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DNA metabolism gene *CDC7* from yeast encodes a serine (threonine) protein kinase

(*Saccharomyces cerevisiae*/cell cycle gene product)

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ABSTRACT **The yeast Cdc7 protein is indispensable to initiation of nuclear DNA replication, based on the phenotype of the conditional, temperature-sensitive (ts) *cdc7* mutants at the restrictive temperature. This protein has likewise been implicated in commitment to meiotic DNA recombination and induced mutagenesis, which may result from error-prone DNA repair. Our previous work revealed sequence similarity between the Cdc7 protein and known protein kinases. To determine whether it possesses kinase activity, we have immunoprecipitated the protein from Cdc7-overproducing yeast cells by using polyclonal antibodies raised against a nondenatured β-galactosidase–Cdc7 fusion protein. In this report, we demonstrate that Cdc7 immune complexes are capable of phosphorylating mammalian histone H1 on serine and/or threonine residues. Immune complexes derived from cells harboring the *cdc7-2* ts mutant gene on a high copy number plasmid possess a thermolabile kinase activity. Thus, we postulate that Cdc7 may regulate the various DNA metabolic pathways by phosphorylating one or more target substrates. Because Cdc7 kinase acts downstream of Cdc28/cdc2 kinase function at "start," the transition from G_1 to S phase in the cell cycle may be the result of a cascade of protein phosphorylation.**

Determination of the function of the yeast Cdc7 protein will help to elucidate the regulation of both cell division and DNA metabolism and thus will enhance our understanding of the various disease states associated with perturbations of these processes. Inactivation of this protein by shift to a restrictive temperature in thermosensitive *cdc7*$^-$ mutants leads to arrest during mitotic cell division. This arrest is correlated to a failure to initiate nuclear DNA replication at the G_1–S phase boundary (1), so this event may be a primary regulatory step in the cell cycle. Initiation is known to be the activating step in replication, since once it occurs replication proceeds to completion. This also is the only step that needs to be altered to divert host cell machinery to replication of the virus genome upon infection by simian virus 40 and polyomavirus.

Besides its involvement in the initiation of nuclear DNA synthesis in mitosis, the Cdc7 protein is thought to be involved in other aspects of DNA metabolism as well. In contrast to the situation in mitosis, premeiotic DNA replication occurs by a Cdc7-independent mechanism, after which Cdc7 is required for commitment to meiotic DNA recombination and formation of synaptonemal complexes (2, 3). Furthermore, the Cdc7 protein may participate in error-prone DNA repair, since the *cdc7-1* mutant yields a reduced level of induced mutagenesis while growing mitotically at the permissive temperature (4).

We previously cloned and sequenced the wild-type *CDC7* gene and found that the predicted sequence of the Cdc7 protein displays strong similarity to known protein kinases, including the Cdc28 and cdc2 protein kinases of *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe*, respectively (5). Recent study of the Cdc28/cdc2 proteins and their homologs in vertebrate cells has brought substantial advances in our understanding of cell cycle regulation (see ref. 6 for review). The Cdc28/cdc2 proteins catalyze an event termed "start," whereby cells become committed to cell division (7, 8) and are needed for entry into mitosis (7, 9). Start occurs in late G_1 phase just prior to the Cdc7-effected initiation of DNA replication. The Cdc28/cdc2 proteins are homologous to mammalian growth-associated histone H1 kinase (22, 34), and the fact that the human *CDC2* gene can complement a mutation in the *Sc. pombe cdc2* gene (10) indicates that key elements of cell cycle regulation are likely to be conserved among all eukaryotes.

In this report, we describe the immunoprecipitation of Cdc7 from yeast cells and demonstrate that Cdc7 immune complexes possess a kinase activity capable of phosphorylating mammalian histone H1. This activity is thermolabile when immune complexes are prepared from cells overproducing the Cdc7-2 mutant protein. These findings suggest that DNA metabolism may be regulated by protein phosphorylation events catalyzed by the Cdc7 protein. They also provide strong evidence supporting the hypothesis that progression through the cell cycle is regulated by a protein phosphorylation cascade involving the Cdc28/cdc2 and the Cdc7 protein kinases (5).

