A Bipartite Yeast SSRP1 Analog Comprised of Pob3 and Nhp6 Proteins Modulates Transcription

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The FACT complex of vertebrate cells, comprising the Cdc68 (Spt16) and SSRP1 proteins, facilitates transcription elongation on a nucleosomal template and modulates the elongation-inhibitory effects of the DSIF complex in vitro. Genetic findings show that the related yeast (Saccharomyces cerevisiae) complex, termed CP, also mediates transcription. The CP components Cdc68 and Pob3 closely resemble the FACT components, except that the C-terminal high-mobility group (HMG) box domain of SSRP1 is not found in the yeast homolog Pob3. We show here that Nhp6a and Nhp6b, small HMG box proteins with overlapping functions in yeast, associate with the CP complex and mediate CP-related genetic effects on transcription. Absence of the Nhp6 proteins causes severe impairment in combination with mutations impairing the Swi-Snf chromatin-remodeling complex and the DSIF (Spt4 plus Spt5) elongation regulator, and sensitizes cells to 6-azauracil, characteristic of elongation effects. An artificial SSRP1-like protein, created by fusing the Pob3 and Nhp6a proteins, provides both Pob3 and Nhp6a functions for transcription, and competition experiments indicate that these functions are exerted in association with Cdc68. This particular Pob3-Nhp6a fusion protein was limited for certain Nhp6 activities, indicating that its Nhp6a function is compromised. These findings suggest that in yeast cells the Cdc68 partners may be both Pob3 and Nhp6, functioning as a bipartite analog of the vertebrate SSRP1 protein.

Transcription of eukaryotic protein-coding genes takes place in the context of chromatin. In chromatin, DNA is associated with histone proteins in a nucleosomal configuration and with other proteins that have structural and/or regulatory roles. This intricate assembly of structural and regulatory proteins with DNA is generally inhibitory for transcription through the interference with transcription activator proteins and general transcription factors (reviewed in reference 57) and obstruction of the RNA polymerase II (RNAP II) elongation complex by nucleosomes (8; reviewed in reference 57). The processivity of elongation complexes is also inhibited by several nonnucleosomal chromatin components (19, 50, 60). Among the proteins regulating both nucleosomal and nonnucleosomal transcriptional inhibition is one named FACT (51).

FACT is an abundant nuclear protein complex that has been purified and characterized based on its ability to allow transcription elongation along a nucleosomal template in vitro (27, 33, 34). In keeping with this activity, the FACT complex (also termed DUF [32]) has been shown to interact with nucleosomes and with histone H2A-H2B dimers and to have DNA-untwisting activity in vitro (32, 34). FACT has also been found to counteract the inhibitory effects on transcription elongation imposed in vitro by the DSIF complex (51). In the budding yeast Saccharomyces cerevisiae, FACT has a structural, and possibly functional, homolog in the form of the CP complex, which has been implicated by genetic evidence in the interaction of chromatin with components of the transcription and replication machineries (5, 13, 29, 30, 39, 55, 56, 60) and in the process of transcription elongation in vivo (34). Thus, genetic and biochemical evidence suggests that FACT or CP facilitates these activities by regulating chromatin. The CP complex can be purified from yeast cells as a 1:1 complex of the Cdc68 protein and the Pob3 protein (5, 56). Similarly, FACT from vertebrate cells is composed of a 1:1 complex of the human version of the Cdc68 protein and SSRP1, the human structural analog of the yeast protein Pob3 (32, 34).

The yeast and human Cdc68 proteins are structurally related throughout their lengths (34). Likewise, throughout its length the yeast Pob3 protein is similar to SSRP1 proteins from various sources (55; our analysis). However, the Pob3 protein does not contain sequences related to the C-terminal portion of SSRP1. These C-terminal SSRP1 sequences absent from Pob3 include a high-mobility group (HMG) box domain, suggesting that HMG sequences not found in Pob3 may be provided to the CP complex by another protein.

Yeast cells do indeed contain an HMG protein that resembles the HMG box domain of SSRP1. This yeast protein, termed Nhp6, is present as two virtually identical isoforms, Nhp6a and Nhp6b, differing slightly in length and encoded by a gene pair (24). Nhp6 is small, consisting of only 93 (Nhp6a) and 99 (Nhp6b) residues, and highly abundant: the combined amounts of Nhp6a and Nhp6b are at least 50,000 copies per haploid nucleus (36), somewhat greater than the abundance of the CP complex (5, 56) and about equal to the number of nucleosomes.

In this report, we describe biochemical and genetic studies showing that the small HMG box protein Nhp6 associates and functions with the yeast complex of Pob3 and Cdc68 proteins and mediates CP-related transcriptional effects in vivo. Our
finding that Pob3 and Nhlp6 associate in the same complex suggests that these two yeast proteins, structurally analogous to different domains of the vertebrate SSR1 protein, may function as a bipartite yeast analog of SSR1.

MATERIALS AND METHODS

Growth conditions and extract preparation. Yeast strains are listed in Table 1. Cells were routinely grown at 30°C or, if temperature sensitive, at 23°C, with orbital shaking in 50 to 100 ml of adenine-supplemented (20 mg/liter) rich medium to 1 × 10^6 to 5 × 10^6 cells/ml; plasmid-bearing cells were grown in enriched selective medium (5). Whole-cell extracts were prepared by pressurizing a suspension of cells (×10^9 cells/ml) in extraction buffer (NaCl or potassium acetate at the indicated concentration, 50 mM HEPES [pH 7.4], 10% glycerol, 1 mM dithiothreitol, 5 mM EDTA, 0.1% Tween 20, protease-inhibitor cocktail [5]) to 16,000 Ib/min in a French press, followed by slow release. The resultant suspension was centrifuged at 13,300 rpm for 10 min in an Eppendorf centrifuge, and the supernatant was saved as a whole-cell extract.

Immunoprecipitation and Western blots. Immunoprecipitations were performed on 0.4-mL volumes (2 to 5 mg of soluble protein/ml) using 6 µl of anti-Cdc68 antiserum or preimmune serum, 5 µg of antithiamin-gluatin (anti-HA) monoclonal antibody (12CA5; Boehringer Mannheim) or 2 µg of anti-V5 monoclonal antibody (Invitrogen) and a 1-h incubation at 4°C. A second 1-h 4°C incubation was then carried out with 25 µl of protein A-agarose or G-Sepharose beads (Sigma) and constant mixing. The resultant bead-bound immunoprotein mix was washed twice with 10 volumes of extraction buffer and then denatured at 90°C for 5 to 10 min in 50 µl of electrophoresis sample buffer. Proteins were visualized by the above antibodies on Western blots (5) of samples resolved electrophoretically on 8, 15, or 6-10-15% (step-gradient) polyacrylamide-sodium dodecyl sulfate gels. Nhlp6 proteins were detected using a 1/1,000 dilution of anti-Nhlp6 antisera (36) and overnight incubation at 4°C.

