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Biochemistry

The CDC7 protein of *Saccharomyces cerevisiae* is a phosphoprotein that contains protein kinase activity

(cell division cycle/S phase/phosphorylation/DNA replication)

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ABSTRACT **The CDC7 protein of *Saccharomyces cerevisiae* may be involved in the G_1/S-phase transition and/or in the initiation of mitotic DNA synthesis. The *CDC7* gene has two in-frame AUG codons as possible translation start sites, which would produce 58- and 56-kDa proteins, respectively. Both p58 and p56 derived from recombinant plasmids complement the temperature-sensitive growth defect of the *cdc7-1* allele. To determine the biochemical function of the CDC7 protein, the *CDC7* gene was cloned and polyclonal antibodies were produced against the CDC7 protein. CDC7 immune complexes prepared from yeast with these antibodies phosphorylate histone H1. Kinase activity is thermolabile in strains carrying the *cdc7-1* temperature-sensitive mutant allele and is elevated >10-fold in strains carrying plasmids overexpressing either p56 or p58, confirming that the kinase in the immunoprecipitates is the *CDC7* gene product. In addition, we show that CDC7 is a phosphoprotein itself. Indirect immunofluorescence and biochemical fractionation show that the CDC7 protein is present at relatively high concentrations in the nucleus compared with the cytoplasm, suggesting that nuclear proteins may be substrates for the CDC7 protein.**

Two major events define the eukaryotic cell cycle: replication of the chromosomes during S phase and segregation of the chromosomes during mitosis. Replication and segregation are separated by two gap periods, G_1 and G_2, during which the cells prepare for these events. There are also two major control points for the cell cycle in G_1 and G_2, the point of commitment to DNA synthesis in G_1 and the regulation of progression into mitosis in G_2. Although we have made great strides in understanding mitotic control in the past three years, relatively little is known about the events in G_1 that lead to S phase. In yeast, the point of commitment to DNA synthesis is defined as "Start", early in G_1. DNA synthesis does not ensue immediately after Start, however, and it is not clear whether the lag is due to assembly of the replication apparatus or to a cascade of controls initiated at Start. *CDC28*, *CDC4*, and *CDC7* define a dependent series of events set in motion at Start (1–3). *CDC28* encodes a protein kinase subunit, the analog of the highly conserved $cdc2^+$/MPF kinase subunit involved in regulation of mitosis (3). *CDC4* is homologous to one subunit of the signal transducing protein, transducin (4). The DNA sequence of the *CDC7* gene predicts a protein that has homology to catalytic domains of the protein kinases CDC2/CDC28, NIM1, and a number of kinase-related transforming proteins (5). The CDC7 sequence differs from that of other protein kinases in the so-called phosphate acceptor domain, however (5, 6).

We have been interested in the role of *CDC7* in the events linking Start and the initiation of DNA replication. Mutations in *CDC7* appear to block a precondition for DNA synthesis because cells carrying these lesions cannot start new rounds of DNA replication after a shift from permissive to nonpermissive temperature (7, 8). The *cdc7* mutation arrests cells at the late G_1/S-phase boundary, prior to the initiation of DNA synthesis, and shows the dumbbell-shaped terminal phenotype typically associated with a DNA synthesis or nuclear division defect (7, 9). Cycloheximide does not inhibit initiation and completion of DNA synthesis when added after return of *cdc7* cells from the restrictive to the permissive temperature (10), suggesting that all protein synthesis late in G_1 essential for initiation of DNA replication can be completed during the *cdc7* block. The phenotype of *cdc7* mutants would be consistent with *CDC7* mediating an important signal for initiation of replication or with *CDC7* participating in initiation itself. The function of the CDC7 protein in the G_1/S transition and/or in the initiation of mitotic DNA replication may be accomplished by its protein kinase activity, for instance by periodically activating a crucial replication protein. This would imply that the function of *CDC7* itself must be periodically regulated. Since overall levels of CDC7 RNA and protein do not seem to be limiting for entry into S phase (11), fluctuation in CDC7 levels does not account for its periodic function. Thus, either the activity of CDC7 itself must change during the cell cycle, or its location in the cell may vary, or there is a periodic change in a protein with which it interacts—for instance, a substrate.

