naumovski, L., and E. C. Friedberg.** 1982. Molecular cloning of eucaryotic genes required for excision repair of UV-irradiated DNA: isolation and partial characterization of the *RAD3* gene of *Saccharomyces cerevisiae*. J. Bacteriol. **152**:323–331.
31. **Njagi, G. D. E., and B. J. Kilbey.** 1982. *cdc7-1*, a temperature sensitive cell-cycle mutant which interferes with induced mutagenesis in *Saccharomyces cerevisiae*. Mol. Gen. Genet. **186**:478–481.
32. **Nurse, P.** 1985. Cell cycle control genes in yeast. Trends Genet. **1**:51–55.
33. **Perkins, D. D.** 1949. Biochemical mutants in the smut fungus *Ustilago maydis*. Genetics **34**:607–626.
34. **Pikielny, C. W., J. L. Teem, and M. Rosbash.** 1983. Evidence for the biochemical role of an internal sequence in yeast nuclear mRNA introns: implications for U1 RNA and metazoan mRNA splicing. Cell **34**:395–403.
35. **Pringle, J. R., and L. H. Hartwell.** 1981. The *Saccharomyces cerevisiae* cell cycle, p. 97–142. *In* J. N. Strathern, E. W. Jones, and J. E. Broach (ed.), The molecular biology of the yeast *Saccharomyces*: life cycle and inheritance. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
36. **Reed, S. I., J. Hadwiger, and A. T. Lorincz.** 1985. Protein kinase activity associated with the product of the yeast cell division cycle gene *CDC28*. Proc. Natl. Acad. Sci. USA **82**:4055–4059.
37. **Rigby, P. W. J., M. Diekmann, C. Rhodes, and P. Berg.** 1977. Labeling deoxyribonucleic acid to high specific activity *in vitro* by nick translation with DNA polymerase I. J. Mol. Biol. **113**:237–251.
38. **Sanger, F., S. Nicklen, and A. R. Coulson.** 1977. DNA sequencing with chain terminating inhibitors. Proc. Natl. Acad. Sci. USA **74**:5463–5467.
39. **Scherer, S., and R. W. Davis.** 1979. Replacement of chromosome segments with altered DNA sequences constructed *in vitro*. Proc. Natl. Acad. Sci. USA **76**:4951–4955.
40. **Schild, D., and B. Byers.** 1978. Meiotic effects of DNA-defective cell division cycle mutations of *Saccharomyces cerevisiae*. Chromosoma **70**:109–130.
41. **Sentenac, A., and B. Hall.** 1982. Yeast nuclear RNA polymerases and their role in transcription, p. 561–606. *In* J. N. Strathern, E. W. Jones, and J. E. Broach (ed.), The molecular biology of the yeast *Saccharomyces*: metabolism and gene expression. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
42. **Sherman, F., G. R. Fink, and J. Hicks.** 1979. Methods in yeast genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
43. **Simchen, G.** 1974. Are mitotic functions required in meiosis? Genetics **76**:745–753.
44. **Stinchcomb, D., C. Mann, E. Selker, and R. Davis.** 1981. DNA sequences that allow the replication and segregation of yeast chromosomes, p. 473–488. *In* D. S. Ray (ed.), The initiation of DNA replication and segregation of yeast chromosomes. Academic Press, Inc., New York.
45. **Struhl, K., D. Stinchcomb, S. Scherer, and R. Davis.** 1979. High frequency transformation of yeast: autonomous replication of hybrid DNA molecules. Proc. Natl. Acad. Sci. USA **76**:1035–1039.
46. **Thomas, P. S.** 1980. Hybridisation of denatured RNA and small DNA fragments transferred to nitrocellulose. Proc. Natl. Acad. Sci. USA **77**:5201–5205.
47. **Tschumper, G., and J. Carbon.** 1980. Sequence of a yeast DNA fragment containing a chromosomal replicator and the *TRP1* gene. Gene **10**:157–166.
48. **Warren, G., and D. Sherratt.** 1978. Incompatibility and transforming efficiency of ColE1 and related plasmids. Mol. Gen. Genet. **161**:39–47.
49. **Zalkin, H., and C. Yanofsky.** 1982. Yeast gene *TRP5*: structure, function, regulation. J. Biol. Chem. **257**:1491–1500.
50. **Zaret, K., and F. Sherman.** 1982. DNA sequence required for efficient transcription termination in yeast. Cell **28**:563–573.