Table 1. Yeast strains

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<th>Strain</th>
<th>Genotype</th>
<th>Source or reference</th>
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<td>MATα leu2-3,112 ura3-52 trp1-1 his4-126 lys2-128B CDC68-200N POB3-200N, expressing HA-Cdc68 and HA-Pob3 proteins</td>
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<td>NB2641</td>
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<td></td>
<td></td>
<td>∆nhlp6a::LEU2</td>
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<td>pRS303 transformant</td>
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<td>MATα ura3-52 his4-126 lys2-128B suc2ΔUAS</td>
<td>F. Winston</td>
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<td>DE4B-20a</td>
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<td>DEAB (12) segregant</td>
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<td>NB3-24a segregant</td>
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<tr>
<td>FY1671</td>
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<td>NB40 segregant transformed with pPNT</td>
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<td>NB249 with trp1-1::HIS3</td>
<td>pTH4 (11) transformant</td>
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POB3-NHP6 fusion gene. Plasmid pYESPEN carries the POB3-NHP6 fusion gene under the control of the GAL1 promoter and was constructed by inserting a 300-bp PCR product containing the NHLP6a open reading frame (ORF), minus the first three codons, into the unique BstEII site of pYES2. A 2-µm-based URA3 plasmid carrying a C-terminally (V5)-tagged POB3 ORF in the pYES2GS vector (Invitrogen). The 300-bp NHLP6PCR product was generated from the plasmid pRJ1361 (61) template using primers 5′-AGCACAGCTCATATTGGCTACCACAAGAGACAAGTTAAGGAAGAACC (containing Ndel and BseHI sites) and 5′-AACGGCGGGGCGGCCGCGTACCGAACCCAAATGTTACCTTCTT (containing BseHI and BsrHI sites). The POB3-NHLP6 integration plasmid pYESPNAC was derived from pYESPEN by removing a 4-kbp ClaI fragment containing the 2-amino sequences and the 5′ portion of the POB3 ORF. A TRP1 version of pYESPNAC was constructed by ligating a 3.1-kbp Scal-ClaI fragment of pYESPEN with a 2.5-kbp Scal-ClaI fragment of pRS304 (47), generating pPNT. Replacement of the chromosomal POB3 gene with the POB3-NHLP6 fusion gene used pPNT linearized at a unique BcI1 site in the POB3 ORF and selection for Trp+ transformants.

NHLP6 plasmid manipulations. Plasmid p314NV5, a pRS314 (47) derivative carrying the NHLP6 promoter and ORF modified to encode C-terminally (V5 + His6)-tagged Nhlp6a protein, was generated by replacing a 300-bp Ndel-BamHI fragment of pRJ3164 with an 800-bp PCR product from pYESPEN, comprising the (V5 + His6)-tagged NHLP6 ORF and the CYC1 transcription terminator. PCR used primer 5′-TCTCTGGATCCCGCGATGTTAAAAGGCTTT-3′ (encoding a BamHI site) and the above-described primer encoding Ndel and BseHI sites. The 1.3-kbp Xhol-BamHI fragment of p314NV5 containing the entire tagged NHLP6A ORF, ~500 bp of 5′ noncoding sequence, and ~500 bp of 3′ plasmid sequences (including the CYC1 transcription terminator) was ligated into pRS424 and pRS423 (9) to generate pI24NV5 and pI23NV5, respectively. Plasmid pHAPN5, which expresses HA-Pob3 and Nhlp6a-V5 His6 proteins from independent genes, was generated by inserting a 1.3-kbp XhoI-BamHI p314NV5 fragment, blunt-ended with mung bean exonuclease, into the SmaI site of pRS424-HAPOB (see below). The NHLP6a-V5-His6 integration plasmid p303-NAXN was generated by blunt-end (exonuclease) ligation after removing a 500-bp XhoI-Ndel sequence from p303-NV5, which was
itself created by inserting a 1.3-kbp XhoI-BamHI NHP6A fragment from p314-NV5 into pRS303 (47). Replacement of the chromosomal NHP6A gene with the V5-tagged version used p303-NΔXN linearized with EcoRV and selection for His⁺ transformants.

**POB3 plasmid manipulations.** Plasmid p424-HAPOB was generated by inserting a 3-kbp KpnI-EcoRI fragment of YEpHAPOB (5), comprising the HA-Pob3 ORF plus 5' and 3' noncoding sequences, into pRS424. The integration plasmid pPH6TI carrying the C-terminal half of the ORF encoding Pob3-V5-His₆ was created by ligating a 2.8-kbp ClaI-ScaI fragment of pYESP with a 2.5-kbp ClaI-ScaI fragment of pRS304. Integration plasmid pYESPΔC was created by removing a 4-kbp ClaI fragment from pYESP. Replacement of the chromosomal POB3 gene with the tagged version used pYESPΔC (URA3) or pPH6TI (TRP1) linearized with BclI.

**RESULTS**

**Nhp6a/b protein associates with the CP complex.** The close structural relationship between the yeast CP complex (Cdc68 plus Pob3) and the analogous vertebrate complexes comprising Cdc68 and the Pob3- and Nhp6-related protein SSRP1 (32, 34) suggests that the yeast Nhp6 and Cdc68 proteins may associate in the same complex. To determine if the Nhp6 proteins do indeed associate physically with Cdc68, Western blots of purified and immunoprecipitated material were probed with anti-V5 antibody. Data are from two independent experiments. (D) Nhp6-associated CP complex associates with high-molecular-weight material. A whole-cell extract from cells containing Nhp6a-V5-His₆ protein (strain NB6241) made in 50 mM potassium acetate buffer was fractionated by centrifugation (1.5 h) at 200,000 × g to yield supernatant (S) and pellet (P) fractions. The pellet fraction was then resuspended in an original volume of 50 mM potassium acetate or 350 mM NaCl extraction buffer (P*). Equal volumes were resolved (6-10-15% gel) and assayed by Western blotting with anti-Cdc68 or anti-V5 antibody. (E) Material immunoprecipitated from equal volumes of the indicated fractions with anti-Cdc68 or anti-V5 antibody was resolved (6-10-15% gels) and assayed by Western blotting using anti-Cdc68 and anti-V5 antibody.