MATERIALS AND METHODS

Strains and Media. *Escherichia coli* strain DH5 (*supE4 hsdR17 recA1 endA1 gyrR96 thi-1 relA1*) was used as the host for routine maintenance and propagation of plasmids. Overproduction of the β-galactosidase–Cdc7 fusion protein was performed with *E. coli* 71-18 [F′ *lacI*^{q} *lacZ* M15 *pro*$^+$/Δ (*lac pro*) *supE*], obtained from Carroll (11), as a host for plasmid pRS343. Bacterial cultures were grown in either L broth or supplemented M9 medium (12) containing ampicillin (50 μg/ml).

Yeast strains used are congenic with A364a. Strain 334 (*Matα leu2 ura3 pep4 prb1*) and strain 236 (*Mata bar1 his6 leu2 trp1 ura3 cdc7-1*) have been described (13, 14). Yeast cultures were grown in supplemented minimal medium or YEPD (5, 15). Plasmids were introduced into yeast cells by spheroplast transformation (13).

Recombinant DNA Techniques and Plasmids. Recombinant DNA manipulations followed described procedures (5, 15).

Plasmid pRS343 was constructed by ligation of the 2.6-kilobase *Nsi* I/*Hin*dIII fragment of the cloned *CDC7* gene (5) into the *Pst* I and *Hin*dIII sites of plasmid pUR290 (11), which was kindly provided by S. Carroll. This creates an in-frame fusion of the 3′-most 855 base pairs of the *CDC7* gene to the 3′ end of the *E. coli lacZ* gene. The *Hin*dIII site in the *CDC7*

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Abbreviation: ts, temperature sensitive.

fragment is located within the yeast *TRP1–ARS1* region, which had been ligated to the *CDC7* region at their common *Eco*RI sites.

Plasmid pRH107 (Fig. 1) was constructed by ligation of the *Cla* I/*Sph* I *CDC7* fragment of pMP201 (5) into the polycloning region of pGEM7 (Promega). In addition, the hybrid *GAL–GAP* promoter, a synthetic combination of the yeast *GAL1,10* promoter (16) and the yeast *GAP1* promoter (17), obtained from G. Stetler (Somatogenetics, Inc.) (details to be published elsewhere), was ligated into the polycloning region just 5′ to *CDC7*. To bring the *GAL–GAP* promoter sequences in close proximity to the in-frame ATG in the *CDC7* sequences, intervening sequences were removed by digestion with *Hin*dIII and then BAL-31 nuclease, followed by creation of blunt ends with Klenow polymerase, addition of an *Xho* I linker (New England Biolabs), and religation. The DNA sequence of the *GAL–GAP* promoter–*CDC7* junction region (Fig. 1) was determined by the chain-termination method (18) with Sequenase 2.0 (United States Biochemical). This *GAL–GAP* promoter–*CDC7* fusion was then recloned into the *Sph* I site of the yeast 2-μm circle-based, high copy number vector pC1/1 (19) to produce pRH107.

Plasmids pRS292, pRS293, and pRS294 were constructed by ligating the 2.6-kilobase genomic *Sph* I fragment containing *CDC7*, *cdc7-1*, or *cdc7-2*, respectively, into the *Sph* I site of the yeast vector YEp13 (20). The *cdc7-1* and *cdc7-2* alleles were cloned as described (14).

Plasmid pRH102 was constructed by inserting the 2.6-kilobase *Sph* I *CDC7* fragment into the *Sph* I site of the shuttle vector pUN70 (21), the generous gift of S. Elledge (Stanford University).

Preparation of Cdc7-Reactive Antibodies. Polyclonal antibodies reactive to the native Cdc7 protein were prepared essentially as described (11). Briefly, native β-galactosidase–Cdc7 fusion protein was overproduced in a 0.5-liter culture by induction of pRS343 with isopropyl β-D-thiogalactopyranoside. Fusion protein was purified from cell lysates by gel filtration through Sephacryl S-300 (Pharmacia) and was then used to immunize rabbits. Cdc7-specific antibodies were purified from serum by addition of 0.1 mg of *E. coli* β-galactosidase (Sigma) per ml of serum, followed by chromatography in which a fusion protein affinity column was used. Antibody preparations were concentrated to ≈0.2 mg of IgG per ml by ultrafiltration through a YM100 Diaflo membrane (Amicon).

