Published in final edited form as:

Biochim Biophys Acta. 2010 ; 1799(1-2): 175. doi:10.1016/j.bbagr.2009.11.010.

Nhp6: A Small but Powerful Effector of Chromatin Structure in *Saccharomyces cerevisiae*

David J. Stillman

Department of Pathology, University of Utah Health Sciences Center, Salt Lake City, UT 84112

Abstract

The small Nhp6 protein from budding yeast is an abundant protein that binds DNA non-specifically and bends DNA sharply. It contains only a single HMGB domain that binds DNA in the minor groove and a basic N-terminal extension that wraps around DNA to contact the major groove. This review describes the genetic and biochemical experiments that indicate Nhp6 functions in promoting RNA pol III transcription, in formation of preinitiation complexes at promoters transcribed by RNA pol II, and in facilitating the activity of chromatin modifying complexes. The FACT complex may provide a paradigm for how Nhp6 functions with chromatin factors, as Nhp6 allows Spt16-Pob3 to bind to and reorganize nucleosomes in vitro.

1. Introduction

Saccharomyces cerevisiae has seven genes expressing HMGB proteins [1]: *HMO1*, *NHP10*, *ABF2*, *ROX1*, *IXR1*, *NHP6A*, and *NHP6B*. *NHP6A* and *NHP6B* encode highly homologous proteins of 93 and 100 amino acids in length. The Nhp6A and Nhp6B proteins differ significantly at their N-termini, but over the 90 amino acid core region they are 89% identical and 96% similar [2].

Nhp6A is an abundant protein, present at 50,000 to 70,000 molecules per haploid cell [3]. This corresponds to one Nhp6A molecule for every 1–2 nucleosomes, which is similar to the value of 1 HMGB1/2 per 3 nucleosomes reported for mammalian cells [4]. ChIP-chip experiments suggest Nhp6 localization in the vicinity of transcription start sites parallels that of nucleosomes [K. Yen and B.F. Pugh, personal communication; 5]. The Nhp6A protein is present at a concentration between three times [6] to ten times [3] higher than Nhp6B, consistent with higher Nhp6A transcript levels [2]. Expression of *NHP6* genes is regulated by the concentration of Nhp6 protein or RNA, since overexpression of Nhp6B protein results in a dramatic decrease in *NHP6A* expression [6]. Additionally, overproduction of Nhp6 is toxic to cells [7].

2. Nhp6 Binds and Bends DNA

Like most HMGB proteins, Nhp6A binds DNA in a sequence-nonspecific fashion [8,9]. Gel shift experiments show multiple Nhp6A complexes on a 98 bp DNA fragment [8]. Nhp6A binds to DNA as a monomer in a stepwise manner with an affinity of 1–10 nM for the initial complex [10–12]. Nhp6A bends DNA sharply, as shown by the ability to supercoil circular

© 2009 Elsevier B.V. All rights reserved.

Phone: 801-581-5429, FAX: 801-581-4517, david.stillman@path.utah.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

DNA [13], and by ligase-mediated circularization assays where Nhp6A promotes circularization of DNA molecules as short as 66 bp [9]. Additionally, Nhp6A binds more tightly and more stably to curved DNA than to linear DNA [8].

3. Nhp6 Structure

The structure of Nhp6A has been solved by NMR [11,14]. Nhp6 adopts the typical L-shaped HMGB fold, both free in solution and when complexed with DNA. The inside of the L binds to DNA in the minor groove resulting in a 70° bend in DNA, similar to the sharp DNA bend seen in other HMGB-DNA structures. Specific Nhp6A side chains intercalate into the DNA minor groove, and studies of Nhp6A bound to cisplatin-modified DNA suggest these intercalating residues are important in inducing the bend in DNA [10]. The N-terminal region (amino acids 8–16) of the protein has numerous basic residues and this region wraps around the DNA to make contacts within the narrowed major groove. Mutations within this basic N-terminal region affect Nhp6 function in vivo [8]. Studies with HMGB chimeras containing the basic N-terminal region of Nhp6A show this region enhances DNA flexibility [15].

4. In vivo studies

Yeast cells with a gene disruption of a single *NHP6* gene, either *NHP6A* or *NHP6B*, are viable and have no visible growth defects. In contrast the *nhp6a nhp6b* double mutant grows slowly at 30°C and does not grow at all at 38°C [16]. Thus Nhp6 is non-essential, but is important for normal growth. While the Nhp6A and Nhp6B proteins are similar and the *nhp6a* and *nhp6b* single mutants grow normally, growth competition experiments [17,18] show that *nhp6a* mutants have a subtle but reproducible growth defect compared to wild type strains, while *nhp6b* mutants compete well with wild type (T. Engar and D.J.S., unpublished results). Cells shifted to the non-permissive temperature display morphological defects including elongated buds and enlarged necks [16]. The temperature sensitive growth defect of *nhp6a nhp6b* double mutants is suppressed by the presence of 1M sorbitol in the medium; this osmoremediation did not suppress the morphological defects, however.

