Characterization of the CP Complex, an Abundant Dimer of Cdc68 and Pob3 Proteins That Regulates Yeast Transcriptional Activation and Chromatin Repression*

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Neil K. Brewster‡§, Gerald C. Johnston§¶, and Richard A. Singer‡¶**

From the Departments of §Biochemistry, ¶Microbiology and Immunology, and ¶¶Medicine, Dalhousie University, Halifax, Nova Scotia B3H 4H7, Canada

The Cdc68 protein of the yeast Saccharomyces cerevisiae is an essential nuclear protein that has been shown to be necessary for the trans-activation of many genes as well as for the maintenance of chromatin-mediated repression in the absence of trans-activation. These activities implicate the Cdc68 protein in the regulation of chromatin structure and/or function. Here we report that Cdc68 is found in association with another essential nuclear protein, Pob3, in what we term the CP complex. This dimer of Cdc68 with Pob3 is stable to partial purification, so that the functions of gene activation and repression that are assigned to Cdc68 are likely to be properties of the CP complex. The CP complex is highly abundant, suggesting that it may be widespread throughout chromatin.

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¶ A Terry Fox Cancer Research Scientist of the National Cancer Institute of Canada.

** To whom correspondence should be addressed: Dept. of Biochemistry, Dalhousie University, Halifax, Nova Scotia B3H 4H7, Canada. Tel.: 902-494-8847; Fax: 902-494-1355; E-mail: rasinger@ns.dal.ca.

transcriptionally active δ-element changes the pattern of transcription (reviewed in Refs. 5 and 6). Functional transcription at these genes can be restored either by a gene mutation or by extra copies of several genes. This "suppressor of Ty" (Spt) effect has been used to identify many proteins that affect chromatin structure, including the histones and the Cdc68/Spt16 protein, which is the subject of this report. The CDC68 gene and the genes encoding histones H2A and H2B are all grouped within the same category of SPT genes; for most of these genes, mutations or increased gene dosage confer similar effects on chromatin repression (6–11). The similar effects of altered activity or abundance for the histones and for Cdc68 are an indication that Cdc68 is involved in chromatin structure and/or chromatin remodeling.

Other genetic tests also implicate Cdc68 in chromatin repression. For example, Cdc68 is necessary for full repression when UAS sequences (trans-activator binding sites) are absent (8, 9, 12, 13). Similarly, Cdc68 maintains the HO gene in a transcriptionally inactive state when the Swi4-Swi6 transcription activator is absent (12). The repressive effects of Cdc68 at several genes can be overcome by the actions of the Swi-Snf complex (8). The mechanism underlying this Cdc68/Swi-Snf antagonism is not understood.

Studies using a temperature-sensitive mutant version of Cdc68 show that at a number of genes (7), including SUC2 and GAL1, the Cdc68 protein facilitates transcription as well as repression. In these temperature-sensitive mutant cells, many mRNAs become depleted, including some that encode essential proteins, a finding in accord with the essential nature of the CDC68 gene (8). The involvement of the Cdc68 protein in transcription as well as chromatin repression suggests that Cdc68 is responsible for maintaining chromatin in a configuration that facilitates proper gene regulation.

The Cdc68 protein is localized to the nucleus (14); we show here by purification and immunoprecipitation experiments that Cdc68 is found exclusively in a complex with another nuclear protein termed Pob3 (15), and we provide evidence that this complex may be a heterodimer of Cdc68 and Pob3. The putative Cdc68-Pob3 heterodimer, designated here the CP complex, is highly abundant, equivalent to the nucleosome in number; this characteristic also supports a role for the CP complex in chromatin structure and/or function. An increased gene dosage of either CDC68 or POB3 can partially overcome the need for the Swi-Snf complex for the expression of several genes, indicating that the stoichiometry of each component of the CP complex, and perhaps of the intact CP complex, with respect to other chromatin components is important for chromatin repression.

1 D. R. H. Evans, N. K. Brewster, Q. Xu, A. Rowley, B. Altheim, G. C. Johnston, and R. A. Singer, manuscript in preparation.
**ExPERIMENTAL PROCEDURES**

**Strains, Growth Conditions, and Extract Preparation—**Yeast strains are listed in Table I; cells were grown and yeast transformations were carried out as described by Xu et al. (13). For whole-cell extracts, 100-ml cultures were grown in YM1 liquid medium (13) and harvested at 2–8 × 10^9 cells/ml; cells were washed once with water and resuspended in one pellet volume of extraction buffer (50 mM NaCl, 40 mM Hepes, pH 7.4, 10% glycerol, 5 mM EDTA, 1 mM dithiothreitol, 0.1% Tween 20, 2 mM benzamidine, 1 mM phenylmethylsulfonyl fluoride, 5 μg/ml aprotinin, and 1 μg/ml concentrations of chymostatin, pepstatin A, and leupeptin) and lysed by glass bead breakage (16); insoluble material was removed by centrifugation at 12,000 rpm for 15 min in a microcentrifuge.