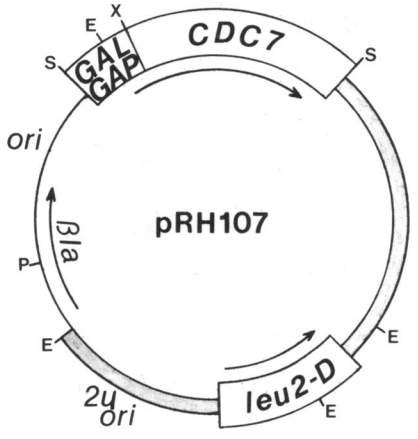

FIG. 1. Map of plasmid pRH107. Plasmid pRH107 is a high copy number *CDC7* expression plasmid. Construction is detailed in *Materials and Methods*. Wide boxes, yeast genomic DNA; narrow boxes, yeast 2-μm circle DNA; lines, pBR322 DNA. Arrows indicate directions of transcription. E, *Eco*RI; P, *Pst* I; S, *Sph* I; X, *Xho* I. The sequence of the *GAL–GAP* promoter–*CDC7* junction region is

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   -20        -10          1          10
. . . TTCGAAATCC TCGAGGAGAG ATG ATA CAG CTC . . .
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The underlined sequence is derived from genomic *CDC7*.

Immunoblotting, *in Vivo* Protein Labeling, and Immunoprecipitation. Yeast cultures used for immunoblot analysis and immunoprecipitation were grown in supplemented minimal medium at 22°C to a density of 10^7 cells per ml and were then harvested. For cells harboring pRH107, galactose was added to 2% as a gratuitous inducer (13) of *CDC7* expression 3.5 hr, or one doubling time, prior to harvest. Yeast spheroplasts were prepared with zymolyase 100T (13), and were lysed in PK buffer (50 mM Tris·HCl, pH 8/50 mM NaCl/0.1% Triton X-100/0.1% Tween 20/1 mM phenylmethylsulfonyl fluoride) (22). Lysates were cleared by centrifugation at 13,000 × *g* for 10 min. Protein concentrations of cell lysates were determined by the method of Bradford (23) using lysozyme as a standard.

Immunoblots were prepared according to described procedures (13). Blots were treated with 0.2 μg of anti-Cdc7 antibody per ml, followed by goat anti-rabbit IgG conjugated with alkaline phosphatase as secondary antibody, and then stained (13).

Yeast cells to be labeled with ^{35}S-labeled amino acids *in vivo* were grown as described above, except that 2 hr before harvesting cells were transferred to minimal medium supplemented with required amino acids and 50 μCi of ^{35}S-labeled protein labeling mix per ml (1 Ci = 37 GBq) (NEN/DuPont).

Immunoprecipitation was accomplished essentially as described (24). Cell lysates (100 μl) were pretreated with 0.1 μl of preimmune rabbit serum and 10 μl of protein A-Sepharose (Sigma) for 1 hr, followed by removal of preimmune complexes by centrifugation at 13,000 × *g* for 1 min. Immune complexes were produced by addition of 20 μg of anti-Cdc7 antibody to the pretreated supernatants followed by addition of 10 μl of protein A-Sepharose, incubating at 4°C for 1 hr for each. Immune complexes were washed five times with RIPA buffer (24) before use in electrophoretic analysis or kinase assays. SDS/PAGE (25) was performed with 10% polyacrylamide/0.8% bisacrylamide, and gels were used for autofluorography.