Some of the residues most highly conserved among HMGB proteins were mutated in Nhp6A and tested for function both in vivo and in vitro [19]. Surprisingly, most of the changes allowed Nhp6A to function normally in supporting growth at 38°C in vivo, and were normal in binding and bending DNA. Interestingly, the F31S and P44L mutations were defective for in vivo functions, but in vitro studies showed these mutant proteins could bind and bend DNA as well as wild type. Based on these results the authors suggest that the biological function of Nhp6 requires interaction with other proteins, a topic that is explored in subsequent sections. Nuclear entry by Nhp6 is via a pathway independent of the Ran import pathway, but requires calmodulin [20,21].

Systematic analysis of the yeast knockout collections [22] has provided a substantial insight as to phenotypes, as have the use of synthetic genetic analysis [23] to identify genetic interactions. This approach does not work well for *nhp6* mutations, as *nhp6a* and *nhp6b* single mutants do not display significant phenotypes, and the *nhp6a nhp6b* double mutant (here referred to as *nhp6ab*) usually must be used in genetic analyses. Studies have shown that *nhp6ab* mutants are sensitive to nitrogen starvation [16], are slightly more resistant to hydrogen peroxide and ultraviolet irradiation [10], and are defective for galactose growth in the S288c strain background [24]. *nhp6ab* mutants have a weak Spt-phenotype and are sensitive to the transcription elongation inhibitor 6-AU [25,26].

A role for Nhp6 in chromatin is seen in genetic studies (Table 1), as a number of chromatin regulators show synthetic lethality or synthetic sickness when combined with *nhp6ab*, including the *GCN5* histone acetyl transferase [24], the Swi/Snf and RSC ATP-dependent

chromatin remodelers [26–29], the *SET2* histone methyltransferase [30], the *SPT4* and *SPT5* elongation factors [25,26], the *REG1* repressor [31], the *NHP10* HMGB factor [29], subunits of the Ccr/Not [32], Ino80 [29], Paf1 [29], NuA4 [29], SAGA [29], and FACT complexes [25,26], as well as mutations affecting histone tails [33]. *nhp6ab* mutants also show synthetic defects when combined with mutations in basal transcription factors such as TFIIA [27]. TBP [34,35], and *MOT1*, a TBP interacting factor [32]. Additive defects are also seen when *nhp6* mutations are combined with promoter mutations in the pol III-transcribed *SNR6* gene [36] and with *RPC40*, a subunit of pol I and pol III [29]. Genetic links are also provided by the genes whose defects can be suppressed by *NHP6* overexpression, including mutations in the RSC and FACT chromatin factors [25,28], and the *SWI6* subunit of the SBF DNA-binding factor [37]. *NHP6* overexpression suppresses a TBP *SWI2* double mutant suggesting a role in preinitiation complex formation [27], and also suppresses a *slk1-1 spa2Δ* double mutant suggesting a role in MAP kinase cascades [16]. Nhp6 therefore has global effects in a range of processes linked to appropriate regulation of transcription through maintenance of normal chromatin structure.

5. Pol III Transcription

Studies have shown a critical role for Nhp6 in transcription by RNA polymerase III, which transcribes tRNAs, 5S RNA, and other small RNA molecules. A screen was conducted to identify multicopy plasmids that can suppress the *nhp6ab* growth defect at 37°C, and this screen identified *BRF1* and *SNR6* [38]. *BRF1* encodes the limiting component of TFIIIB, one of the basal factors required for pol III transcription, and *SNR6* is a pol III-transcribed gene that encodes the U6 spliceosomal RNA. Another genetic screen used a *SNR6* gene with a promoter mutation causing inefficient expression and growth defects [39]. These authors screened for multicopy suppressors and identified plasmids with *NHP6A*, *NHP6B*, and the limiting *BRF1* factor [39]. Expression of U6 snRNA in vivo is reduced in *nhp6ab* mutants, and Nhp6 can stimulate pol III transcription in vitro in studies with purified systems [38,39]. Thus Nhp6 plays an important role in *SNR6* expression, and the *nhp6ab* temperature sensitive phenotype is due, at least in part, to decreased *SNR6* expression. The *nhp6ab* defect can be suppressed by increasing the number *SNR6* templates, and both the *nhp6ab* defect and the *SNR6* promoter mutations can be suppressed by overexpressing *BRF1*, the limiting component. Combining *nhp6ab* mutations with mutations in the *SNR6* promoter, either at the 5' TATA element or the 3' flanking A and B block region, results in lethality [36]. MNase digestion experiments show that there is a specific chromatin structure at the *SNR6* TATA region, and this is altered in an *nhp6ab* mutant [39]. TBP is a subunit of TFIIIB that binds at the 5' flanking TATA element of pol III-transcribed genes, and chromatin immunoprecipitation (ChIP) experiments show reduced TBP binding to the *SNR6* promoter in *nhp6ab* mutant [35]. Nhp6 provides transcriptional initiation fidelity to tRNA genes in a highly purified in vitro system, and in vivo studies show changes in transcriptional start sites at tRNA genes in an *nhp6ab* mutant [40]. The RSC chromatin remodeler interacts with Nhp6 [28], and global ChIP-chip studies show high RSC occupancy at pol III-transcribed genes [41]. Finally, tRNA genes can provide a barrier to the spread of heterochromatin, and this barrier function is compromised in an *nhp6ab* mutant [42].