**Plasmids—** E. coli transformants were obtained by electroporation (17) and selected on 2× YT solid medium (13) containing 50 μg/ml ampicillin. Plasmid pETCDC68 comprises the 3.65-kbp 100-base pair restriction fragment from pGTEP encoding the HA epitope at its C-terminus. The 5′-TTAGCGCGCCGCTTGTAAAGAAGCTTTGAAACCC (containing the NotI site) and 5′-TCTAGCTTCTTCAATGCATTGACAAATTG (formed by PCR with the primers 5′-AGGTCACTTCGAACCCGCTGTTGCTTCAG-3′ and pJW18 5′-AGGTCACTTCGAACCCGCTGTTGCTTCAG-3′, respectively, obtained from plasmids pJW18 and pJW19 (15), between the KpnI and XbaI sites of YEpPOB3 (16). Plasmid pGADPOB3 was constructed by inserting a 1.9-kbp KpnI–XbaI fragment and a 2-kbp XbaI–SpI fragment, respectively, obtained from plasmids pJW18 and pJW19 (15), between the KpnI and SpI sites of YEPR352 (16). Plasmid pGADPOB3 was constructed by inserting a 1.9-kbp PCR product encoding the complete open reading frame of POB3 between the EcoRI and BamHI sites of pGAD424 (20). The POB3 PCR product was generated using primers 5′-TATGAGCAGACTCGAGGATCCCTAGAGGCAGCCAGCCAGCCACAGCTAGGAGGATC-3′ and pJW18 5′-TATGAGCAGACTCGAGGATCCCTAGAGGCAGCCAGCCACAGCTAGGAGGATC-3′, respectively.

**Immunoprecipitation and Western Analysis—** Immunoprecipitations were carried out (21) on whole-cell extracts or partially purified CP complex. For whole-cell extracts, 200 μl (2–3 mg of soluble protein) was first incubated with protein A beads (40 μl; Sigma) for 15 min at 4 °C, which were then removed by centrifugation. The extract cleared in this way of nonspecific binding material was then incubated at 4 °C for 20 min with 20–40 μl of protein A-agarose coupled to preimmune serum at a ratio of 1:2 to remove nonspecific IgG-binding material. The supernatant of this incubation was then treated with 20–40 μl of protein A-agarose coupled to preimmune serum at a ratio of 1:2 to remove nonspecific IgG-binding material. The supernatant of this incubation was then treated with 20–40 μl of protein A-agarose coupled to preimmune serum at a ratio of 1:2 to remove nonspecific IgG-binding material. The supernatant of this incubation was then treated with 20–40 μl of protein A-agarose coupled to preimmune serum at a ratio of 1:2 to remove nonspecific IgG-binding material. The supernatant of this incubation was then treated with 20–40 μl of protein A-agarose coupled to preimmune serum at a ratio of 1:2 to remove nonspecific IgG-binding material.