Kinase Assays and Analysis of Phosphorylated Amino Acids. Immune complexes were washed and then resuspended in kinase buffer (50 mM Tris·HCl, pH 7.6/50 mM sodium fluoride/1 mM phenylmethylsulfonyl fluoride/10 mM $MgCl_2$). [γ-^{32}P]ATP (NEN/DuPont) was added to 1.0 μCi/μl and nonradioactive ATP was added to bring the final ATP concentration to 300 μM. Histone H1 from calf thymus was added as the kinase substrate to 0.5 mg/ml. The total reaction vol was 30 μl. Reactions were allowed to proceed for 30 min at 22°C unless otherwise noted, and were terminated by boiling in gel loading buffer. SDS electrophoresis through 15% polyacrylamide/0.1% bisacrylamide followed by autoradiography was used to visualize reaction products. The gels were boiled in 5% trichloroacetic acid for 30 min prior to autoradiography to reduce the background of noncovalently ^{32}P-labeled material (24). Kinase assays were repeated with 0.5 μCi of [γ-^{32}P]ATP per ml and no trichloroacetic acid boiling, and results similar to those shown in Fig. 4 were produced. To determine which amino acid residues were phosphorylated, histone H1 phosphorylated by immunoprecipitates was subjected to acid degradation followed by paper electrophoresis and autoradiography with phosphoserine, phosphothreonine, and phosphotyrosine standards as markers (26).

RESULTS

Cdc7-Specific Antibodies Detect Cdc7 Protein in Overproducer Yeast Cells. Cdc7-specific antibodies were affinity purified and tested for their ability to detect wild-type levels of Cdc7 protein in yeast cell lysates by immunoblot analysis. When this proved unsuccessful, various plasmids capable of

overproducing the protein were constructed and used to transform yeast. The function of these plasmids was verified by their ability to complement the *cdc7-1* and *cdc7-2* temperature-sensitive (ts) mutants (data not shown). However, only when the gene was expressed on a high copy number plasmid was the protein detected by immunoblot (see Fig. 2). Overexpression of the *CDC7* gene with these plasmids produced no apparent change in the phenotype of the transformed cells.

Plasmid pRH107 (Fig. 1) is a yeast 2-μm circle-based, high copy number plasmid bearing a *CDC7* gene under the transcriptional control of the inducible *GAL–GAP* hybrid promoter. The plasmid-borne *CDC7* gene has been truncated such that only the second of two in-frame ATG translation start codons found in the wild-type gene (5) is present. We previously have shown that a similar *CDC7* fragment is able to complement the defects in mitotic replication (5), induced mutagenesis (27), and meiotic recombination (unpublished observations). It is interesting to note that this plasmid is capable of complementing *cdc7* mutants regardless of galactose induction, indicating that a low level of transcription occurs from the *GAL–GAP* promoter in the uninduced state, and that this produces enough Cdc7 protein to complement the mutants. However, using this plasmid, a single, 56-kDa protein was detected only in lysates of cells grown in the presence of galactose (Fig. 2, lane 3). This is the size expected for the protein produced using the second ATG codon; a 58-Kda protein would be produced using the first ATG codon (5). Cells grown in the absence of galactose (lane 2), or grown in the presence of galactose but harboring a control plasmid identical to pRH107 except for lack of the *CDC7* sequences (lane 1), contained no protein detectable by our Cdc7-specific antibodies.

To determine the ability of the antibodies to bind to mutant Cdc7 proteins, plasmids pRS292, pRS293, and pRS294 were constructed in which several different alleles of the full-sized gene including its own promoter were inserted into the high copy number yeast vector YEp13 (20). Cells transformed with such plasmids bearing either the *CDC7* wild-type sequence or the *cdc7-1* or *cdc7-2* mutant sequences, all contained an ≈58-kDa protein that could be detected by Cdc7-specific immunoblotting (Fig. 2, lanes 5–7). These plasmids all yield equal amounts of antibody-detectable protein, and this amount is roughly one-fourth the amount of protein detected by using plasmid pRH107 when equal numbers of cells are analyzed. No bands corresponding to the 56-kDa band seen using pRH107 (lane 3) were visible in these lanes.