To investigate whether the activity of CDC7 changes during the cell cycle, we wished to define its biochemical activity and to devise an assay that could be used to follow CDC7 activity through the cell cycle. In this paper, we show that the CDC7 protein contains protein kinase activity that can be measured specifically in nuclear extracts using histone H1 as substrate. We also show that the CDC7 protein is modified by phosphorylation and that it is located in the nucleus.

MATERIALS AND METHODS

Strains. *Saccharomyces cerevisiae* strains were SEY6210 (*MATα his3-Δ200 lys2-801 trp1-Δ901 ura3-52 suc2-Δ9 leu2-3,112*) and YC7379 (*MATα ade1 his7 lys2 tyr1 ura3-52 cdc7-1*). Strain SEY6210 was obtained from S. D. Emr (California Institute of Technology, Pasadena, CA) and strain YC7379 was constructed for this work.

Production of CDC7 Antisera. The *CDC7* gene was introduced into *Escherichia coli* using the p*tac* expression vector, pKK223-3 (12). Briefly, the gene was brought under the control of the *tac* promoter by inserting the *Cla* I/*Eco*RI fragment from CDC7 into the *Eco*RI site of the vector, and reconstituting the CDC7 coding sequence with the oligonucleotide 5′-AA TTC ATG ACA AGC AAA ACG AAG AAT AT-3′. The protein was partially purified by chromatography on DEAE-cellulose (Whatman) and heparin-agarose col-

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Abbreviation: PMSF, phenylmethylsulfonyl fluoride.

umns. Bands corresponding to the CDC7 protein were excised from SDS/polyacrylamide gels and used for immunization of two rabbits whose sera were free of anti-yeast IgG. Multiple injections were given until the CDC7 protein was detected on immunoblots of yeast extracts. A 1:600 dilution of antibody is sufficient to detect 50 ng of CDC7 protein by protein blotting.

Indirect Immunofluorescence of Yeast Cells. This procedure is based on the techniques developed by Kilmartin and coworkers for whole-mount yeast cells (13, 14) and modified from the procedure as described (15).

Isolation of Yeast Nuclei. Yeast nuclei were prepared by the method of Hurt *et al.* (16). The nuclear pellet was resuspended in 50 ml of Percoll gradient buffer containing 50% Percoll (Pharmacia), 40 mM Pipes (pH 6.5), 5 mM $MgCl_2$, 0.5 mM phenylmethylsulfonyl fluoride (PMSF) and centrifuged in a Beckman VTi50 rotor at 21,000 rpm for 40 min. Three bands were shown after centrifugation. The nuclear band was in the middle of the gradient and was collected by inserting a needle into that position. The nuclear fraction was diluted with 3 vol of 40 mM Pipes, pH 6.5/5 mM $MgCl_2$/0.5 mM PMSF and centrifuged at 12,000 rpm for 20 min to remove Percoll.