**Immunoprecipitation experiments also used the monoclonal anti-HA antibody 12CA5 (Boehringer Mannheim).**

**RESULTS**

**Yeast Chromatin CP (Cdc68-Pob3) Dimer**

**Table I Yeast strains used in this study**

| Strain            | Genotype* or phenotype | Source |
|-------------------|------------------------|--------|
| 21R               | MATa ura3–52 leu2–3,112 ade1 | Ref. 7 |
| FY56              | MATa ura3–52 his4–9125 lys2–1286 | Ref. 8 |
| FY712             | MATa snf5::URA3 ura3–52 leu2–3,112 his4–9125 lys2–1286 | F. Winston |
| DE4B–17b          | MATa cd68–392–32 leu2–3,112 trp1–1 his4–9125 lys2–1286 | This laboratory |
| DE4B–17c          | MATa cd68–392–392 leu2–3,112 trp1–1 his4–9125 lys2–1286 | This laboratory |
| DE4B–17d          | MATa leu2–3,112 ura3–52 trp1 his4–9125 lys2–1286 | FY712 × DE4B–17b segregant |
| SH711             | MATa leu2–3,112 ura3–52 trp1 his4–9125 lys2–1286 | Ref. 15 |
| 4053–5–4          | MATa leu2 ura3 trp1 his7 POB3–200[N]URA3 | Integrative transformant of 4053–5–4 |
| 4053HA            | MATa leu2 ura3 trp1 his7 POB3–200[N]URA3 | 4053HA × D858–11c |
| 68507A            | MATa cd68–1 ura3–52 Ade | Ref. 7 |
| QX8611            | MATa cd68–101::LEU2[CDC68–200N TRP1] leu2–3,112 trp1–1 his4–9125 lys2–1286 | Ref. 14 |
| QX8611            | MATa cd68–101::LEU2[CDC68–200N TRP1] leu2–3,112 trp1–1 his4–9125 lys2–1286 | Integrative transformant of QX8611 |
| NB687A            | MATaMATa cd68–101::LEU2[CDC68–200N TRP1] leu2–3,112 trp1–1 his4–9125 lys2–1286 | qX8611 × 68507A |
| 2041–3–2          | MATa leu2 ura3 trp1 his7 pob3::TRP1 (pJWA) | Strain 2041 segregant |
| 2041–3–2 (pGADPOB) | MATa leu2 ura3 trp1 his7 pob3::TRP1 (pGADPOB) | pGADPOB in place of pJWA |
| 2041–3–2 (YEppPOB) | MATa leu2 ura3 trp1 his7 pob3::TRP1 (YEppPOB) | YEppPOB in place of pGADPOB |

* Square brackets enclose plasmid-derived sequences integrated in single copy.
chymotrypsinogen (Kirkegaard & Perry Laboratories). Antibody 12CA5 was used at a 1:1000 dilution and was detected with horseradish peroxidase-linked anti-mouse IgG (Kirkegaard & Perry) at 1:5000.

**Gel Filtration and Ion Exchange Chromatography**—Gel filtration was performed using S-300 Sephacryl (Pharmacia-Uppjohn) in a 2.6 × 100-cm column. Whole-cell extract was clarified by centrifugation at 10,000 × g for 15 min and then at 100,000 × g for 3 h. Five mg (0.25 ml) of clarified extract was loaded onto the column and eluted at 0.5 ml/min into 1-ml fractions. Material from fractions was concentrated by precipitation with 18% trichloroacetic acid, washed with 100% acetone, redissolved in SDS-polyacrylamide gel electrophoresis loading buffer, and resolved electrophoretically through an 8% SDS-polyacrylamide gel in preparation for Western blot analysis.

For ion exchange chromatography, 250–500 mg of soluble protein was loaded onto a 10-ml DEAE-Sepharose column equilibrated with extraction buffer modified to contain 200 mM NaCl. The column was then washed with 10 column volumes of this buffer and eluted with 2 column volumes of a 0.3–0.5 M NaCl gradient in extraction buffer. Fractions (0.5 ml) were collected and assayed by Western blot analysis. For certain immunoprecipitation experiments, whole-cell extracts were fractionated by batch chromatography; DEAE-Sepharose beads (0.5 ml), equilibrated in extraction buffer containing 0.2 M NaCl, were mixed with 1 ml of whole-cell extract (10–20 mg), harvested by centrifugation, washed with 4 volumes of extraction buffer, and eluted with 0.5–1 ml of extraction buffer containing 0.5 M NaCl.

**Preparation of Recombinant Cdc68 Protein**—E. coli cells harboring pETCDC68 were grown in 2YT medium under ampicillin selection, induced at OD 0.8 by incubation for 3 h at 37 °C with 1 mM isopropyl-1-thio-β-D-galactopyranoside, and harvested for the preparation of inclusion bodies containing recombinant Cdc68 protein (18, 25). Inclusion body protein was solubilized in SDS-polyacrylamide gel electrophoresis loading buffer (23), resolved by electrophoresis through an 8% denaturing polyacrylamide gel, and visualized by Coomassie staining without fixative. Recombinant Cdc68 polypeptide was electroeluted from the gel in Laemmli buffer (23) and made up in 400 μl of 10 mM Tris, pH 7.5, 1 mM EDTA. Cdc68 protein purified in this way was resolved by 8% SDS-polyacrylamide gel electrophoresis, stained with Coomassie Blue, and quantified by comparing the intensities of the recombinant Cdc68 bands with those of known amounts of bovine serum albumin similarly resolved and stained.