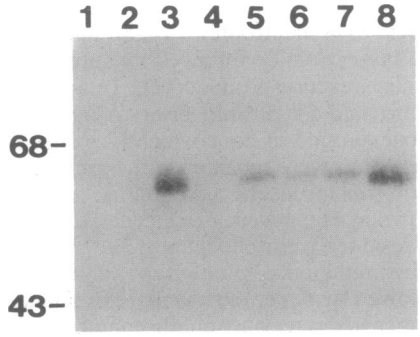

FIG. 2. Immunoblot of Cdc7-overproducer cell lysates. Cleared lysate proteins (50 μg) were loaded in each lane. Lanes: 1, 334 cells transformed with vector pC1/1 (19) grown without galactose; 2, 334 cells with pRH107, no galactose; 3, 334 cells with pRH107, with galactose; 4, 236 cells with YEp13 (20); 5, 236 cells with pRS292 (*CDC7*$^+$); 6, 236 cells with pRS293 (*cdc7-1*); 7, 236 cells with pRS294 (*cdc7-2*); 8, 236 cells with pRS292 and 334 cells with pRH107, with galactose. Molecular mass markers: bovine serum albumin, 68 kDa; ovalbumin, 43 kDa.

Because the predicted size of the protein product using the first of the two in-frame ATG start codons is 58 kDa, this start site may be used exclusively for translation *in vivo*.

The ability of the antibodies to bind native Cdc7, as opposed to the denatured protein used for immunoblotting, was established by immunoprecipitation. The antibodies immunoprecipitated a 58-kDa ^{35}S-labeled protein only from cells transformed with one of the *CDC7* high copy number plasmids pRS292, pRS293, or pRS294 (Fig. 3). The Cdc7-1 (lane 3) and Cdc7-2 (lane 4) mutant proteins were precipitated about as well as the wild-type protein (lane 2). The other bands that appear on the autofluorogram may represent other proteins with which the antibodies cross-react during immunoprecipitation. Immune complexes produced with preimmune rabbit serum contained no detectable 56- or 58-kDa proteins (lane 5).

Cdc7 Immune Complexes Possess Histone H1 Kinase Activity. Using calf thymus histone H1 as a protein kinase substrate, we assayed the ability of Cdc7 immune complexes to transfer phosphate from [γ-^{32}P]ATP to a target protein. Such activity was detected when immune complexes were derived from pRH107-containing cells grown in the presence of the inducer galactose (Fig. 4, lane 5), but not from the same grown in the absence of galactose (lane 4). Kinase activity was also detected when plasmid pRS292 was used to overproduce full-sized Cdc7 (see description of Table 1 below). A weak H1 kinase activity was present in immune complexes produced by using preimmune rabbit serum, but this activity was not galactose inducible (lanes 1 and 2). The detection of this non-Cdc7-specific activity is not surprising, since yeast cells are known to contain many protein kinases (28). It is worth noting that Cdc7 autophosphorylation is not detected by our assay, although such activity may occur at a level below the detection limit of our assay. These results are similar to that found for yeast Cdc28 kinase (24).

Analysis of phosphorylated amino acids was performed on histone H1 that had been phosphorylated by Cdc7 immune complexes from pRH107-containing cells grown in galactose (Fig. 4). Only radiolabeled phosphoserine and phosphothreonine were detected (Fig. 5). This amino acid specificity was ex-

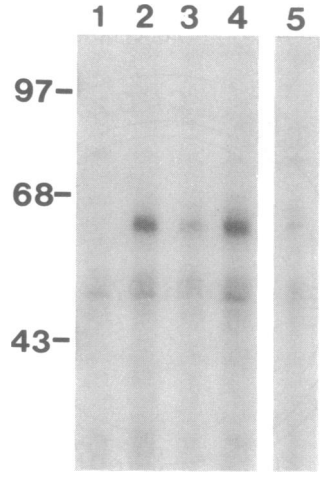

FIG. 3. Immunoprecipitation using Cdc7-overproducer cell lysates. Autofluorogram of ^{35}S-labeled Cdc7-specific immune complexes. Complexes were derived from strain 236 with either plasmid YEp13 (lane 1), pRS292 (*CDC7*$^+$) (lane 2), pRS293 (*cdc7-1*) (lane 3), or pRS294 (*cdc7-2*) (lane 4) were denatured by boiling in gel loading buffer (25). Lane 5, ^{35}S-labeled preimmune complexes derived from strain 236 with plasmid pRS292. Gel was soaked in Fluoro-Hance (Research Products International), dried, and exposed to Kodak XAR5 film with an intensifying screen. Molecular mass markers: phosphorylase B, 97 kDa; bovine serum albumin, 68 kDa; ovalbumin, 43 kDa.