**FIG. 1.** Nhp6 protein physically associates with the CP complex. (A) Material immunoprecipitated by anti-Cdc68 antibody (P) from a whole-cell extract (strain W303-1a) and that remaining in the supernatant (S) were resolved electrophoretically on a 15% polyacrylamide–sodium dodecyl sulfate gel and analyzed by Western blotting with anti-Nhp6a antibody. The buffer for extract preparation and immunoprecipitation contained 350 mM NaCl, 50 mM potassium acetate, or 100 mM potassium acetate as indicated. Recombinant Nhp6a protein (25 ng) served as the marker. (B) Material immunoprecipitated (P) or remaining in the supernatant (S) after treatment of whole-cell extracts of cells containing HA-tagged Cdc68 and Pob3 proteins (strain NQ6811) with anti-HA monoclonal antibody was resolved (8 and 15% gels) and analyzed by Western blotting with anti-HA and anti-Nhp6a antibodies. The negative control (designated C) comprised a whole-cell extract of untagged cells (strain W303-1a) treated as described above. Immunoprecipitations are shown for two independent experiments. (C) Nhp6a and the CP complex associate in vitro. Nhp6a-V5-His₆ protein and CP containing Pob3-V5-His₆ were separately purified (from strains RJY6249 harboring p314NV5 and NB6244) using metal affinity resin as described in the text, mixed, and immunoprecipitated with anti-Cdc68 antibody. Resulting Western blots of purified and immunoprecipitated material were probed with anti-V5 antibody. Data are from two independent experiments. (D) Nhp6-associated CP complex associates with high-molecular-weight material. A whole-cell extract from cells containing Nhp6a-V5-His₆ protein (strain NB6241) made in 50 mM potassium acetate buffer was fractionated by centrifugation (1.5 h) at 200,000 × g to yield supernatant (S) and pellet (P) fractions. The pellet fraction was then resuspended in an original volume of 50 mM potassium acetate or 350 mM NaCl extraction buffer (P*). Equal volumes were resolved (6-10-15% gel) and assayed by Western blotting with anti-Cdc68 or anti-V5 antibody. (E) Material immunoprecipitated from equal volumes of the indicated fractions with anti-Cdc68 or anti-V5 antibody was resolved (6-10-15% gels) and assayed by Western blotting using anti-Cdc68 and anti-V5 antibody.
found to coimmunoprecipitate with each of the two different HA-tagged versions of the CP complex (data not shown). From an extract of doubly tagged HA-Cdc68 HA-Pob3 cells (5), approximately 50% of both Cdc68 and Pob3 were immunoprecipitated by anti-HA antibody (Fig. 1B). In contrast, only 5% of total Nhp6a/b protein was present in the anti-HA immunoprecipitate (Fig. 1B). These values indicate that ~10% of the total Nhp6a/b protein pool of 50,000 copies/cell (36) can be found in association with the CP complex, whose abundance is estimated at 10,000 to 50,000 copies/cell (5, 56). Based on these abundance estimates, from 10% to as much as 50% of the CP complexes are associated with Nhp6a/b protein.

The association of Nhp6a/b with CP was also assessed for CP complex affinity purified from cells in which Pob3 protein is linked to a V5 epitope and a run of six histidine residues. This tagged version of Pob3 provides essential Pob3 function in vivo, as indicated by robust cell growth (data not shown). An extract was prepared from these cells using 50 mM potassium acetate buffer and treated with metal affinity resin, which binds the His<sub>6</sub> tag at the Pob3 C terminus. Western blot analysis of the bound versus unbound material again showed approximately 10% of the total amount of Nhp6a/b associated with CP (data not shown). We conclude that the CP complex physically associates with Nhp6a/b in vivo.

Association of Nhp6a with CP was reproduced in vitro. CP complex containing Pob3-V5-His<sub>6</sub> was affinity purified using 350 mM NaCl buffer, eluted in 50 mM potassium acetate buffer, and mixed with (V5+His<sub>6</sub>)-tagged Nhp6a protein similarly purified. Under these buffer conditions, Nhp6a, identified by its V5 tag, was coimmunoprecipitated with anti-Cdc68 antibody (Fig. 1C).

Centrifugation at 200,000 × g was used to fractionate a whole-cell extract (50 mM potassium acetate buffer) of cells expressing the Nhp6a-V5-His<sub>6</sub> protein from the chromosomal NHP6A locus into soluble and high-molecular-weight (pelleted) material; approximately 40% of the total amounts of Cdc68 and Nhp6a proteins were pelleted (Fig. 1D). A similar distribution of Cdc68 between soluble and pelleted, histone-containing material has been reported for a related fractionation procedure (56). In contrast, the CP complex in whole-cell extracts made using buffer containing 350 mM NaCl is at least 90% soluble, as defined by high-speed (100,000 × g) centrifugation (5). Therefore the 50 mM potassium acetate buffer, which allows the identification of CP-Nhp6 interactions, also alters the centrifugation characteristics of the CP complex. Association of Cdc68 with the high-molecular-weight material was also found for extracts of Δnhp6a/b mutant cells and is therefore independent of Nhp6 protein (data not shown). The soluble fraction and the high-molecular-weight material, resuspended in extraction buffer containing either 50 mM potassium acetate or 350 mM NaCl, were then subjected to immunoprecipitation. Similar amounts of Cdc68 protein immunoprecipitated with anti-Cdc68 antibody from all three fractions. Probing the same blot with anti-V5 antibody showed that Nhp6a coimmunoprecipitated with Cdc68 predominantly from the high-molecular-weight fraction resuspended in 50 mM potassium acetate buffer (Fig. 1E). The presence of ethidium bromide during resuspension and immunoprecipitation did not decrease this coimmunoprecipitation (data not shown), suggesting that protein-protein interactions rather than protein-DNA interactions mediate the association of Nhp6 with Cdc68 (25). As found when using whole-cell extracts, coimmunoprecipitation of Nhp6a protein with Cdc68 was markedly decreased when using high-molecular-weight material resuspended in 350 mM NaCl buffer, indicating that these conditions destabilize interactions responsible for Cdc68-Nhp6a association (Fig. 1E). Despite the similar amounts of Nhp6a in all three fractions, Cdc68 was effectively coimmunoprecipitated with Nhp6a (anti-V5 antibody) only from the high-molecular-weight fraction resuspended in 50 mM potassium acetate buffer, thus mirroring the anti-Cdc68 coimmunoprecipitation results (Fig. 1E). These findings verify the association of Nhp6a protein with the CP complex and suggest that this association may be stabilized by alterations to the CP complex and/or Nhp6a which facilitate CP complex and/or Nhp6a interactions with other proteins.

**Nhp6 protein mediates promoter repression.** Genetic and biochemical assays have implicated both the CP complex and the Nhp6 proteins in transcriptional regulation (13, 29, 30, 36). The Nhp6a and Nhp6b proteins are functionally redundant and important yet not essential for growth (10, 36), allowing the use of null alleles of the NHP6A and NHP6B genes (nhp6AΔ::URA3 and nhp6BΔ::LEU2) (36). Studies were carried out using nhp6aΔnhp6bΔ double-mutant null cells (designated here as Δnhp6a/b cells) and the plasmid-borne NHP6A gene, which encodes the more abundant Nhp6 protein (36).