**RESULTS**

**A Cdc68 Complex**—For investigations of Cdc68 interactions, we raised polyclonal antibody against the entire Cdc68 polypeptide. These antibodies were then used to detect Cdc68 protein, by immunoblotting, in material from whole-cell extracts that had been resolved on a sizing column. This analysis suggested that the Cdc68 protein is part of a complex (Fig. 1A). Compared with the elution pattern of standard proteins (indicated in Fig. 1A) the Cdc68 complex migrates at approximately 400 kDa. Virtually none of the 118-kDa Cdc68 protein was detected in monomeric form (Fig. 1A). The Cdc68 protein therefore functions as part of a complex to regulate chromatin.

**A 70-kDa Polypeptide Is in the Cdc68 Complex**—To identify other components of the Cdc68 complex, we employed a co-immunoprecipitation approach, using anti-Cdc68 antibody to precipitate proteins from whole-cell extracts. Along with the Cdc68 protein a 70-kDa polypeptide (p70) was also immunoprecipitated (Fig. 2A, lane 2). Neither Cdc68 nor p70 was precipitated by the preimmune control serum (lane 1), indicating that this immunoprecipitation was specific for Cdc68 and associated proteins. A Western blot of these immunoprecipitates probed with polyclonal anti-Cdc68 antibody identified the intact 118-kDa Cdc68 protein but not p70; thus p70 is a novel protein rather than a proteolytic fragment of Cdc68 (data not shown). As an additional test of the specificity of p70 interaction with Cdc68, we determined if p70 interacts with a truncated Cdc68 protein missing residues 6–306. This protein, the product of the cdc68-Δ922 gene,1 co-immunoprecipitated p70 with an efficiency equivalent to that of full-length Cdc68 (Fig. 2B, lanes 2 and 3). Therefore, interaction with p70 is a property of the C-terminal ½ of the Cdc68 protein, which supplies all essential Cdc68 functions.1

The Cdc68 complex was partially purified by ion exchange and molecular sieve chromatography (Fig. 1B). We found that Cdc68 has a high affinity for DEAE-Sepahcel, remaining bound to this matrix in 0.2 M NaCl. Protein was eluted from the DEAE column using a NaCl gradient from 0.3 to 0.5 M, and fractions containing Cdc68 protein were pooled and resolved by gel filtration. Cdc68 partially purified in this way still eluted in the 400-kDa size range, indicating that the Cdc68 complex is stable under these ionic conditions (Fig. 1B). Analysis of the material in the 400-kDa size range (fractions 35–38) showed that Cdc68 and p70 were the only proteins detected by immunoprecipitation with anti-Cdc68 antibodies (Fig. 1B, lanes 1 and 2). These observations suggested that p70 remains associated with Cdc68 during this purification.

To determine whether the migration of p70 in the 400-kDa size range depends on the presence of Cdc68 protein, we used a strain that harbors a mutant Cdc68 protein with temperature-
sensitive function (7). This mutant protein, Cdc68-1, is intrinsically unstable at high temperatures, resulting in loss of Cdc68-1 protein from the cell (14). A population of these mutant cells was grown at 23 °C, a temperature at which the Cdc68-1 mutant protein is functional, and half of this growing culture was then shifted to 37 °C for a further 1-h incubation to allow the depletion of the Cdc68-1 mutant protein. The amount of immunoprecipitated Cdc68-1 protein was markedly decreased in the 37 °C extract compared with the 23 °C extract (Fig. 2A, lanes 6 and 8). Moreover, the amount of immunoprecipitated p70 decreased in parallel with the abundance of the Cdc68-1 mutant protein (Fig. 2A, lanes 6 and 8), showing that the immunoprecipitation of p70 depends on the presence of Cdc68. The 23 °C and 37 °C cell extracts were then resolved by gel filtration as described above to determine whether p70 continues to migrate in the 400-kDa size range in the absence of Cdc68. Immunoblotting showed that the Cdc68-1 protein in the 23 °C extract fractionated in the 400-kDa range, while as expected the equivalent fractions of the 37 °C extract had markedly less Cdc68-1 protein (Fig. 3A, fractions 30–33). We then analyzed the protein content of both sets of fractions of the 400-kDa range (fractions 30–33) on a silver-stained polyacrylamide gel. As shown in Fig. 3B, p70 was present in the 400-kDa material from the 23 °C extract but absent from the equivalent set of fractions of the 37 °C extract that was also devoid of Cdc68-1 protein. We conclude that incorporation of p70 into large complexes depends on the presence of Cdc68 protein.