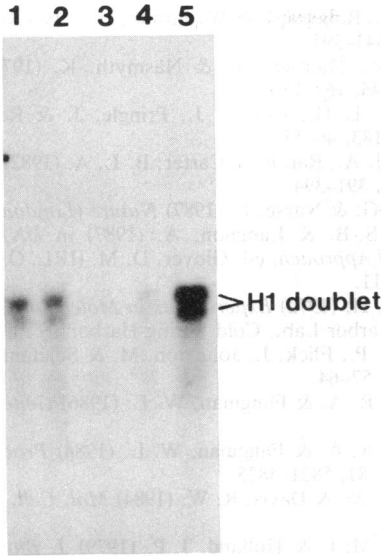

FIG. 4. Protein kinase assay of Cdc7 immune complexes. Autoradiogram of denatured reaction products from kinase assays. Reactions used preimmune complexes from 334 cells with plasmid pRH107 grown in the absence (lane 1) or presence (lane 2) of galactose, or Cdc7-specific immune complexes from 334 cells with plasmid pC1/1 (19) grown in galactose (lane 3), or from 334 cells with plasmid pRH107 grown in the absence (lane 4) or presence (lane 5) of galactose. Gel was dried and used to expose Kodak XAR5 film with an intensifying screen. H1 doublet, position of the calf thymus histone H1 bands.

pected, because sequence comparisons predict that Cdc7 is a member of the serine/threonine family of protein kinases (29).

Cdc7-2 Mutant Protein Kinase Is Thermosensitive *in Vitro*. To further delineate the connection between kinase activity and the Cdc7 protein, we sought to determine whether Cdc7 mutant protein immune complexes possess a thermolabile kinase activity. Immune complexes derived from the same cells used to produce the immunoprecipitation results shown in Fig. 3 were tested for kinase activity at 22°C and 40°C. Complexes were preincubated at these temperatures for 30 min prior to kinase assays to ensure that temperature influences had taken effect. Histone H1 kinase activity at 22°C using Cdc7-2 complexes was reduced ≈4-fold compared to that using Cdc7 wild-type complexes (Table 1). It is not uncommon for ts mutant enzymes to be less active *in vitro* than their wild-type counterparts, even at the permissive temperature (15, 22). Kinase activity was reduced an additional 4-fold at 40°C when these mutant protein complexes were used (Table 1). In contrast, the activity of the wild-type complexes was ≈2-fold higher at 40°C than at 22°C (Table 1). This latter result argues against the possibility that thermolability of kinase activity is due to some factor other than the mutant Cdc7-2 protein itself, such as a ts inhibitor or phosphatase. This result also justifies the use of 40°C as the restrictive temperature for these assays.

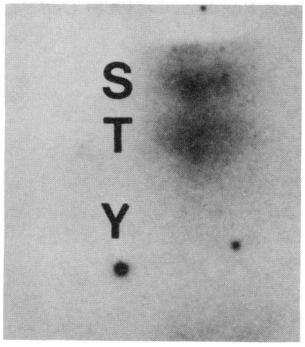

FIG. 5. Analysis of histone H1 phosphorylated by Cdc7 protein kinase. Histone H1 was phosphorylated by Cdc7 immunoprecipitates from 334 cells with plasmid pRH107 grown in the presence of galactose (see Fig. 4, lane 5), purified, subjected to acid degradation, and analyzed by paper electrophoresis and autoradiography (26). The positions at which phosphoserine (S), phosphothreonine (T), and phosphotyrosine (Y) standards migrated are indicated.

Table 1. Thermolability of Cdc7-2 immune complexes

Kinase	% kinase activity		-fold increase (40°C/22°C)
	At 22°C	At 40°C	
$Cdc7^+$	63 ± 9.0	100	1.6 ± 0.25
Cdc7-2	15.4 ± 3.0	4.2 ± 1.5	0.27 ± 0.09

Histone H1 kinase assays were performed with $Cdc7^+$ wild-type or Cdc7-2 mutant immune complexes (see Fig. 4). The resulting autoradiograms of the gels were quantitated by densitometry. The intensity of the ^{32}P-labeled histone H1 bands in the lane containing Cdc7 wild-type kinase reaction products performed at 40°C was set as 100% activity for each of two separate experiments. The -fold increase is activity at 40°C divided by activity at 22°C. Numbers listed are mean values ± SD for the two experiments.