Immunoprecipitation and Protein Kinase Assays. Nuclear fractions were prepared as described above except the Percoll gradient step was not performed. Crude nuclei were resuspended in 50 mM Tris·HCl, pH 7.5/10 mM $MgCl_2$/1 mM dithiothreitol/1 mM PMSF, and 4 M $(NH_4)_2SO_4$ was added to 0.4 M final concentration. The lysate was spun at 100,000 × *g* for 30 min. The supernatant was precipitated with ammonium sulfate (70% saturation) and centrifuged at 100,000 × *g* for 20 min. The pellet was dissolved in 3 ml of 15% (wt/vol) sucrose/50 mM Tris·HCl, pH 7.5/50 mM NaCl/0.1% Triton X-100/1 mM dithiothreitol/0.5 mM PMSF, dialyzed against the same buffer, and frozen in aliquots at −70°C until needed. Immunoprecipitations and protein kinase assays were performed as described by Reed *et al.* (3) with some modifications. Ten microliters of preimmune serum was added to 20 μl of a protein A-Sepharose (100 mg/ml) suspension and incubated for 1 hr at 4°C. The beads were collected by centrifugation and resuspended with 0.5 mg of cytosolic or nuclear fractions from various yeast cultures. After 1 hr at 4°C, the suspension was centrifuged and the supernatant was transferred to another tube. The pellet contained preimmune complexes and was saved for further assays. The supernatant was used for a second immunoprecipitation with 10 μl of CDC7 antisera. Then, the preimmune and CDC7 immune complexes were washed twice in 50 mM Tris·HCl, pH 7.5/150 mM NaCl/0.5% Tween 20/0.2 mM EDTA, followed by two washes in 0.5 M LiCl/0.1 M Tris·HCl, pH 7.5, and finally by two washes with 25 mM Tris·HCl, pH 7.5/5 mM NaF/10 mM $MgCl_2$/0.1 mM EGTA/0.5 mM PMSF (reaction buffer). The beads were resuspended in 30 μl of the reaction buffer. For kinase assays, 2 μg of histone H1 (Boehringer Mannheim) was added to 30 μl of redissolved immunoprecipitate along with 300 μM [γ-^{32}P]ATP (0.3 μCi/nmol; 1 Ci = 37 GBq). After 15 min at 37°C, reactions were terminated by the addition of SDS sample buffer, and phosphorylated products were analyzed by electrophoresis on SDS/polyacrylamide gels.

RESULTS

Overexpression of *CDC7* in Yeast. The *CDC7* gene was isolated by transformation of a *ura3-52 cdc7-1* strain of *S. cerevisiae* with a YCp50 yeast genomic library constructed in our lab (17). To define the catalytic activity of the CDC7 protein, we used the cloned gene to facilitate isolation of the CDC7 protein from *E. coli* and yeast. The CDC7 protein expressed in *E. coli* using three different expression systems was not active as a protein kinase with any substrates tested (data not shown). Since we were unable to demonstrate CDC7 kinase activity in *E. coli*, we reasoned that there might be a specific modification of the protein or a specific cofactor only found in yeast. Therefore, we next undertook to isolate the protein from yeast. For this purpose, we cloned CDC7 in a GAL10 expression vector. The nucleotide sequence of CDC7 contains two in-frame ATG codons, one at nucleotide 1 and the other at nucleotide 55. The gene product arising from the first ATG in CDC7 is called p58, and the one from its second ATG is called p56. Since it is not known which ATG is used *in vivo*, we produced both p58 and p56. The entire *CDC7* gene was inserted into the unique *Eco*RI site of the GAL expression vector pSEY18·Gal (S. D. Emr), a high copy number vector that allows inducible expression of the CDC7 protein. The fragment carrying the *CDC7* gene was created by ligation of a synthetic linker (*Eco*RI ATG ACA AGC AAA ACG AAG AAT *Cla* I), representing the first seven amino acids of CDC7, to the 2.4-kilobase *Cla* I/*Eco*RI fragment of CDC7. To express p56, the first ATG was removed by cutting at the *Cla* I site, which lies between the two initiation codons, followed by further deletion with BAL-31 of the DNA between that *Cla* I site and the second ATG. The plasmids encoding p58 and p56 were named pSYC758 and pSYC756, respectively. Both plasmids were able to complement the mitotic growth defect in *cdc7-1* cells at the nonpermissive temperature.

CDC7 protein expressed from these plasmids was analyzed by protein blotting and immunoprecipitation of ^{35}S-labeled yeast cells. As shown in Fig. 1, yeast strains carrying pSYC758 and pSYC756 produced *CDC7*-encoded proteins of 58 and 56 kDa, respectively. Strong signals were obtained only after galactose induction (lanes e, f, and h). Thus, the CDC7 protein may be present at very low levels in wild-type cells.