Pob3 Is the 70-kDa Protein That Associates with Cdc68—An approximately 70-kDa protein that affects the function of mutant Cdc68 proteins is San1 (13); however, extracts of san1Δ null mutant cells still gave co-immunoprecipitation of p70 (data not shown), suggesting that San1 is not a Cdc68-associated p70 protein. Another potential Cdc68-associated p70 protein, Pob3, was initially identified by its interaction with the catalytic (α-) subunit of DNA polymerase I, the same study also found this interaction for Cdc68 (15). We tested whether p70 is Pob3 by creating a detectable variant of the Pob3 protein for this we used the activation domain (AD) of the Gal4 transcription factor to tag Pob3 at its N terminus. The resultant AD-Pob3 chimeric polypeptide, expressed from the multicopy plasmid pGADPOB3, was first assessed for in vivo function, using cells in which the chromosomal POB3 gene had been replaced with the TRP1 gene (pob3Δ:TRP1). POB3 is an essential gene (15), so the viability of these pob3Δ mutant cells was maintained by the presence of the wild-type POB3 gene on a low copy plasmid (pRSPOB3). Using a plasmid-shuffling approach (26), we demonstrated that the pGADPOB3 plasmid could replace the wild-type POB3 plasmid and sustain the viability of the pob3Δ mutant cells. Indeed, the growth of pob3Δ mutant cells harboring pGADPOB3 was indistinguishable from that of wild-type POB3 cells and of pob3Δ mutant cells harboring the wild-type POB3 plasmid. Thus, the AD-Pob3 protein provides Pob3 function.

We then assessed the physical association of AD-Pob3 with Cdc68. Extracts prepared from pob3Δ cells harboring pGADPOB3 were partially purified by ion exchange chromatography and treated with anti-Cdc68 polyclonal antibodies, and the immunoprecipitated proteins were resolved electrophoretically and visualized by silver staining. The co-immunoprecipitating p70 protein detectable in this way from extracts of wild-type cells or of pob3Δ mutant cells expressing wild-type POB3 from plasmid YEpPOB3 (Fig. 4A, lane 2) was not evident in extracts from pob3Δ mutant cells harboring pGADPOB3. Instead, a polypeptide of approximately 80 kDa, the expected size of AD-Pob3, was found to co-immunoprecipitate with Cdc68 (Fig. 4A, lane 1). An analogous co-immunoprecipitation of Pob3 by our anti-Cdc68 antibody has been reported (15). These results show that the p70 protein that co-immunoprecipitates with Cdc68 is Pob3.

To confirm that Pob3 associates with Cdc68, we carried out
immunoprecipitation experiments using another modified version of Pob3, one containing an N-terminal extension that encodes the HA epitope. The HA-tagged version of Pob3 used for this analysis was expressed from the \textit{POB3} chromosomal locus; expression was verified by detecting HA-Pob3 in immunoblots of whole-cell extracts using the anti-HA monoclonal antibody 12CA5 (data not shown). Cells expressing HA-Pob3 rather than Pob3 itself were indistinguishable from wild-type cells, demonstrating that this modified Pob3 protein supplies essential Pob3 functions. The HA-Pob3 protein could be immunoprecipitated from a whole-cell extract using anti-HA antibody, verifying the specificity of these immunoprecipitations (Fig. 4). As expected, neither untagged Pob3 nor untagged Cdc68 was immunoprecipitated with the anti-HA antibody, verifying the specificity of these immunoprecipitations (Fig. 4B). This co-immunoprecipitation confirms that Pob3 is the p70 component of the Cdc68 complex, which we henceforth refer to as the CP (Cdc68-Pob3) complex. The CP Complex Is a Heterodimer of Cdc68 and Pob3—The CP complex resolves on a sizing column at 400 kDa, a size greater than the sum of Cdc68 (118.5 kDa) and Pob3 (63 kDa). We therefore analyzed the composition of the CP complex by first investigating the stoichiometry of Cdc68 in the CP complex. For this we used an HA-tagged version of Cdc68 that supplies Cdc68 function (14). A diploid strain was constructed to contain both HA-tagged and untagged versions of Cdc68. These different functional forms of Cdc68 can be distinguished electrophoretically, because the HA tag increases the size of the Cdc68 protein by approximately 4.5 kDa. Using a whole-cell extract prepared from diploid cells containing both tagged and untagged Cdc68, we found that anti-Cdc68 antibody immunoprecipitated both Cdc68 and HA-Cdc68, and that both versions of Cdc68 were of equal abundance (Fig. 5A, lane 4). HA-tagged Cdc68 and untagged Cdc68 were also immunoprecipitated with anti-Cdc68 antibody from extracts of haploid cells to facilitate identification of each form of Cdc68; the smaller Cdc68 protein in the diploid extract had the electrophoretic mobility of untagged Cdc68 protein (Fig. 5A, lane 3), whereas the larger version co-migrated with HA-Cdc68 (Fig. 5A, lane 1). We then immunoprecipitated HA-Cdc68 from the diploid extract with the anti-HA antibody and looked for untagged Cdc68 in the HA-Cdc68 immunoprecipitate. Using polyclonal anti-Cdc68 antibody to probe a blot of the HA-Cdc68 immunoprecipitate, we detected HA-Cdc68 protein, as expected, but not untagged Cdc68 protein (Fig. 5A, lane 2). To obtain better resolution of the HA-tagged and untagged forms of Cdc68 protein, an analogous experiment was performed using the truncated \textit{cdc68-D922} form of Cdc68, with identical results; immunoprecipitation with anti-HA antibody did not co-immunoprecipitate the untagged Cdc68 protein present in the same cell (Fig. 5B). These findings suggest that there is only one molecule of Cdc68 protein in each CP complex.