Two informative reporter genes for transcription have been his4-912Δ and lys2-128Δ (53, 54). Transcription from each of these genes is altered by the insertion of a Ty 1 retrotransposon long terminal repeat (Δ element); as a result, cells harboring these mutant alleles are unable to make functional his4-912Δ and lys2-128Δ transcripts and are auxotrophic for histidine and lysine. Restoration of functional transcription at these alleles allows growth on medium lacking histidine and/or lysine. This effect (known as the Spt<sup>−</sup> phenotype) has been demonstrated for several mutations that affect chromatin function, including cdc68Δ and pob3Δ mutations (13, 30, 45, 58).

Growth of haploid Δnhp6a/b his4-912Δ lys2-128Δ segregants on medium lacking histidine was significant at 23°C, although less robust than the growth of Δnhp6a/b HIS4 segregants from the same cross. Similarly, Δnhp6a/b lys2-128Δ segregants grew on medium lacking lysine but in a temperature-dependent fashion, showing better growth at 30 than at 23°C (data not shown). Suppression of the His<sup>−</sup> phenotype of his4-912Δ was seen only in segregants containing both the nhp6aΔ and nhp6bΔ mutations (Fig. 2A). Transformation of Δnhp6a/b his4-912Δ lys2-128Δ cells with a low-copy-number NHP6A plasmid restored the histidine and lysine auxotrophies (data not shown), confirming that repression of functional transcription at his4-912Δ and lys2-128Δ is mediated by Nhp6 protein.

In cells that have Nhp6 protein, increased dosage of the CDC68 gene allows growth of his4-912Δ lys2-128Δ cells on medium lacking histidine and lysine (5, 30). In contrast, neither a high-copy NHP6A plasmid nor the increased dosage of both NHP6A and POB3 together had any effect on the His<sup>−</sup> lys<sup>−</sup> phenotype of his4-912Δ lys2-128Δ cells (data not shown). Furthermore, neither increased dosage of NHP6A nor increased dosage of both NHP6A and POB3 suppressed the Spt<sup>−</sup> (His<sup>−</sup> Lys<sup>−</sup>) phenotype of his4-912Δ lys2-128Δ cells arising from increased CDC68 dosage (data not shown). The benign effect of
excess Nhp6a protein is similar to the lack of effect of increased POB3 gene dosage on the his4-912b and lys2-1286 reporter genes (5).

Chromatin is thought to maintain repression at the SUC2 gene promoter in the absence of SUC2 upstream activating sequences (UAS). Transcription from the core promoter of this suc2ΔUAS mutant allele, as indicated by growth on solid medium containing sucrose or raffinose as the sole carbon source, can be increased by mutating Cdc68 (13, 30, 39, 58) and proteins that alter chromatin (39). On sucrose medium at 30°C, Δnhp6a/b suc2ΔUAS segregants grew significantly better than did NHP6A NHP6B suc2ΔUAS segregants from the same genetic cross and showed only marginally less growth than NHP6A NHP6B SUC2 segregants. A low-copy NHP64 plasmid in Δnhp6a/b suc2ΔUAS cells prevented growth on sucrose medium, indicating that Nhp6a protein helps to maintain chromatin repression at the suc2ΔUAS core promoter (Fig. 2B). The degree of suppression of the suc2ΔUAS Suc+ phenotype for cells lacking the Nhp6 proteins was similar to that for NHP6A NHP6B suc2ΔUAS cells harboring the cdc68ΔΔ22 mutation (13 and data not shown). Therefore Nhp6 protein, like Cdc68, helps to maintain repression at a core promoter.

**Nhp6 protein facilitates transcription.** Like CP (13, 29, 43), Nhp6 protein facilitates activated transcription, in vivo and in vitro (36, 46). The interactions of Nhp6 with different transcription modulators were therefore investigated genetically.

Transcription of a subset of genes is brought about in part through the actions of the Swi-Snf complex (reviewed in reference 23), a chromatin-remodeling complex that interacts genetically with the CP complex (13, 30). Cells missing the Swi-Snf component Snf5 are unable to assemble a functional Swi-Snf complex (37); as a consequence, snf5 mutant cells exhibit a mild growth impairment on rich medium. Using standard genetic techniques, the nhp6a Δ::URA3, nhp6b Δ::LEU2, and snf5 Δ::URA3 mutant alleles were reassorted in meiotic tetrads, and initial analysis focused on a tetrad in which the UAS reporter gene dosage on the his4-912b reporter gene. The lower panels indicate NHP6A/B genotypes as indicated by Ura and Leu phenotypes. (B) Representative segregants from AW11-9a × NB6040 with genotypes suc2ΔUAS Δnhp6a/b harboring the NHP64 plasmid p314-NVS or the pRS314 vector, SUC2 Δnhp6a/b, and suc2ΔUAS NHP64 NHP6B were grown on solid rich medium at 28°C, replica plated to rich medium containing glucose or sucrose (plus antimycin A) as the carbon source, and incubated 3 days at 23°C. (C) Representative segregants from FY711 × NB39-18a with genotypes snf5 Δ::URA3 Δnhp6a/b with or without p314-NVS, SNF5 NHP64 NHP6B, and snf5 Δ::URA3 NHP64 NHP6B were grown on solid rich medium at 23°C, replica plated to rich medium, with or without 1 M sorbitol, containing glucose or sucrose (plus antimycin A) as the carbon source, and incubated for 6 days at 23 and 33°C. (D) Representative segregants from FY300 × NB40-1aF with genotypes Δnhp6a Δ::URA3 Δnhp6b Δ::LEU2, Δnhp6a Δ::URA3 NHP6B, and Δnhp6a Δ::URA3 Δnhp6b Δ::LEU2 with POB3 NHP64 in place of POB3 were replica plated to uracil-free medium containing 6AU (100 μg/ml) and incubated at 28°C.
snf5 cells was partially alleviated by the inclusion of sorbitol in the growth medium (Fig. 2C), a situation which also partially alleviates the high-temperature (38°C) growth defect of \(\Delta\text{nhp6a/b} \) mutant cells (10). These observations indicate that Nhp6 protein and the Swi-Snf complex similarly facilitate transcription important for growth at 32.5°C; consequently, the absence of Nhp6 protein imposes a need for the Swi-Snf complex at certain genes (and vice versa).

The Swi-Snf complex facilitates transcription of the SUC2 gene (1, 21), as evidenced by the poor growth of snf5 mutant cells using sucrose as a carbon source. We noted that \(\Delta\text{nhp6a/b} \) snf5 cells grow poorly on sucrose medium even at 23°C (data not shown), but the general growth impairment of these mutant cells, and its incomplete remediation by sorbitol, complicated our attempts to assess genetic interactions in SUC2 transcription. In cells with Swi-Snf function, the absence of Nhp6 protein did not impair growth on sucrose medium (data not shown), indicating that Nhp6 is dispensable for Swi-Snf-activated SUC2 transcription.