Protein Kinase Activity of the *CDC7* Gene Product Isolated from Yeast. Because of the low levels of CDC7 protein present in whole cell extracts of wild-type cells and because the overproduced protein was difficult to solubilize, conventional purification was inefficient. CDC7 protein was therefore immunoprecipitated from both nuclear and cytosolic fractions of various yeast strains, and the redissolved immunoprecipitates were used in protein kinase assays with calf thymus histone H1 as the substrate. As shown in Fig. 2, histone H1 is clearly phosphorylated by immunoprecipitates prepared from nuclear extracts with CDC7 antisera, suggesting that the CDC7 protein contains protein kinase activity.

Since to preserve enzymatic activity immunoprecipitates must be prepared under conditions in which additional proteins may coprecipitate (although we removed proteins interacting nonspecifically with the rabbit IgG by first precipitating with preimmune sera), to ensure that the kinase activity observed was due to the *CDC7*-encoded protein and not to a contaminating kinase, two control experiments were carried out. First, we demonstrated overproduction of kinase activity in extracts of *cdc7-1* mutant strains carrying plasmids pSYC758 and pSYC756 compared with extracts of the same strain carrying vector alone. Comparison of lanes c, d, and e in Fig. 2 indicates that the level of kinase activity was >10-fold higher in the CDC7 overproducer than in a strain containing vector alone. The magnitude of overproduction was estimated from four independent experiments and densitometer tracings of the autoradiograms. Since these assays measure extents of phosphorylation rather than initial rates, this is a minimum estimate of the actual overproduction. Second, we demonstrated that the kinase activity was thermolabile in immunoprecipitates prepared from *cdc7-1* mutants. Comparison of lanes b and c in Fig. 2 shows that the kinase activity is thermolabile in the strain carrying vector alone (Q_{23}^{37} = 0.2). Conversely, in the same strains carrying

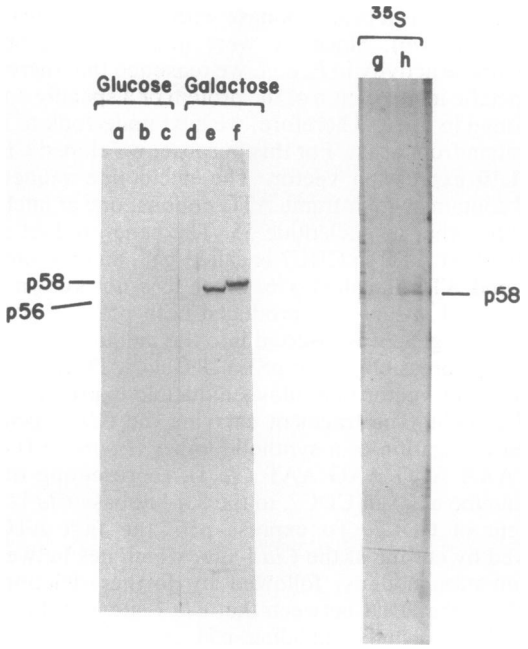

FIG. 1. Characterization of CDC7 antibody by immunoblot and immunoprecipitation. (*Left*) Stationary cultures of yeast strain SEY6210/pG12/pSEY18·Gal (lanes a and d), and the CDC7-overproducing strains SEY6210/pG12/pSYC756 (lanes b and e), and SEY6210/pG12/pSYC758 (lanes c and f) grown in synthetic complete (SC) medium (minus uracil and leucine) plus 2% glucose were diluted 1:10 in SC medium (minus uracil and leucine) containing either 2% glucose (lanes a–c) or 2% galactose (lanes d–f). Plasmid pG12 provides the GAL4 protein essential for expression from the GAL10 promoter (18). After 8 hr at 30°C, whole yeast lysates were prepared by disruption of the cells with glass beads and clarified by centrifugation. The pellets were resuspended in buffer containing 1% SDS and analyzed on a SDS/10% polyacrylamide gel. Proteins were then electroblotted to a nitrocellulose membrane. This immunoblot was stained with anti-CDC7 antiserum at a dilution of 1:250 and visualized by using the Bio-Rad Immun-Blot assay kit (goat anti-rabbit IgG horseradish peroxidase conjugate). (*Right*) For labeling with [^{35}S]methionine (Tran^{35}S-label, ICN), strain SEY6210/pG12/pSYC758 was grown in SC medium (minus uracil, leucine, and methionine) plus 2% glucose, to 2×10^7 cells per ml. Cells were harvested, washed twice, and resuspended in the same medium containing 2% galactose. After 1 hr at 30°C, 250 μCi of [^{35}S]methionine was added and the incubation continued for 40 min. Cells were lysed in 100 μl of 50 mM Tris·HCl, pH 7.5/1 mM EDTA/1% SDS by Vortex mixing in the presence of glass beads followed by boiling for 5 min. The lysate was diluted by the addition of 1 ml of buffer (50 mM Tris·HCl, pH 7.5/150 mM NaCl/0.1 mM EDTA/0.5% Tween 20) and centrifuged, and the supernatant was used for immunoprecipitation with 5 μl of preimmune serum (lane g) or 5 μl of CDC7 antiserum (lane h). Samples were analyzed by electrophoresis in SDS/10% polyacrylamide gel.