The silver-stained gel of purified CP complex (Fig. 1B) suggests a 1:1 stoichiometry of Cdc68 and Pob3. The molar ratio of Cdc68 to Pob3 in the CP complex was also determined by a different method. From haploid cells that contained chromosomal genes expressing HA-Cdc68 and HA-Pob3 instead of
untagged Cdc68 and Pob3, an extract was prepared and treated with anti-Cdc68 antibody. Replicate Western blots of the anti-Cdc68 immunoprecipitate, using several transfer times (data not shown), were then probed with anti-HA monoclonal antibody to detect both HA-Cdc68 and HA-Pob3. In every case, the signal generated by the anti-HA antibody for HA-Pob3 was no stronger than that for HA-Cdc68 in the anti-Cdc68 immunoprecipitate (Fig. 5C, lanes CP and in total cell extract (Fig. 5C, lanes WCE). These analyses suggest that Cdc68 and Pob3 are present in the CP complex in a 1:1 molar ratio.

Additional CP components were sought. Chemical cross-linking experiments did not suggest the presence of additional proteins or indicate dimer interactions (data not shown). When the CP complex was immunoprecipitated from extracts of cells grown for several generations in medium containing [35S]methionine, we readily observed [35S]-labeled Cdc68 and Pob3 in the anti-Cdc68 immunoprecipitate using autoradiographic procedures but did not detect any other proteins specific to the Cdc68 immunoprecipitate (data not shown). Moreover, there is no other protein in the CP complex of a size similar to that of Cdc68 or Pob3. The replacement of Pob3 with the larger AD-Pob3 protein did not unmask any other 70-kDa component in anti-Cdc68 immunoprecipitates (Fig. 4A), and we did not see any 120-kDa proteins in silver-stained anti-Cdc68 immunoprecipitates when the only Cdc68 protein was the functional but truncated 90-kDa version of Cdc68 (Fig. 2B). Despite previous indications (15), the polymerase I protein was not immunoprecipitated by our anti-Cdc68 antibody, although it was detected in the supernatant (data not shown). Neither extraction and immunoprecipitation under less stringent conditions, using buffer of lower ionic strength (Fig. 2A, lanes 3 and 4), nor the replacement of NaCl with ammonium sulfate or potassium acetate, affected the ratio of Cdc68 to Pob3 or led to the identification of additional CP components (data not shown). Thus, under the conditions used here, the CP complex is most likely a heterodimer of Cdc68 and Pob3.

The CP Complex Is Abundant—the Cdc68 protein potentiates global transcriptional activation and also relieves chromatin repression at several core promoters (8). It is a multiprotein complex that has chromatin-remodeling activity in vitro and in vivo (2). Several yeast genes depend on the Swi-Snf complex for effective expression in response to various conditions (5, 12). Several yeast genes depend on the Swi-Snf complex for effective expression in response to various conditions (5, 12). The Cdc68 protein potentiates global transcriptional activation and also relieves chromatin repression at several core promoters (8). It is a multiprotein complex that has chromatin-remodeling activity in vitro and in vivo (2). Several yeast genes depend on the Swi-Snf complex for effective expression in response to various conditions (5, 12).