The synthetic lethality seen for \(\Delta\text{nhp6a/b} \) snf5 cells contrasts with the suppression of the effects of snf5 and another Swi-Snf mutation by alteration of the CP complex (13, 30). This difference suggests that the CP complex and Nhp6 interact functionally with the Swi-Snf complex in different ways. Gene dosage effects also indicate a functional difference. An increased dosage of the CDC68 and/or POB3 genes suppresses several effects of Swi-Snf dysfunction (5, 30), whereas an increased dosage of the NHP6A gene did not alleviate the Suc- and Ino- consequences of the snf5 mutation (data not shown).

Another transcription modulator is the complex containing the Spt4 and Spt5 proteins (19, 50). This complex, like CP, has both positive and negative effects on yeast cell growth (19), while the mammalian Spt4-Spt5 complex, termed DSIF, has been found to modulate the acquisition of elongation competence in vitro by the transcriptionally engaged RNAP II enzyme (50). The absence of Nhp6 protein produces a synthetic lethality with the spt5-194 mutation (19) (Fig. 2D), implicating Nhp6 in transcription elongation.

Mutations that affect transcription elongation, such as \(\Delta\text{ppr2} \), which eliminates sequences encoding transcription elongation factor TFIIS (15, 31), may confer sensitivity to the analog 6-azauracil (6AU). We found that \(\Delta\text{nhp6a/b} \) mutant cells were sensitive to 6AU (Fig. 2E), consistent with a role for Nhp6 protein in modulating transcription elongation. Over a range of 6AU concentrations, the sensitivity of \(\Delta\text{nhp6a/b} \) \(\Delta\text{ppr2} \) triple-mutant cells was no greater than that of \(\Delta\text{nhp6a/b} \) or \(\Delta\text{ppr2} \) cells (data not shown), consistent with Nhp6 modulation of 6AU resistance through TFIIS function. Thus, several indicators implicate Nhp6 in transcription, specifically in elongation.

\textit{cdc68-\(\Delta\text{922} \)} \textit{has effects resembling those caused by the absence of Nhp6 protein}. Cells lacking both Nhp6a and Nhp6b proteins are temperature sensitive at 38°C, as are cells harboring the \(\text{cdc68-\(\Delta\text{922} \)} \) allele (10, 13, 36). Cells lacking Nhp6 are also sensitive to nitrogen starvation, as indicated by a loss of viability during incubation under nitrogen-deficient conditions (10); similarly, \(\text{cdc68-\(\Delta\text{922} \)} \) mutant cells were also found to be sensitive to nitrogen starvation. Cells were replica plated to synthetic medium without ammonium sulfate and incubated at 23°C for starvation, and then replica plated to rich medium for further incubation at 23°C to assess cell growth as a measure of cell survival. After 4 days of nitrogen starvation, the \(\text{cdc68-\(\Delta\text{922} \)} \) mutant cells exhibited significantly less growth on rich medium than did wild-type cells (Fig. 3A), indicating that many of the starved \(\text{cdc68-\(\Delta\text{922} \)} \) cells lost the capacity for colony formation. The \(\text{cdc68-\(\Delta\text{922} \)} \) colonies that did form were not the consequence of additional mutations, as cells from several \(\text{cdc68-\(\Delta\text{922} \)} \) colonies that formed after nitrogen starvation displayed the same overall sensitivity to nitrogen starvation as that of the original \(\text{cdc68-\(\Delta\text{922} \)} \) cells (data not shown). Survival decreased with starvation time, as indicated by the virtual absence of regrowth of \(\text{cdc68-\(\Delta\text{922} \)} \) cells, and of \(\Delta\text{nhp6a/b} \) cells, after 5 days of nitrogen starvation; in contrast, wild-type cells similarly starved showed significant regrowth (data not shown). Thus, cells with the \(\text{cdc68-\(\Delta\text{922} \)} \) mutant version of the CP complex display a growth phenotype resembling that caused by the absence of Nhp6 protein.

The temperature sensitivity and the starvation sensitivity of \(\Delta\text{nhp6a/b} \) mutant cells are partially alleviated by the presence of the osmotic stabilizer sorbitol (10; our observations). However, sorbitol (1 M) did not alleviate the temperature sensitivity or the starvation sensitivity of \(\text{cdc68-\(\Delta\text{922} \)} \) mutant cells (data not shown). This observation suggests that the temperature and starvation sensitivities of \(\Delta\text{nhp6a/b} \) and of \(\text{cdc68-\(\Delta\text{922} \)} \) are due to different effects.

Nhp6 protein facilitates CP activity in vivo. A cross between \(\Delta\text{nhp6a/b} \) and \(\text{cdc68-\(\Delta\text{922} \)} \) parental strains showed that \(\Delta\text{nhp6a/b} \) \(\text{cdc68-\(\Delta\text{922} \)} \) triple-mutant segregants were compromised for growth and survival compared to \(\text{cdc68-\(\Delta\text{922} \)} \) segregants with either Nhp6a or Nhp6b function and to parental cells. The \(\Delta\text{nhp6a/b} \) \(\text{cdc68-\(\Delta\text{922} \)} \) triple-mutant cells were temperature sensitive for growth at 34°C, a temperature at which the \(\Delta\text{nhp6a/b} \) and \(\text{cdc68-\(\Delta\text{922} \)} \) parental strains and segregants with these genotypes show good growth (Fig. 3B), and lost viability more rapidly upon nitrogen starvation (Fig. 3C). The more severe temperature and starvation sensitivities of \(\Delta\text{nhp6a/b} \) \(\text{cdc68-\(\Delta\text{922} \)} \) cells were alleviated by a low-copy-number NHP6A plasmid (Fig. 3B and C) or a low-copy-number CDC68 plasmid (data not shown). Thus, the absence of Nhp6 protein is synthetically lethal with the \(\text{cdc68-\(\Delta\text{922} \)} \) mutation, a finding that strengthens the view that Nhp6 and the CP complex have related effects on transcription. In contrast to what is seen for the temperature sensitivity of \(\Delta\text{nhp6a/b} \) CDC68 cells, 1 M sorbitol did not alleviate the synthetically enhanced temperature sensitivity of \(\Delta\text{nhp6a/b} \) \(\text{cdc68-\(\Delta\text{922} \)} \) cells (data not shown). This synthetic enhancement is therefore a likely consequence of an impaired CP complex, rather than the absence of Nhp6 protein, so that CP-related temperature sensitivity is exacerbated in \(\Delta\text{nhp6a/b} \) \(\text{cdc68-\(\Delta\text{922} \)} \) triple-mutant cells. Thus Nhp6 facilitates high-temperature growth when the CP complex is compromised by mutation.