CDC7-containing plasmids, kinase activity at 37°C is at least 2-fold higher than at 23°C [lanes f and g ($Q_{23}^{37} = 2$)], indicating that the thermolability observed in lanes b and c is not due to nonspecific factors in the strain background affecting kinase activity at 37°C vs. 23°C and that the kinase is associated with the *CDC7* gene product. We could not detect CDC7-dependent phosphorylation of histones with preimmune complexes (data not shown).

CDC7 Is a Phosphoprotein. In immunoprecipitates from strains overproducing p58 and p56, in addition to phosphorylation of histone H1, a protein of 58 or 56 kDa, respectively, was also phosphorylated. As shown in Fig. 3, phosphorylation of these 56- and 58-kDa proteins was detectable only in nuclear and not in cytoplasmic extracts. It is likely that these proteins are the *CDC7* gene product. The 48-kDa band that appears in this experiment was also seen in immunoprecipitates prepared with preserum and may represent a contaminant (such as IgG), or it may be an activator or a substrate of CDC7.

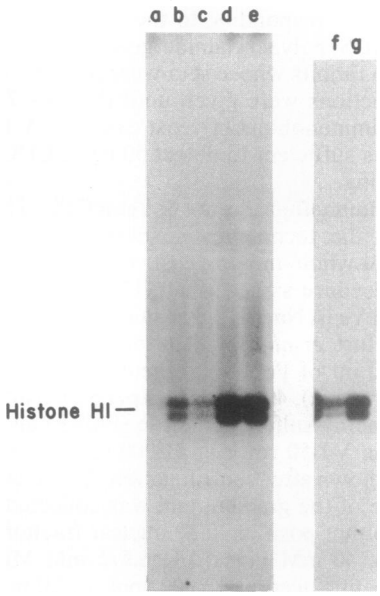

FIG. 2. CDC7-dependent phosphorylation of histone H1. Nuclear extracts were prepared from strain YC7379 (*cdc7-1*), carrying pSEY18·Gal, pSYC756, or pSYC758. Cells were grown at 23°C and proteins (0.5 mg) were immunoprecipitated twice: proteins interacting nonspecifically with the rabbit IgG were removed by precipitating with preimmune serum and the supernatant was subjected to a second immunoprecipitation with CDC7 immune antiserum. The final pellet was preincubated at 23°C (lanes b and f) and 37°C (lanes a, c, d, e, and g). After 30 min, 2 μg of histone H1 and 300 μM [γ-^{32}P]ATP were added and incubation continued for 15 min. Reaction products were analyzed on a SDS/10% polyacrylamide gel, stained with Coomassie blue, dried, and autoradiographed. Lanes: a, histone H1 alone; b, YC7379/pSEY18·Gal, 23°C; c, YC7379/pSEY18·Gal, 37°C; d, YC7379/pSYC756, 37°C; e, YC7379/pSYC758, 37°C; f, YC7379/pSYC758, 23°C; g, YC7379/pSYC758, 37°C.