6. Regulation of Pol II Transcription

Strains with mutations in both *NHP6A* and *NHP6B* display significant defects in transcriptional activation by RNA polymerase II. Paull et al. [3] demonstrated that activation of a number of inducible promoters is reduced in *nhp6ab* mutants, including copper induction of *CUP1*, low glucose induction of *CYC1*, DNA damage induction of *DDR2*, and galactose induction of *GAL1* [3]. The *GAL1* defect is consistent with the *nhp6ab* defect in growth on galactose media in some strain backgrounds [24]. The *nhp6ab* mutations did not affect *PHO5*, and thus not all

inducible genes are affected [3]. It was subsequently shown that *nhp6ab* mutants are also defective for induction of the *CHA1* gene by serine [43], the *SUC2* gene by sucrose [44], and the *FRE2* gene by low iron [45]. Chromatin structure at the *CHA1* locus was altered in the *nhp6ab* strain [43]. My laboratory showed that expression of the *HO* gene is reduced in an *nhp6ab* mutant [24], consistent with identification of *NHP6* as a multicopy suppressor that allows *HO* expression despite a mutant Swi6 activator [37]. While *HO* is not induced by a metabolite, its expression is tightly cell cycle regulated. Genetic analysis showed that the *nhp6ab* defect in *HO* expression could be suppressed by a number of mutations, including *sin3* (which affects the Rpd3(L) histone deacetylase), *spt3* (a component of the SAGA histone or protein acetyltransferase complex), and *sin4* (a component of the Pol II Mediator complex) [24]. These latter results suggest Nhp6 functions at the chromatin level in regulating gene expression, an idea supported by the observation that a multicopy plasmid with *NHP6* allows *HO* expression in the absence of the normally required *GCN5* histone acetyltransferase [24]. Microarray experiments conducted to examine gene expression globally have not identified major defects in gene expression in *nhp6ab* mutants [P. Eriksson and D.J.S, unpublished; 43], although modest effects of the *nhp6ab* mutations were observed on expression of methionine biosynthetic genes, pheromone-regulated genes, and genes involved in the mating response were seen [43]. These results are consistent with the interpretation that Nhp6 plays an important supportive role in global regulation of transcription rather than an essential role at a subset of genes.

7. Basal Pol II Transcription Factors

Genetic and biochemical experiments both suggest that one function of Nhp6 is to facilitate preinitiation complex formation at promoters. In vivo studies with chimeric promoters suggested that Nhp6 has effects at both the UAS and core regions of promoters [3]. In vitro binding experiments showed that Nhp6A promotes TFIIB to a DNA-TBP-TFIIA complex in vitro [3], and that Nhp6A can facilitate the in vitro interaction of TBP with DNA, especially in the presence of TFIIA [27].

A series of genetic studies provide strong support for the idea of interactions between Nhp6 and basal pol II factors. Overexpression of TBP suppresses the *nhp6ab* defect in *HO* expression as well as the *nhp6ab* temperature sensitive growth defect [34]. Strains with an epitope tag on TBP at either the N- or C-terminus are viable and healthy, but these TBP-tags are lethal in an *nhp6ab* mutant [35]. This result led to a screen to identify point mutations in TBP that are viable on their own, but lethal in combination with *nhp6ab* [35]. Interestingly, the TBP mutations identified this way cluster on the protein surface and particularly affect residues known to interact with the TFIIB and TFIIA basal factors. Many of these TBP alleles are also lethal when combined with a mutation in either the *SWI2* subunit of Swi/Snf or the *GCN5* histone acetyltransferase subunit of SAGA [27]. Some of these synthetic lethal interactions can be suppressed by multicopy plasmids with either TFIIA or *NHP6A* [27]. *nhp6ab* is also lethal when combined with TFIIA mutations that affect its interaction with TBP that are otherwise tolerated [27].