Increased Abundance of Pob3 or Cdc68 Decreases the Need for the Swi-Snf Chromatin-remodeling Complex—The CDC68 gene is one of several genes that can affect gene expression through gene dosage effects. Plasmid-borne CDC68, when present in multiple copies in wild-type cells, can restore functional transcription to certain genes that have been inactivated by the insertion of the retrotransposon Ty1 long terminal repeat (8) into promoter regions (8). In an attempt to understand the molecular basis of this Spt’ effect (5, 6), caused by excess Cdc68 protein, we assessed levels of CP complex components under multicity CDC68 conditions. From wild-type cells harboring the CDC68 gene on a multicopy plasmid, which increases Cdc68 abundance by approximately 10-fold (Fig. 6, lanes 3 and 4), greater Cdc68 protein amounts were immunoprecipitated by anti-Cdc68 antibody, but these increased Cdc68 levels were not accompanied by increased amounts of immunoprecipitated Pob3 (data not shown). Therefore, the effects of CDC68 gene dosage are most likely due to excess Cdc68 protein in a non-CP context, which may inhibit or sequester an interacting component. Whereas a multicopy CDC68 plasmid produced the expected Spt’ effect and restored His’ and Lys’ growth to cells harboring the his4–912 dys2–1286 strain DE4B-17d harboring the CDC68 plasmid pSH65, the POB3 plasmid YEpPOBKE, the vector YEp352, or both pH SH55 and YEPOBKE were spread on complete synthetic medium lacking either histidine or lysine and incubated at 30 °C for 3 days.
for SUC2 expression (8). Increased CDC68 gene dosage also overcomes the effects of a suc2-snf2 mutation for expression of the HO gene.3 We therefore determined the ability of CDC68 and POB3 gene dosage to overcome transcriptional defects caused by decreased Swi-Snf function. Swi-Snf mutations such as snf5Δ generate an inositol auxotrophy due to impaired expression of the INO1 gene (27) and prevent effective growth using sucrose or raffinose as a carbon source due to impaired SUC2 expression (28). The increased gene dosage of either POB3 or CDC68 was able to mitigate the effects of a snf5Δ mutation and restore growth in the absence of exogenous inositol (Fig. 7A). Similarly, either POB3 or CDC68 in increased dosage diminished the effects of a snf5Δ mutation for SUC2 expression, an effect seen as increased growth using sucrose as the carbon source (Fig. 7A). The AD-Pob3 derivative expressed from a multicopy plasmid also restored Ino+ and Suc+ growth to snf5Δ mutant cells and thus has effects similar to those of Pob3 itself (data not shown). These findings indicate that excess Pob3, like excess Cdc68, does indeed affect gene expression.

In this light, the inability of excess Pob3 to exert an Spt− effect suggests that the Spt− effect of excess Cdc68 and the Swi-Snf “bypass” brought about both by Pob3 and by Cdc68 may reflect related but different effects on chromatin.

DISCUSSION

The Cdc68 protein of yeast is an essential nuclear protein that allows trans-activators to function at a number of genes and also maintains chromatin-mediated repression in the absence of trans-activation (7, 8, 12, 14).1 These activities implicate the Cdc68 protein in the regulation of chromatin structure and/or function. Here we report that Cdc68 is found associated with another essential nuclear protein termed Pob3; we have named this heterodimer the CP complex. Both Cdc68 and Pob3 have structural homologs in mammalian cells and diverse eukaryotes1,4 (15); therefore, the CP complex may be a general eukaryotic feature. The association of Cdc68 with Pob3 is relatively stable; therefore, the functions in gene activation and repression that are associated with Cdc68 are likely to be properties of the CP complex. Neither Cdc68 nor Pob3 has been found in any other transcription complex, including the RNA polymerase II holoenzyme, which was specifically tested for the presence of Cdc68 with our anti-Cdc68 antibody.5 Therefore the CP complex probably functions as an independent nuclear regulator. The abundance of the CP complex suggests that it may be widespread throughout chromatin. Indeed, the CP complex, at ~50,000 copies/haploid nucleus, is approximately as abundant as the nucleosome.

The CP heterodimer of Cdc68-Pob3 has a calculated size of 182 kDa, less than the apparent size of 400 kDa that is indicated by molecular sieve chromatography. Our assessment of the composition of the CP complex indicates that the significantly larger apparent size is unlikely to be due to the presence of additional proteins in the CP complex, while stoichiometry estimations suggest that the CP complex contains only one molecule each of Cdc68 and of Pob3. The difference between actual and apparent sizes therefore suggests that the CP complex may adopt a nonspherical shape, with the inherently larger Stokes radius of a nonspherical particle accounting for the larger hydrodynamic size that determines migration during gel filtration. These findings relate to the predominant form of the CP complex; less abundant proteins may well associate with the basic CP complex, leading to minor CP variants.