DISCUSSION

We have successfully produced antibodies that specifically bind the yeast Cdc7 protein. These antibodies react with either a 56- or 58-kDa protein, depending on which of two in-frame ATG start codons (5) is available for initiation of translation. This binding occurred when either a denatured version of the protein was tested (Fig. 2) or when the native protein was tested (Fig. 3). Only a 58-kDa protein was detected when a full-sized *CDC7* gene was expressed from its own promoter on a high copy number plasmid, even though the second ATG codon was present in the *CDC7* sequences. This indicates that the 58-kDa protein is the major, if not the sole, species synthesized *in vivo*. If the first ATG is removed, then translation from the second produces a 56-kDa protein. These findings are in contrast to those of Bahman *et al.* (30), who were unable to obtain $p58^{CDC7}$ in the absence of $p56^{CDC7}$ when using the full-sized version of the gene. We believe that this discord is due to the fact that Bahman *et al.* (30) synthesized the Cdc7 protein by transcription and translation *in vitro*, which may differ from synthesis *in vivo*, as was done in this study.

It is interesting to note that the Cdc7 protein is detected by immunoblotting or in immunoprecipitates only when the *CDC7* gene is overexpressed on a high copy number plasmid. No protein is detectable by our assays in extracts of wild-type cells or of cells harboring a single copy *CDC7* plasmid. Also, the inducible plasmid pRH107 complements $cdc7^-$ mutants even in the absence of the inducer galactose, probably because of a low level of constitutive transcription from the *GAL–GAP* hybrid promoter, although this level is not high enough to permit detection of the protein by immunoblot analysis. We interpret these two findings to imply that the Cdc7 protein is present and is required in very low levels in the cell.

Protein kinase assays performed on Cdc7-specific immune complexes reveal that these complexes possess an activity capable of transferring phosphate from ATP to serine and/or threonine residues in histone H1. This activity was predicted by the sequence similarity between the Cdc7 protein and known protein kinases (5, 29), although the Cdc7 kinase would differ in primary structure by virtue of unusually long regions of nonhomology interrupting its putative catalytic domains (5). The demonstration that Cdc7 immune complexes do possess kinase activity suggests that Cdc7 has a unique structure among protein kinases, with the nonhomol-

ogous inserts likely forming loops that bring the conserved regions into juxtaposition (29). Because removal of one of these inserts destroys the ability to complement the *cdc7-1* mutation, this suggests that the unusual structure of the Cdc7 kinase is essential for at least one of its cellular functions (30).

CDC7-dependent kinase activity has also been detected in a high molecular weight DNA-replicative complex (31) and in the protein mixture resulting from the *in vitro* transcription and translation of the cloned *CDC7* gene (30). The results described here demonstrate that immune complexes containing the authentic, *in vivo*-synthesized Cdc7 protein also possess kinase activity. This kinase activity is inherent to Cdc7 itself, because the activity of Cdc7-2 ts mutant immune complexes is thermolabile *in vitro*.

The association between the functions of Cdc-7 *in vivo* and its kinase activity *in vitro* has yet to be determined. Nevertheless, it appears likely that the Cdc7 protein acts to regulate several aspects of DNA metabolism by phosphorylating one or more target substrates. It also appears to comprise a link between DNA replication and progression through the cell division cycle, since inactivation of Cdc7 causes cell cycle arrest. This progression may be controlled by a cascade of coupled phosphorylation events involving the Cdc7 kinase and the well-characterized Cdc28/cdc2 kinases, which control the entry into the cell cycle just prior to the Cdc7 execution point. At the very least, DNA replication is not initiated until start has occurred. Cdc7 kinase may be a substrate for Cdc28/cdc2 kinase because Cdc7 contains a consensus phosphorylation site, Ser-Pro-Gln-Arg (32). Most interestingly, Ser-72 in c-src tyrosine kinase is phosphorylated by Cdc28/cdc2 kinase at this same site (33). To clarify these issues, the regulation of Cdc7 protein kinase activity by phosphorylation and the nature of its substrates need to be investigated.

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