There are also genetic interactions between *NHP6* and factors known to interact with TBP. The Spt3 subunit of the SAGA complex shows allele-specific interactions with TBP [46], and an *spt3* mutation also suppresses both the *nhp6ab* defect in *HO* expression and the temperature sensitive growth defect [34]. An *spt3* mutation suppresses some of the *nhp6ab* TBP synthetic lethal interactions [35], as well as the synthetic lethality between *nhp6ab* and the *SPT16* subunit of FACT [27]. Mot1 also interacts with TBP [47,48], and *mot1* mutations are synthetically defective with *nhp6ab* [32]. This *mot1 nhp6ab* synthetic lethality can be suppressed by multicopy plasmids containing the genes for TBP or TFIIB [32]. Similarly, *nhp6ab* is

synthetically defective with a *ccr4* mutation affecting the Ccr4/Not complex, and this can be suppressed by a multicopy plasmid with TBP [32].

One study detected an interaction between Nhp6 and Mediator using the split-ubiquitin two hybrid system, and showed that overexpression of the Rpb4 subunit of RNA pol II suppressed the 6-AU sensitivity of *nhp6ab* mutants [49]. The 6-AU sensitivity of a *nhp6ab* mutant can also be suppressed by an *spt3* mutation or by multicopy *SNR6* [35].

These experiments all support the idea that Nhp6 works in the same pathway as the Swi/Snf chromatin remodeling complex and the Gcn5 histone acetyltransferase in promoting transcription by facilitating the interaction of TBP and TFIIA on promoter DNA (Fig 1). The synthetic lethal interactions are consistent with this idea: While gene deletions eliminating *SWI2*, *GCN5* or *NHP6AB* are tolerated, inactivating any two of these pathways, in the *swi2 gcn5*, the *swi2 nhp6ab*, or the *gcn5 nhp6ab* mutant, results in synthetic lethality. Mutations that compromise TBP or TFIIA interaction can be tolerated, but are lethal when *NHP6*, *SWI2* or *GCN5* are mutated.

8. Interaction of Nhp6 with Chromatin Modifiers

Nhp6 has been shown to interact with a variety of chromatin modifiers, including FACT, Swi/Snf, RSC, Ssn6, and Spt6 (Table 1). By far the most work has been done on how Nhp6 stimulates FACT activity, and I will discuss FACT first. The FACT chromatin reorganizer plays important roles in DNA replication, transcription elongation, and transcription initiation [for review see 50]. Recent work has shown that FACT is required for redeposition of histones evicted by elongating RNA polymerase [51] and for nucleosome eviction at certain promoters [52–55]. In vitro studies show FACT changes the accessibility of DNA within nucleosomes without repositioning the histone octamer core relative to the DNA [12,25,52]. FACT affects chromatin structure without hydrolyzing ATP, and thus FACT functions quite differently from the Swi/Snf family of ATP-dependent chromatin remodeling factors.

FACT is a heterodimer composed of two subunits, Spt16-Pob3 in yeast, and Spt16-SSRP1 in metazoans. The Spt16 protein is conserved along its entire length from yeast to humans. In contrast, while Pob3 is conserved over most of its length, the fungal forms lack the HMGB motif present at the C-terminus of the metazoan homologs. The evidence suggests that the Nhp6 HMGB protein provides a DNA-binding (or DNA-bending) activity for the yeast Spt16/Pob3 complex. The Spt16-Pob3 heterodimer does not bind to DNA or nucleosomes in vitro; addition of Nhp6, however, permits the formation of a FACT – nucleosome complex [25]. The structure of nucleosomes, as assayed by sensitivity to DNaseI and restriction enzymes, is altered by FACT (Spt16-Pob3-Nhp6) [25,52]. Nhp6 does not stably associate with Spt16-Pob3 [25], although an association has been described using an immunoprecipitation protocol conducted at 50 mM potassium acetate [26].

Careful studies examining the amount of Nhp6 required to affect nucleosome structure in vitro have been informative [12]. Adding increasing concentrations of Nhp6 to in vitro binding reactions results in discrete complexes that differ by one Nhp6 monomer, whether the DNA is free in solution [8] or present in a nucleosome [12]. When sufficient Nhp6 is present the nucleosomes become sensitive to DNaseI cleavage or hydroxyl radical damage and also can bind Spt16-Pob3 [12,52]. Importantly, these changes all occur at similar Nhp6 concentrations, suggesting the effects are related. Additionally, the concentration of Nhp6 needed for these effects is 10-fold higher than that needed for simple Nhp6 binding. It has been suggested that HMGB proteins function as "shape chaperones," repeatedly binding to and releasing from DNA and thus allowing the DNA to form other shapes [56,57]. Thus Nhp6 may repeatedly bind to nucleosomal DNA and thus promote formation of an altered nucleosome as assayed by nuclease sensitivity, and this altered nucleosome is then receptive to Spt16-Pob3 binding. The

stoichiometry of Nhp6, compared to Spt16-Pob3, shows that multiple Nhp6 molecules are needed for a FACT-nucleosome complex to form. The authors propose that nucleosome reorganization by FACT is a two step process (Fig 2), with the concerted action of multiple Nhp6 molecules converting nucleosomes as the first step, and the second step requiring Spt16-Pob3 [12].