The observed phosphorylation of p56 and p58 by the CDC7 immunoprecipitates could be due either to autophosphorylation or to a coprecipitating kinase. The results suggested, however, that CDC7 might occur *in vivo* as a phosphoprotein. To investigate the latter possibility, cells carrying pSYC758 were grown on galactose, labeled with $^{32}P_i$, and immunoprecipitated with CDC7 antisera. As shown in Fig. 3 (lane i), strains overproducing p58 do contain a 58-kDa phosphoprotein. This protein is not observed in the absence of overproduction (lane j) or in extracts precipitated with preimmune serum (lane k).

Localization of the *CDC7* Gene Product by Indirect Immunofluorescence and Subcellular Fractionation. Knowledge of the subcellular localization of the CDC7 protein may be useful in establishing its molecular function by narrowing the range of potential substrates and cellular proteins with which it interacts. Using indirect immunofluorescence microscopy with CDC7 antisera, we found intense immunofluorescence in the nuclei of CDC7-overproducing cells (Fig. 4*A*). Fainter but distinct immunofluorescence was also seen in the cytoplasm. In the experiment shown, cells were also stained with Hoechst 33258 to verify the position of the nuclei (Fig. 4*B*). However, identical staining patterns were observed in other experiments in which only anti-CDC7 staining and Nomarsky optics were used, and therefore the patterns are not due to leakage from the additional dye. Thus, the CDC7 protein appears to be present in both the cytoplasm and nucleus but to be relatively concentrated in the nucleus. Only faint

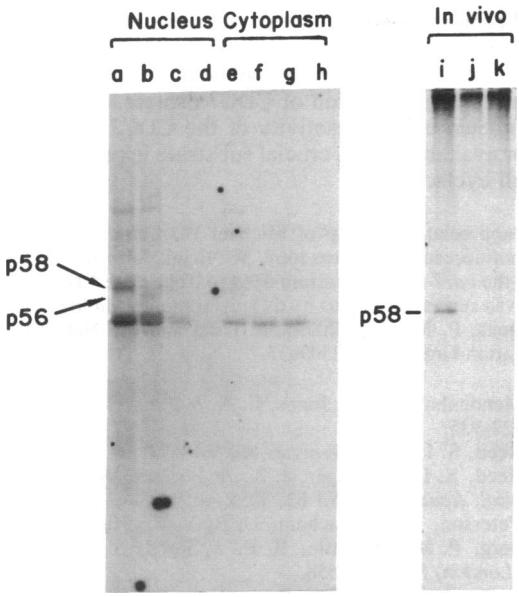

FIG. 3. Phosphorylation of the CDC7 protein *in vivo* and *in vitro*. (*Left*) Proteins (0.5 mg) of nuclear (lanes a–d) and cytosolic (lanes e–h) fractions were immunoprecipitated twice as described in Fig. 2 and incubated for 30 min at 37°C in the presence of 10 μCi of [γ-^{32}P]ATP (3000 Ci/mmol; Amersham). Samples were analyzed on a SDS/10% polyacrylamide gel. Lanes: a and e, SEY6210/pG12/pSYC758; b and f, SEY6210/pG12/pSYC756; c and g, SEY6210/pG12/pSEY18·Gal; d and h, YC7379/pSEY18·Gal. (*Right*) ^{32}P-labeled CDC7 immunoprecipitates were prepared as follows: 5×10^7 cells of strain SEY6210/pG12/pSEY18·Gal (lane j) or SEY6210/pG12/pSYC758 (lanes i and k) were labeled for 1 hr with 0.5 mCi of [^{32}P]orthophosphate (New England Nuclear) in reduced phosphate minimal medium (19) containing 2% galactose. Cell lysis and immunoprecipitation were performed as described in the legend to Fig. 1 except that either 10 μl of preimmune serum (lane k) or 10 μl of *CDC7* immune antiserum (lanes i and j) was used.

CDC7-specific immunofluorescence was detected when cells carrying the vector pSEY18·Gal were stained with CDC7 antisera in an identical manner (data not shown). The difficulty in visualizing CDC7 antigen in the absence of overproduction suggests that the levels of CDC7 protein in yeast are low, in accord with the immunoblot analysis (Fig. 1, lanes a and d).