Pob3-Nhp6a fusion creates a yeast version of SSRP1 with Pob3 function. The biochemical and genetic findings discussed above suggest that the Nhp6 protein may function in association with the CP complex of Cdc68 and Pob3 and that the yeast Pob3 and Nhp6 proteins, structurally homologous to N-terminal and C-terminal regions, respectively, of the vertebrate SSRP1 protein, may function together. To test these ideas, the Nhp6a protein was covalently attached to the C terminus of Pob3, resulting in a Pob3-Nhp6a fusion protein that resembles SSRP1 (in primary sequence). The Pob3-Nhp6a fusion protein
contains the entire sequence of Pob3, a 7-residue spacer, the entire Nhp6a sequence minus the first three amino acids (which are dispensable for Nhp6a function [61]), and the V5 epitope and His6 tag at its C terminus (Fig. 4A).

To determine if the Pob3-Nhp6a fusion protein provides essential Pob3 function, the POB3-NHP6A gene was placed under the control of the regulated GAL1 promoter on a plasmid and transformed into Dpob3 mutant cells kept alive by the POB3 plasmid pGADPOB (5). Transformants were then grown in selective medium containing galactose and screened for leucine auxotrophy, indicating loss of the pGADPOB plasmid. The Dpob3 mutant cells that had lost pGADPOB but retained the POB3-NHP6A plasmid showed robust growth on galactose, indicating that the Pob3-Nhp6a fusion protein provides Pob3 function (data not shown). On glucose medium, which represses transcription of the plasmid-borne POB3-NHP6A gene, growth was greatly inhibited (data not shown), indicating that the Pob3-Nhp6a protein provides the essential function(s) of Pob3.

The chromosomal copy of the POB3 gene was replaced with the POB3-NHP6A gene under the control of POB3 transcription regulatory sequences. These transformants, containing Pob3-Nhp6a instead of Pob3 protein, grew as robustly as wild-type cells, indicating that the POB3-NHP6A gene, expressed at normal POB3 levels, provides wild-type Pob3 function (see Fig. 5). The Pob3-Nhp6a fusion protein in these cells migrated at an apparent size consistent with the addition of the Nhp6a-V5-His9 polypeptide to Pob3 protein (70 kDa) (5). Moreover, the Pob3-Nhp6a fusion protein is fully intact in vivo (Fig. 4B); therefore, biological activity of the POB3-NHP6A gene can be attributed to full-length Pob3-Nhp6a protein.
Pob3-Nhp6a protein provides Nhp6a activity. The chromosomal replacement of \textit{POB3} with \textit{POB3-NHP6A} was made in cells lacking Nhp6 protein (Table 1). The replacement of the \textit{POB3} gene with \textit{POB3-NHP6A} in \textit{Dnhp6a/b} mutant cells restores growth at 38°C (Fig. 5) and sensitivity to 6AU (Fig. 2E), while \textit{POB3-NHP6A} expression in \textit{Dnhp6a/b} cells alleviates synthetic interaction with \textit{spt5-194} (data not shown). The Pob3-Nhp6a protein also allows \textit{Dnhp6a/b} cells to survive nitrogen starvation, although growth of \textit{Dnhp6a/b POB3-NHP6A} cells after starvation was less substantial than that of wild-type cells (Fig. 5). Another test of Nhp6 function is growth on galactose. Cells lacking Nhp6a and Nhp6b are impaired for \textit{GAL1} induction (36) and unable to grow effectively using galactose as the carbon source (62). Similarly, we saw an extended (7-day) lag for \textit{Dnhp6a/b} mutant cells on galactose solid medium at 23°C, and only low-level growth was ever achieved (Fig. 5). In contrast, robust galactose-dependent growth was evident after a 2-day incubation for \textit{Dnhp6a/b} cells that contained the Pob3-Nhp6a protein in place of Pob3 (Fig. 5) and was indistinguishable from the growth of \textit{Dnhp6a/b} cells harboring the wild-type \textit{NHP6A} gene on a low-copy-number plasmid (data not shown). Thus, by several criteria the Pob3-Nhp6a fusion protein supplies both Pob3 and Nhp6a function in vivo.

\textbf{Pob3-Nhp6a fusion protein associates with Cdc68 in the CP complex.} A high-speed supernatant fraction from \textit{Dnhp6a/b POB3-NHP6A} cells was size-fractionated and assayed for the distributions of Cdc68 and Pob3-Nhp6a. As shown in Fig. 6A, both proteins cofractionated in the size range of the Cdc68 + Pob3 (CP) complex (5), suggesting that the Cdc68 and Pob3-Nhp6a proteins form an in vivo complex analogous to the CP complex. To verify this association, the proteins were separately immunoprecipitated from a clarified whole-cell extract using anti-V5 and anti-Cdc68 antisera. The anti-V5 antibody immunoprecipitated Pob3-Nhp6a, as expected, and coimmunoprecipitated Cdc68 (Fig. 6B). The reciprocal experiment using anti-Cdc68 antiserum to immunoprecipitate Cdc68 from the same clarified whole-cell extract showed that Pob3-Nhp6a coimmunoprecipitated with Cdc68 (Fig. 6B). Comparisons of the amounts of immunoprecipitated material versus residual soluble material (Fig. 6B) indicated that Cdc68 and Pob3-Nhp6a are present in the complex in equal stoichiometric amounts, as previously demonstrated for the CP complex of Cdc68 and wild-type Pob3 (5). These observations suggest that the Nhp6 activity attributed above to the Pob3-Nhp6a fusion...

**FIG. 5.** The Pob3-Nhp6a protein provides Pob3 and Nhp6a activity in vivo. Cells of the \textit{Dnhp6a/b POB3-NHP6A-V5-His6} strain NB6249 (designated \textit{Dnhp6a/b POB3-NHP6a}), the \textit{Dnhp6a/b POB3} strain RY6249 (designated \textit{Dnhp6a/b}), and the \textit{NHP6A NHP6B POB3-V5-His6} strain NB6244 (designated \textit{wt}) were grown on solid rich glucose medium at 23 and 38°C, on rich galactose medium at 23°C, and on rich glucose medium at 23°C following a 5-day incubation at 23°C on enriched medium lacking (NH$_4$)$_2$SO$_4$ (designated N-starvation).

**FIG. 6.** The Pob3-Nhp6a protein forms a stable complex with Cdc68. (A) Material in the 200,000 g supernatant fraction from cells of strain NB6249 was size fractionated by gel filtration (4) and analyzed by Western blotting using anti-Cdc68 and anti-V5 antibodies. Size markers (Bio-Rad) were cofractionated with the extract. (B) Equal volumes of the same 200,000 g supernatant were treated with anti-Cdc68 or anti-V5 antibody and the resultant immunoprecipitates and corresponding supernatants were resolved (8% gel) and analyzed by Western blotting with anti-Cdc68 and anti-V5 antibody.
protein is largely due to Nhp6a in covalent association with the CP complex.