The biochemical analysis suggests Spt16-Pob3 require Nhp6 for activity, and there is also strong genetic support for this idea. *SPT16* and *POB3* mutations are synthetically lethal with *nhp6ab*, and Nhp6 overexpression suppresses mutations in these FACT subunits [25,26]. *SPT16* and *POB3* are both essential genes, raising the question of how an *nhp6a nhp6b* double mutant is viable. It is possible that other proteins besides Nhp6 can provide the necessary function for Spt16-Pob3 in vivo. Candidates for this activity would be other HMGB proteins such as Hmo1 and Nhp10; however, the *nhp6a nhp6b hmo1 nhp10* quadruple deletion strain is viable [58]. Thus Spt16-Pob3 might be able to reorganize nucleosomes in vivo without Nhp6, albeit less efficiently, or possibly using other types of DNA-binding proteins.

It seems likely that Nhp6 works with other chromatin modifiers besides FACT. Nhp6 overexpression suppresses mutations in subunits of the Swi/Snf and Rsc ATP-dependent chromatin organizers [27,28], and there are synthetic interactions between *nhp6ab* and either Swi/Snf or Rsc [26–29]. It is intriguing that mammalian Swi/Snf contains an HMG domain that is absent in yeast Swi/Snf [59], a situation analogous to mammalian and yeast FACT. In vitro experiments show RSC stimulates Nhp6 binding to nucleosomes [28], and Nhp6 stimulates nucleosome sliding by RSC and Swi/Snf (M. Hepp, X. Steinberg, and J.L. Gutierrez, personal communication). Nhp6 also promotes nucleosome binding in vitro by the Spt6 histone chaperone (H. Xin and T. Formosa personal communication), analogous to the loading of Spt16-Pob3 by Nhp6. Finally, genetic experiments suggest Nhp6 facilitates recruitment of the Ssn6 coactivators to the *FRE2* genes [45]. This suggests that Nhp6 alters nucleosome structure globally, and this affects the activity of many factors that act upon nucleosomes. The complex genetic interactions observed could be explained solely by an indirect effect in which failure of Nhp6 to maintain a permissive nucleosome structure forces a broad range of factors that must overcome nucleosomal barriers to act with full efficiency. Alternatively, The local concentration of Nhp6 could be increased by specific protein-protein interactions like the weak binding detected with Spt16-Pob3 [25]. Finally, it is intriguing that mammalian HMGB facilitates nucleosome sliding by the ACF/CHRAC remodeling factor [60], analogous to the activity of Nhp6.

A recent paper shows that the Msh2-Msh6 DNA mismatch repair factor stimulates binding of Nhp6 to DNA in vitro [61]. Additionally, Nhp6 reduces the nonspecific binding of Msh2-Msh6 to homoduplex DNA, but Nhp6 does not affect Msh2-Msh6 binding to mismatched DNA. Thus Nhp6 modulates the binding activity of the Msh2-Msh6 DNA mismatch repair complex towards targets with heteroduplex DNA.

9. Summary

The 11 kD Nhp6 protein is small in size, but is required for normal growth of budding yeast. It is an abundant protein that binds DNA non-specifically and bends DNA sharply. Genetic experiments suggest Nhp6 interacts with variety of chromatin modifiers, including FACT, Swi/Snf, RSC, and Ssn6. Biochemical experiments with FACT suggest that Nhp6 bends the DNA within a nucleosome, allowing recruitment of Spt16-Pob3 and then reorganization of a nucleosome as assayed by nuclease sensitivity. Preliminary biochemical work indicates Nhp6 can affect the activity of the Swi/Snf and RSC remodelers and of the Spt6 histone chaperone in vitro, perhaps through a similar mechanism. Nhp6 is also required for efficient transcription by RNA polymerase III, particularly at the *SNR6* gene, and also for formation of the

preinitiation complex pol II basal factors. It is possible that these latter effects are indirect, due to the *nhp6ab* mutation affecting the structure of nucleosomes or the activity of chromatin complexes such as FACT.

Acknowledgments

I thank Tim Formosa, Jose Gutierrez, and Frank Pugh for granting permission to mention unpublished results. I especially thank Tim Formosa for many helpful discussions and for comments on the manuscript. This work was supported by grants R01-GM39067 and R01-GM64649 from the National Institutes of Health.