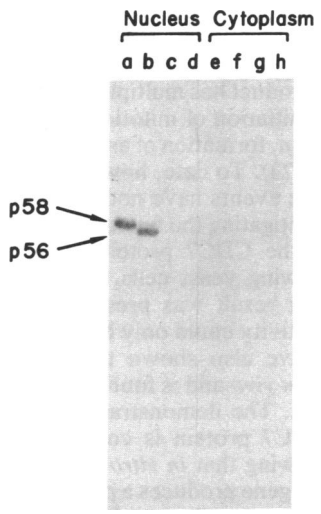

FIG. 5. Localization of CDC7 by subcellular fractionation. Proteins (100 μg) of nuclear (lanes a–d) and cytosolic (lanes e–h) fractions were separated by a SDS/10% polyacrylamide gel, electroblotted to nitrocellulose, and stained with anti-CDC7 antiserum (diluted 1:250). Arrows on the left indicate the immunoblot bands for CDC7-p58 and CDC7-p56, respectively. Lanes: a and e, SEY6210/pG12/pSYC758; b and f, SEY6210/pG12/pSYC756; c and g, SEY6210/pG12/pSEY18·Gal; d and h, YC7379/pSEY18·Gal.

The nuclear localization of the CDC7 protein was corroborated by biochemical fractionation. Fig. 5 shows immunoblot analysis of CDC7 protein in cytosolic and nuclear fractions. Equal amounts of protein, rather than equal numbers of cell equivalents, were loaded in each lane, because of the difficulty of quantitating cell equivalents due to uncertainty in measurement of losses experienced during the purification of nuclei. These results demonstrate, however, that CDC7 is relatively concentrated in the Percoll gradient-purified nuclei. When 5-fold excess of cytosolic protein is analyzed, CDC7 is detectable (data not shown). We estimate that both p58 and p56 were present in at least 20-fold higher concentrations in nuclear fractions than in cytosolic fractions (Fig. 5). ABF1 and SSB1, nuclear proteins previously studied in this lab (20, 21), were also observed in our nuclear fractions only (data not shown). Comparison of the CDC7 sequence with the nuclear targeting signal consensus revealed putative nuclear localization signals in at least six different positions.