**Nhp6 functions of Pob3-Nhp6a protein may depend on Cdc68 association.** Both Pob3 and the Pob3-Nhp6a fusion protein can associate with Cdc68; Pob3-Nhp6a protein, however, has the added ability to provide Nhp6a function. Pob3-Nhp6a protein may even be physically associated with Cdc68 for this Nhp6a function. To test this idea, the ability of Pob3 protein to displace Pob3-Nhp6a from Cdc68 was assessed under growth conditions that necessitate the Nhp6a activity of the Pob3-Nhp6a protein.

For this experiment, Δnhp6a/b POB3-NHP6A cells were transformed with a multicopy plasmid expressing HA-Pob3 from the POB3 promoter. In parallel, the same Δnhp6a/b POB3-NHP6A cells were transformed with a multicopy plasmid expressing both POB3 (HA-Pob3) and NHP6A (Nhp6a-V5-His6) as separate genes. Both transformant types, plus a vector-transformant control, were grown at 37°C, a temperature at which Nhp6 function is necessary for robust growth, and harvested in mid-log phase for the preparation of whole-cell extracts. Anti-Cdc68 antiserum was used to immunoprecipitate the CP complex, and levels of coimmunoprecipitating Pob3-Nhp6a protein were determined. Under these 37°C growth conditions, Pob3-Nhp6a was associated with Cdc68 in all cases but in amounts twofold greater in the absence of the individual Nhp6a and HA-Pob3 proteins than in their presence (Fig. 7A, compare lanes 1 and 3). The presence of HA-Pob3 alone did not decrease the amount of Pob3-Nhp6a associated with Cdc68 (Fig. 7A, lane 2). These observations indicate that growth at 37°C favors the association of Cdc68 with Pob3-Nhp6a rather than Pob3 (in the absence of native Nhp6 protein). To show that HA-Pob3 displaces Pob3-Nhp6a from Cdc68 when Nhp6a is present, the same blot was probed with anti-HA to assess the level of HA-Pob3 in the CP complex. Although at 37°C a small fraction of HA-Pob3 was incorporated into the CP complex in the absence of Nhp6a (Fig. 7A, lane 2), there was a marked increase in the level of HA-Pob3 in the CP complex when Nhp6a was present in the cell (Fig. 7A, lane 3). The increased amount of Cdc68-associated HA-Pob3 was consistent with the degree of displacement of Pob3-Nhp6a under those conditions. Similar experiments suggest that the association of Pob3-Nhp6a with Cdc68 also facilitates growth on galactose (data not shown). These findings therefore suggest that for some aspects of Nhp6a function, including 37°C growth and galactose catabolism, the Nhp6a protein may have to be associated with the CP complex, a situation that can be satisfied by either covalent (Pob3-Nhp6a) or noncovalent interactions.

**Pob3-Nhp6a is compromised for certain Nhp6a functions.** Despite the above findings, Δnhp6a/b POB3-NHP6A cells do not grow as well as cells in which native Nhp6a protein is present, suggesting that the Pob3-Nhp6a protein lacks full Nhp6a activity. Inadequacy is also evident for Nhp6a activities related to chromatin repression: the Pob3-Nhp6a protein could not suppress the His+ Lys+ phenotype or the SUC2 UAS reporter of Δnhp6a/b his4-912 lys2-1288 suc2ΔUAS mutant cells. On the other hand, the Pob3-Nhp6a protein in place of Pob3 does not diminish promoter repression at the his4-912, lys2-1288, and suc2ΔUAS loci in cells containing the Nhp6a/b proteins (data not shown), and Nhp6a protein restores robust growth to Δnhp6a/b POB3-NHP6A cells (data not shown), indicating that the Pob3-Nhp6a protein does not interfere with the interactions of native Nhp6a protein. In Δnhp6a/b POB3 cells, expression of the POB3-NHP6A gene from the GAL1 promoter on a multicopy plasmid improves cell growth as expected but still does not suppress the His+ Lys+ phenotype (data not shown), making it unlikely that the chromosomal POB3-NHP6A gene simply produces insufficient protein for all Nhp6 activities. We considered the idea that the Nhp6a portion of the Pob3-Nhp6a fusion protein may not properly associate with the CP complex, in which case Nhp6a may be able to interact with the Pob3-Nhp6a version of the CP complex to restore full function. To test this idea, the CP complex was immunoprecipitated with anti-Cdc68 antibody from Δnhp6a/b POB3 cells and Δnhp6a/b POB3-NHP6A cells that also harbored a low-copy-number plasmid expressing (V5+His+)tagged Nhp6a protein and the amount of tagged Nhp6a coim-

![Image](http://mcb.asm.org/)

**FIG. 7. Interactions of the complex containing Pob3-Nhp6a and Cdc68.** (A) An Nhp6a function of the Pob3-Nhp6a protein depends on Cdc68 association. Cells of the Δnhp6a/b POB3-NHP6A-V5-His6, strain NB6251 harboring the pRS424 vector (lane 1) or the POB3 plasmid p424-HAPOB (lane 2) or the POB3 + NHP6A plasmid pHAPNV5 (lane 3) were grown at 37°C in enriched selective medium to ~10^7 cells/ml. Whole-cell extracts were treated with anti-Cdc68 antibody, and the immunoprecipitates were resolved (8% gel) and analyzed for communoprecipitation of Pob3-Nhp6a-V5 and HA-Pob3 using anti-V5 and anti-HA antibodies. On the same blot (but in different lanes), whole-cell extracts (input) were probed with anti-V5 antibody to indicate the relative amounts of extract used for immunoprecipitation. (B) The assemblage of Cdc68 and Pob3-Nhp6a protein binds Nhp6a. Anti-Cdc68 immunoprecipitates from whole-cell extracts of Δnhp6a/b POB3 cells (strain RJY6249) and Δnhp6a/b POB3-NHP6A-V5-His6 cells (strain NB6251), each housing p314-NV5 (lanes 2 and 4) or the pRS314 vector (lanes 1 and 3), were resolved (6-10-15% gel) and analyzed by Western blotting with anti-V5 antibody.
munoprecipitating with Cdc68 was determined. These results showed that Nhp6a communoprecipitates equally well with the (Cdc68 + Pob3) and the (Cdc68 + Pob3-Nhp6a) complexes (Fig. 7B). The amount of Nhp6a associated with each of these versions of the CP complex was not increased by an increased dosage of the NHP6A gene, suggesting that the Nhp6a protein interacts with a specific binding site associated with both versions of the complex (data not shown). The Nhp6a portion of Pob3-Nhp6a may not properly occupy an Nhp6-binding site associated with the CP complex, but can nonetheless provide certain Nhp6a functions by virtue of its physical attachment to Pob3.