References

1. Bustin M. Revised nomenclature for high mobility group (HMG) chromosomal proteins. *Trends Biochem Sci* 2001;26:152–153. [PubMed: 11246012]
2. Kolodrubetz D, Burgum A. Duplicated NHP6 genes of *Saccharomyces cerevisiae* encode proteins homologous to bovine high mobility group protein 1. *J Biol Chem* 1990;265:3234–3239. [PubMed: 2406250]
3. Paull TT, Carey M, Johnson RC. Yeast HMG proteins NHP6A/B potentiate promoter-specific transcriptional activation in vivo and assembly of preinitiation complexes in vitro. *Genes Dev* 1996;10:2769–2781. [PubMed: 8946917]
4. Kuehl L, Salmond B, Tran L. Concentrations of high-mobility-group proteins in the nucleus and cytoplasm of several rat tissues. *J Cell Biol* 1984;99:648–654. [PubMed: 6235236]
5. Mavrich TN, Ioshikhes IP, Venters BJ, Jiang C, Tomsho LP, Qi J, Schuster SC, Albert I, Pugh BF. A barrier nucleosome model for statistical positioning of nucleosomes throughout the yeast genome. *Genome Res* 2008;18:1073–1083. [PubMed: 18550805]
6. Kolodrubetz D, Kruppa M, Burgum A. Gene dosage affects the expression of the duplicated NHP6 genes of *Saccharomyces cerevisiae*. *Gene* 2001;272:93–101. [PubMed: 11470514]
7. Espinet C, de la Torre MA, Aldea M, Herrero E. An efficient method to isolate yeast genes causing overexpression-mediated growth arrest. *Yeast* 1995;11:25–32. [PubMed: 7762298]
8. Yen YM, Wong B, Johnson RC. Determinants of DNA binding and bending by the *Saccharomyces cerevisiae* high mobility group protein NHP6A that are important for its biological activities. Role of the unique N terminus and putative intercalating methionine. *J Biol Chem* 1998;273:4424–4435. [PubMed: 9468494]
9. Paull TT, Johnson RC. DNA looping by *Saccharomyces cerevisiae* high mobility group proteins NHP6A/B. Consequences for nucleoprotein complex assembly and chromatin condensation. *J Biol Chem* 1995;270:8744–8754. [PubMed: 7721780]
10. Wong B, Masse JE, Yen YM, Giannikopoulos P, Feigon J, Johnson RC. Binding to cisplatin-modified DNA by the *Saccharomyces cerevisiae* HMGB protein Nhp6A. *Biochemistry* 2002;41:5404–5414. [PubMed: 11969400]
11. Allain FH, Yen YM, Masse JE, Schultze P, Dieckmann T, Johnson RC, Feigon J. Solution structure of the HMG protein NHP6A and its interaction with DNA reveals the structural determinants for non-sequence-specific binding. *EMBO J* 1999;18:2563–2579. [PubMed: 10228169]
12. Ruone S, Rhoades AR, Formosa T. Multiple Nhp6 molecules are required to recruit Spt16-Pob3 to form yFACT complexes and to reorganize nucleosomes. *J Biol Chem* 2003;278:45288–45295. [PubMed: 12952948]
13. Kao LR, Megraw TL, Chae CB. Essential role of the HMG domain in the function of yeast mitochondrial histone HM: functional complementation of HM by the nuclear nonhistone protein NHP6A. *Proc Natl Acad Sci U S A* 1993;90:5598–5602. [PubMed: 8516306]
14. Masse JE, Wong B, Yen YM, Allain FH, Johnson RC, Feigon J. The *S. cerevisiae* architectural HMGB protein NHP6A complexed with DNA: DNA and protein conformational changes upon binding. *J Mol Biol* 2002;323:263–284. [PubMed: 12381320]
15. Sebastian NT, Bystry EM, Becker NA, Maher LJ. Enhancement of DNA flexibility in vitro and in vivo by HMGB Box A proteins carrying box B residues. *Biochemistry* 2009;48:2125–2134. [PubMed: 19236006]