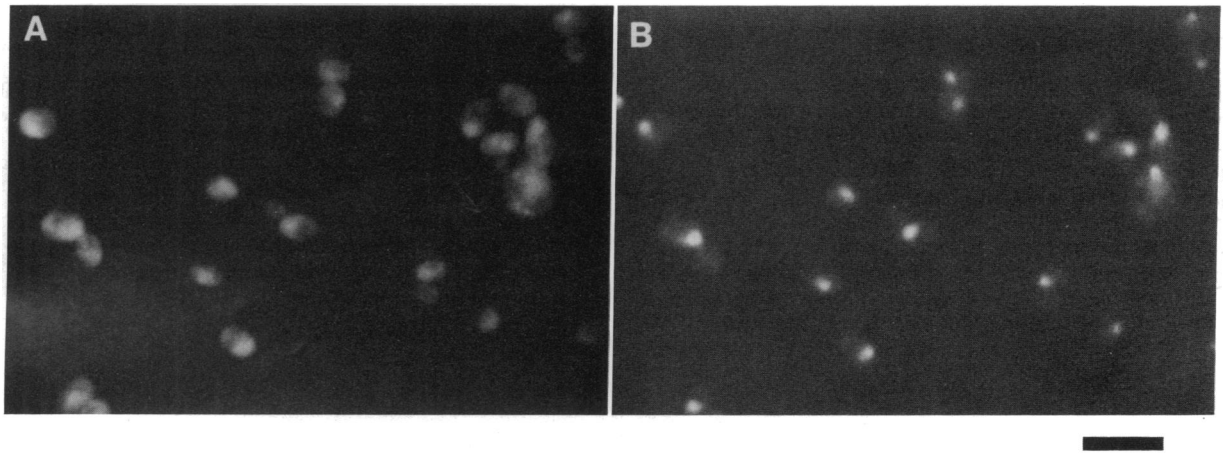

FIG. 4. Subcellular localization of the CDC7 protein. Yeast strain SEY6210/pG12/pSYC758 was grown to stationary phase in synthetic complete (SC) medium (minus uracil and leucine) plus 2% glucose. Cells were harvested, washed twice with SC medium (minus uracil and leucine) containing 2% galactose, and resuspended in the same medium. At OD_{600} = 0.4, cells were fixed with formaldehyde, digested with β-glucuronidase and zymolyase 100T, immobilized on polylysine-coated slides, and stained with polyclonal anti-CDC7 antiserum (*A*) and Hoechst 33258 (*B*). (*A*) Viewing was under rhodamine excitation wavelength. (*B*) Yeast nuclei were visualized by staining with the dye Hoechst 33258. (Bar = 10 μm.)

DISCUSSION

The phenotype of *S. cerevisiae cdc7* mutants suggests that the *CDC7* gene product has multiple functions. CDC7 may be involved in the initiation of mitotic DNA synthesis, premeiotic recombination, formation of ascospores, and error-prone DNA repair (22, 23). To date, however, the molecular roles of CDC7 in those events have not been characterized. As a step toward investigating the functions of CDC7 in yeast, we have identified the CDC7 protein as a protein kinase in vegetatively growing yeast cells. [While our work was in review, a similar result was presented by others (24), although kinase activity could only be observed after overproduction.] We have also shown that the CDC7 protein is phosphorylated *in vivo* and is found in the nucleus as well as in the cytoplasm. The demonstration of protein kinase activity in the CDC7 protein is consistent with preliminary experiments showing that *in vitro* transcription and translation of the *CDC7* gene produces a protein kinase activity (25). CDC7 appears to be present at low abundance in yeast as predicted from the codon utilization analysis of the CDC7 open reading frame. We have, however, found a very faint band of p58 in immunoblot analysis performed with nuclear extracts of wild-type yeast strains when alkaline phosphatase conjugates were used to visualize the CDC7 protein (unpublished data).

As suggested by the amino acid sequence, the CDC7 protein seems to carry a protein kinase activity. Using exogenous histone H1 as a substrate, we demonstrated protein kinase activity in CDC7 immunoprecipitates of nuclear extracts of wild-type cells without overproduction. The histone phosphorylation was thermolabile in *cdc7-1* extracts and phosphorylation was proportional to the amount of p58 present. Elevated kinase levels in the strain overproducing p56 suggest that p56, as well as p58, is active as a kinase. In addition to acting as protein kinases, both p56 and p58 also appear to be substrates of a phosphorylating activity in the immunoprecipitates. It is not yet clear whether this represents autophosphorylation or coprecipitation of another kinase. Our finding that CDC7 protein is a phosphoprotein *in vivo* suggests that the *in vitro* phosphorylation may be physiologically significant.

According to indirect immunofluorescence and subcellular fractionation studies, both forms of CDC7 are concentrated in the nuclei of CDC7-overproducing cells. We are not sure whether the staining patterns reflect a role for CDC7 in both the cytoplasm and nucleus or if they simply result from the delocalization of the exclusively nuclear protein due to overproduction. The demonstration that CDC7 is associated with the nuclei of mitotic cells suggests that CDC7 may function in the mitotic cell cycle by phosphorylating proteins involved in the initiation of DNA synthesis and/or in the G_1/S-phase transition.

Although basal level expression requires an element in the promoter that is homologous to a c-fos-activating sequence, CDC7 mRNA remains at a constant level throughout the cell cycle when measured in cells released from α-factor arrest (11, 26). Furthermore, indirect evidence suggests that the CDC7 protein exists in excess of the amount required for a single mitotic cell division (11). Yet CDC7 is required at a discrete point in the cycle. It will be of interest to investigate whether phosphorylation of CDC7 protein, translocation to the nucleus, catalytic activity of the CDC7 protein kinase, and/or availability of a crucial substrate is regulated through the cell cycle.

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