**DISCUSSION**

The budding yeast *S. cerevisiae* contains a nuclear protein complex that is related structurally and functionally to the FACT complex of human cells and the DUF complex of *Xenopus* (32, 34). Both the yeast and vertebrate complexes contain the Cdc68 (Spt16) protein, and both also contain the structurally related proteins SSRP1 (vertebrate) and Pob3 (yeast). The yeast Pob3 protein does not contain the HMG box domain found at the C terminus of SSRP1; however, yeast cells have several small HMG box proteins. Here we provide evidence that members of a functionally redundant pair of small HMG box proteins, Nhp6a and Nhp6b, associate physically with the yeast complex of Pob3 and Cdc68 proteins and have effects on transcription that are related to those of the CP (Cdc68-Pob3) complex. These conclusions are derived in part from a functional analysis of a Pob3-Nhp6a fusion protein constructed to resemble an SSRP1 protein. Our findings suggest that Nhp6 protein (Nhp6a and/or Nhp6b) may associate with and function along with the Pob3 protein to make up a functional, but bipartite, yeast analog of the vertebrate SSRP1 protein.

Here we have demonstrated a physical association of Nhp6 with the CP complex by communoprecipitation and affinity purification. Both procedures showed that a significant amount of Nhp6 protein could be isolated along with the CP complex, and that this CP-Nhp6 ensemble is for the most part associated with high-molecular-weight material that pellets at 200,000 × g; this material would include chromatin. In contrast, the CP that remained soluble during this fractionation associated poorly with Nhp6. Association of CP with Nhp6 was not improved by Nhp6a overexpression, suggesting that the affinity of Nhp6 for CP may be regulated and correlated with retention of CP and/or Nhp6 in high-molecular-weight material. Nhp6 has sequence-nonspecific DNA-binding ability (35, 61), but this DNA-binding activity is not solely responsible for the association of CP with high-molecular-weight material, for CP was also found in the 200,000 × g pellet made from extracts of ∆nhp6a/b mutant cells. The molecular bases for the high-molecular-weight associations of CP and the differences in CP-Nhp6 affinity, and indeed whether the association between Nhp6 and CP is direct or indirect, remain to be determined.

The human FACT complex can facilitate transcription elongation on a nucleosomal template in vitro (27, 33), while genetic findings from yeast are consistent with an analogous role in vivo for the CP complex (34). The Cdc68 and Pob3 proteins constituting CP are both essential, as might be expected for global modulators of transcription (30, 55). In contrast, the Nhp6a-Nhp6b protein pair is dispensable for life and for the transcription of many genes (10, 36). Is this dispensability accounted for by the function of another homolog? Another small HMG box protein that resembles the C terminus of SSRP1 is Nhp10 (28). However, the Nhp10 protein is unlikely to have Nhp6-like function, because synthetic interactions between ∆nhp6a/b and the complete absence of Nhp10 protein could not be detected (our unpublished observations). Likewise, no synthetic interactions were detected between ∆nhp6a/b and the complete absence of Hmo1, another HMG box protein (28). These negative findings suggest that an HMG box domain is dispensable for some of the transcription functions of the CP complex.

Despite the dispensability of Nhp6 protein for some CP functions, our findings indicate a direct involvement of CP in Nhp6-mediated transcription. Nhp6 proteins do facilitate the transcription of a subset of genes (36, 46), and the galactose auxotrophy that we and others (62) find for ∆nhp6a/b cells supports an important role for Nhp6 in *GAL* gene transcription. For effective growth on galactose, Nhp6 activity can be supplied by the Pob3-Nhp6a fusion protein, in association with Cdc68. Thus Nhp6 may function in a gene-specific manner through association with the CP complex.

The nature of one transcriptional role for Nhp6 may be indicated by the genetic relationship between Nhp6 and the Swi-Snf complex, a chromatin-remodeling machine that facilitates the transcription of several genes (reviewed in reference 23). We find that the absence of Nhp6 proteins increases the reliance on the Swi-Snf complex for overall growth, especially at elevated temperatures. These findings indicate that Nhp6 and the Swi-Snf complex affect the same process but in different ways.

Genetic interactions analogous to those reported here between Nhp6 and Swi-Snf are also found for Nhp6 proteins and the Gcn5 protein (62), which is the catalytic subunit of several histone acetyltransferase complexes (16, 38, 44), and for Gcn5 and Swi-Snf (3, 12, 18, 38, 41, 42, 48). Therefore, Nhp6 protein, the Swi-Snf complex, and Gcn5-mediated histone acetylation have mutually complementary effects that facilitate transcription. These common effects may assist transcriptional activators. Nhp6 can facilitate the binding of the TATA-binding protein component of TFIID to TATA sequences in vitro (36), and overexpression of Nhp6 protein increases transcription activation by mutated forms of the activator SBF (Swi4-Swi6) that retain sequence-specific DNA-binding ability (14, 46). Swi-Snf facilitates activator function through the remodeling of chromatin, whereas the chromatin-remodeling ability of Nhp6, in association with the CP complex or independently, is not known. Nhp6 proteins do, however, bend and partially unwind DNA by binding in a sequence-nonspecific manner in the minor groove (2, 35, 61). Negative supercoiling, as would result from Nhp6 binding, is correlated with disruption of nucleosome structure (26) and may therefore be involved in the facilitation of transcription by Nhp6 protein. Yeast Swi-Snf and mammalian Swi-Snf-like complexes can also bind the minor groove of DNA in a relatively sequence-nonspecific manner, with an affinity for bent and partially unwound DNA (40, 52), but this binding induces positive DNA supercoiling and may therefore have different consequences. Therefore, Nhp6...
Nhp6 and Swi-Snf may facilitate the binding of general transcription factors by different mechanisms.

Our findings indicate that the Nhp6 protein has transcriptional effects similar to those of CP (13, 30) and can physically associate with CP. However, several Nhp6 functions are not provided by the Pob3-Nhp6a fusion protein. Our finding that native Nhp6a protein can associate with the Cdc68 + Pob3-Nhp6a complex is evidence that this particular fusion protein is not constructed to allow optimal Nhp6a interaction with CP-associated material, a situation which may prevent the full spectrum of Nhp6a activity. Alternatively, tethering Nhp6 to CP via Pob3-Nhp6a may interfere with other Nhp6 associations.

Our biochemical findings are consistent with this possibility, in that not all native Nhp6 protein can be found to be associated with the CP complex. There are many examples of transcription-related proteins seen in multiple complexes. Those related to RAP1 II activity include several components common to the SAGA, ADA and TFIIH complexes (12, 17); the Anc1 (Swp29, Tfg3, or Taf30) protein that is a component of Swi-Snf (6), the NuA3 histone acetyltransferase (22), the Swi-Snf (6), the NuA3 histone acetyltransferase (22), the transcription-related proteins seen in multiple complexes. There are many examples of transcription-related proteins seen in multiple complexes. Those related to RAP1 II activity include several components common to the SAGA, ADA and TFIIH complexes (12, 17); the Anc1 (Swp29, Tfg3, or Taf30) protein that is a component of Swi-Snf (6), the NuA3 histone acetyltransferase (22), the transcription-related proteins seen in multiple complexes. There are many examples of transcription-related proteins seen in multiple complexes.

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