16. Costigan C, Kolodrubetz D, Snyder M. NHP6A and NHP6B, which encode HMG1-like proteins, are candidates for downstream components of the yeast SLT2 mitogen-activated protein kinase pathway. *Mol Cell Biol* 1994;14:2391–2403. [PubMed: 8139543]
17. Smith V, Chou KN, Lashkari D, Botstein D, Brown PO. Functional analysis of the genes of yeast chromosome V by genetic footprinting. *Science* 1996;274:2069–2074. [PubMed: 8953036]
18. Thatcher JW, Shaw JM, Dickinson WJ. Marginal fitness contributions of nonessential genes in yeast. *Proc Natl Acad Sci U S A* 1998;95:253–257. [PubMed: 9419362]
19. Kruppa M, Kolodrubetz D. Mutations in the yeast Nhp6 protein can differentially affect its in vivo functions. *Biochem Biophys Res Commun* 2001;280:1292–1299. [PubMed: 11162669]
20. Yen YM, Roberts PM, Johnson RC. Nuclear localization of the *Saccharomyces cerevisiae* HMG protein NHP6A occurs by a Ran-independent nonclassical pathway. *Traffic* 2001;2:449–464. [PubMed: 11422939]
21. Hanover JA, Love DC, DeAngelis N, O'Kane ME, Lima-Miranda R, Schulz T, Yen YM, Johnson RC, Prinz WA. The High Mobility Group Box Transcription Factor Nhp6Ap enters the nucleus by a calmodulin-dependent Ran-independent pathway. *J Biol Chem* 2007;282:33743–33751. [PubMed: 17878171]
22. Giaever G, Chu AM, Ni L, Connelly C, Riles L, Veronneau S, Dow S, Lucau-Danila A, Anderson K, Andre B, Arkin AP, Astromoff A, El-Bakkoury M, Bangham R, Benito R, Brachat S, Campanaro S, Curtiss M, Davis K, Deutschbauer A, Entian KD, Flaherty P, Foury F, Garfinkel DJ, Gerstein M, Gotte D, Guldener U, Hegemann JH, Hempel S, Herman Z, Jaramillo DF, Kelly DE, Kelly SL, Kotter P, LaBonte D, Lamb DC, Lan N, Liang H, Liao H, Liu L, Luo C, Lussier M, Mao R, Menard P, Ooi SL, Revuelta JL, Roberts CJ, Rose M, Ross-Macdonald P, Scherens B, Schimmack G, Shafer B, Shoemaker DD, Sookhai-Mahadeo S, Storms RK, Strathern JN, Valle G, Voet M, Volckaert G, Wang CY, Ward TR, Wilhelm J, Winzeler EA, Yang Y, Yen G, Youngman E, Yu K, Bussey H, Boeke JD, Snyder M, Philippsen P, Davis RW, Johnston M. Functional profiling of the *Saccharomyces cerevisiae* genome. *Nature* 2002;418:387–391. [PubMed: 12140549]
23. Boone C, Bussey H, Andrews BJ. Exploring genetic interactions and networks with yeast. *Nat Rev Genet* 2007;8:437–449. [PubMed: 17510664]
24. Yu Y, Eriksson P, Stillman DJ. Architectural transcription factors and the SAGA complex function in parallel pathways to activate transcription. *Mol Cell Biol* 2000;20:2350–2357. [PubMed: 10713159]
25. Formosa T, Eriksson P, Wittmeyer J, Ginn J, Yu Y, Stillman DJ. Spt16-Pob3 and the HMG protein Nhp6 combine to form the nucleosome-binding factor SPN. *EMBO J* 2001;20:3506–3517. [PubMed: 11432837]
26. Brewster NK, Johnston GC, Singer RA. A bipartite yeast SSRP1 analog comprised of Pob3 and Nhp6 proteins modulates transcription. *Mol Cell Biol* 2001;21:3491–3502. [PubMed: 11313475]
27. Biswas D, Imbalzano AN, Eriksson P, Yu Y, Stillman DJ. Role for Nhp6, Gcn5, and the Swi/Snf complex in stimulating formation of the TATA-binding protein-TFIIA-DNA complex. *Mol Cell Biol* 2004;24:8312–8321. [PubMed: 15340090]
28. Szerlong H, Saha A, Cairns BR. The nuclear actin-related proteins Arp7 and Arp9: a dimeric module that cooperates with architectural proteins for chromatin remodeling. *EMBO J* 2003;22:3175–3187. [PubMed: 12805231]
29. Collins SR, Miller KM, Maas NL, Roguev A, Fillingham J, Chu CS, Schuldiner M, Gebbia M, Recht J, Shales M, Ding H, Xu H, Han J, Ingvarsdotir K, Cheng B, Andrews B, Boone C, Berger SL, Hieter P, Zhang Z, Brown GW, Ingles CJ, Emili A, Allis CD, Toczycki DP, Weissman JS, Greenblatt JF, Krogan NJ. Functional dissection of protein complexes involved in yeast chromosome biology using a genetic interaction map. *Nature* 2007;446:806–810. [PubMed: 17314980]
30. Biswas D, Dutta-Biswas R, Mitra D, Shibata Y, Strahl BD, Formosa T, Stillman DJ. Opposing roles for Set2 and yFACT in regulating TBP binding at promoters. *EMBO J* 2006;25:4479–4489. [PubMed: 16977311]
31. Laser H, Bongards C, Schuller J, Heck S, Johnsson N, Lehming N. A new screen for protein interactions reveals that the *Saccharomyces cerevisiae* high mobility group proteins Nhp6A/B are involved in the regulation of the GAL1 promoter. *Proc Natl Acad Sci U S A* 2000;97:13732–13737. [PubMed: 11095729]