3 Q. Xu, G. C. Johnston, and R. A. Singer, unpublished observations.
4 G. Orphanides and D. Reinberg, personal communication.
5 D. Chao and R. A. Young, personal communication.

The transcription effects of increased CDC68 and POB3 gene dosage are intriguing yet poorly understood mechanistically. An excess of Cdc68 or Pob3, as shown here, does not compromise the ability of CP to allow trans-activator function. In contrast, the ability of the CP complex to maintain a repressive conformation of chromatin is compromised by excess Cdc68 or Pob3. This effect is indicated by the restoration, by excess Cdc68 or Pob3, of transcription in cells with a mutagenically impaired Swi-Snf chromatin-remodeling complex. The effects of excess CP components may be analogous to the “squelching” effects on transcription that can be exerted by the overexpression of strong trans-activators and consequent sequestration of other factors needed for transcription initiation (29). Cdc68 or Pob3 in excess may sequester factors needed for chromatin repression; the appropriation of such repressive factors by excess, probably nonchromatin, CP components may alter chromatin conformation in ways related to Swi-Snf-mediated chromatin remodeling. Transcription in the absence of Swi-Snf activity is also seen when Cdc68 and Pob3 are both present in excess. Under these conditions, most of the excess protein may exist as the stable Cdc68-Pob3 complex. The gene dosage effects in this situation suggest that the hypothesized sequestration of factors may not take place through surfaces dedicated to Cdc68-Pob3 interaction but instead may reflect bona fide interactions that occur normally with the CP complex. An analogous restoration of transcription from the normal promoter at the his4–9125 δ-insertion allele may also be a manifestation of sequestration. HIS4 transcription is facilitated by the Swi-Snf complex (30, 31), raising the possibility that sequestration of a Swi-Snf target by Cdc68 protein may be responsible for the functional his4–9125 transcription seen in Cdc68 excess. However, excess Pob3 is unable to exert a similar effect, suggesting that excess CP components may alleviate the need for the Swi-Snf complex and restore functional transcription to the δ-insertion alleles by different mechanisms.

The CP complex resembles other nuclear proteins in structure and/or function. Foremost among these are the yeast Spt5 and Spt6 proteins. Like Cdc68 and Pob3, Spt5 and Spt6 are both essential proteins, and each has a domain that is highly acidic, although the acidic domains are at the N terminus for Spt5 and Spt6 (32–34) rather than at the C terminus as found for both CP components (7, 15). As found for the CP components, structural homologs of Spt5 and Spt6 exist in vertebrates and other eukaryotes (35–38). Similarities may also extend to function, as suggested by the effects of mutations. Like cdc68 mutations, spt5 and spt6 mutations allow functional expression of the his4–9125 and lys2–1288 mutant alleles, decrease the need for the Swi-Snf complex for the expression of several genes, and allow transcription from core promoters (9, 32–34, 39–42). These effects implicate these Spt proteins in chromatin repression (5). Consistent with a chromatin role, the Spt6 protein interacts directly with histone H3, and spt6 mutations can alter chromatin structure (43). Recent evidence indicates a role for the Spt5 and Spt6 proteins in the elongation phase of transcription (44, 45); it remains to be seen if the CP complex has a similar function. Gene dosage effects suggest that the CP complex and these Spt proteins may be functionally distinct. For example, while increased SPT6 gene dosage allows transcription from core promoters (32), neither CDC68 nor POB3 in increased dosage has been found to exert this effect.6 Moreover, increased SPT5 or SPT6 dosage restores functional transcription of his4–9125 or lys2–1288, while increased POB3 dosage does not. Nonetheless, as found for the

6 N. K. Brewster, G. C. Johnston, and R. A. Singer, unpublished observations.
CDC68 gene, increased dosage of the SPT5 or SPT6 gene can decrease the need for the Swi-Snf complex and restore functional transcription at the his4–9125 and lys2–1286 alleles (32–34). Therefore, the CP complex and the Spt proteins may work in overlapping but distinct ways to regulate chromatin structure.

Functions of the CP complex may extend beyond transcription. Both components of the CP complex can be pulled out of cell extracts by physical interaction with the catalytic subunit of DNA polymerase α, and Cdc68 has been implicated genetically in the function of DNA polymerase (15). These interactions suggest a role for the CP complex in modulating the accessibility of DNA to DNA polymerase, analogous to a role for the CP complex in modulating the accessibility of DNA to RNA polymerase II. The CP complex may generally control access to chromatin-associated DNA.

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