32. Biswas D, Yu Y, Mitra D, Stillman DJ. Genetic interactions between Nhp6 and Gcn5 with Mot1 and the Ccr4-Not complex that regulate binding of TATA-binding protein in *Saccharomyces cerevisiae*. *Genetics* 2006;172:837–849. [PubMed: 16272410]
33. Formosa T, Ruone S, Adams MD, Olsen AE, Eriksson P, Yu Y, Rhoades AR, Kaufman PD, Stillman DJ. Defects in SPT16 or POB3 (yFACT) in *Saccharomyces cerevisiae* cause dependence on the Hir/Hpc pathway: polymerase passage may degrade chromatin structure. *Genetics* 2002;162:1557–1571. [PubMed: 12524332]
34. Yu Y, Eriksson P, Bhoite LT, Stillman DJ. Regulation of TATA-binding protein binding by the SAGA complex and the Nhp6 high-mobility group protein. *Mol Cell Biol* 2003;23:1910–1921. [PubMed: 12612066]
35. Eriksson P, Biswas D, Yu Y, Stewart JM, Stillman DJ. TATA-binding protein mutants that are lethal in the absence of the Nhp6 high-mobility-group protein. *Mol Cell Biol* 2004;24:6419–6429. [PubMed: 15226442]
36. Martin MP, Gerlach VL, Brow DA. A novel upstream RNA polymerase III promoter element becomes essential when the chromatin structure of the yeast U6 RNA gene is altered. *Mol Cell Biol* 2001;21:6429–6439. [PubMed: 11533232]
37. Sidorova J, Breeden L. The MSN1 and NHP6A genes suppress SWI6 defects in *Saccharomyces cerevisiae*. *Genetics* 1999;151:45–55. [PubMed: 9872947]
38. Kruppa M, Moir RD, Kolodrubetz D, Willis IM. Nhp6, an HMG1 protein, functions in SNR6 transcription by RNA polymerase III in *S. cerevisiae*. *Mol Cell Biol* 2001;21:309–318. [PubMed: 11239460]
39. Lopez S, Livingstone-Zatchej M, Jourdain S, Thoma F, Sentenac A, Marsolier MC. High-mobility-group proteins NHP6A and NHP6B participate in activation of the RNA polymerase III SNR6 gene. *Mol Cell Biol* 2001;21:3096–3104. [PubMed: 11287614]
40. Kassavetis GA, Steiner DF. Nhp6 is a transcriptional initiation fidelity factor for RNA polymerase III transcription in vitro and in vivo. *J Biol Chem* 2006;281:7445–7451. [PubMed: 16407207]
41. Parnell TJ, Huff JT, Cairns BR. RSC regulates nucleosome positioning at Pol II genes and density at Pol III genes. *EMBO J* 2008;27:100–110. [PubMed: 18059476]
42. Braglia P, Dugas SL, Donze D, Dieci G. Requirement of Nhp6 proteins for transcription of a subset of tRNA genes and heterochromatin barrier function in *Saccharomyces cerevisiae*. *Mol Cell Biol* 2007;27:1545–1557. [PubMed: 17178828]
43. Moreira JM, Holmberg S. Chromatin-mediated transcriptional regulation by the yeast architectural factors NHP6A and NHP6B. *EMBO J* 2000;19:6804–6813. [PubMed: 11118215]
44. Turkel S. Non-histone proteins Nhp6A and Nhp6B are required for the regulated expression of SUC2 gene of *Saccharomyces cerevisiae*. *J Biosci Bioeng* 2004;98:9–13. [PubMed: 16233659]
45. Fragiadakis GS, Tzamarias D, Alexandraki D. Nhp6 facilitates Aft1 binding and Ssn6 recruitment, both essential for FRE2 transcriptional activation. *EMBO J* 2004;23:333–342. [PubMed: 14739928]
46. Eisenmann DM, Arndt KM, Ricupero SL, Rooney JW, Winston F. SPT3 interacts with TFIID to allow normal transcription in *Saccharomyces cerevisiae*. *Genes Dev* 1992;6:1319–1331. [PubMed: 1628834]
47. Auble DT, Hansen KE, Mueller CG, Lane WS, Thorner J, Hahn S. Mot1, a global repressor of RNA polymerase II transcription, inhibits TBP binding to DNA by an ATP-dependent mechanism. *Genes Dev* 1994;8:1920–1934. [PubMed: 7958867]
48. Poon D, Campbell AM, Bai Y, Weil PA. Yeast Taf170 is encoded by MOT1 and exists in a TATA box-binding protein (TBP)-TBP-associated factor complex distinct from transcription factor IID. *J Biol Chem* 1994;269:23135–23140. [PubMed: 8083216]
49. Xue X, Lehming N. Nhp6p and Med3p regulate gene expression by controlling the local subunit composition of RNA polymerase II. *J Mol Biol* 2008;379:212–230. [PubMed: 18448120]
50. Formosa T. FACT and the reorganized nucleosome. *Mol Biosyst* 2008;4:1085–1093. [PubMed: 18931784]
51. Jamai A, Puglisi A, Strubin M. Histone chaperone spt16 promotes redeposition of the original h3-h4 histones evicted by elongating RNA polymerase. *Mol Cell* 2009;35:377–383. [PubMed: 19683500]

52. Xin H, Takahata S, Blanksma M, McCullough L, Stillman DJ, Formosa T. yFACT induces global accessibility of nucleosomal DNA without H2A-H2B displacement. *Mol Cell* 2009;35:365–376. [PubMed: 19683499]
53. Takahata S, Yu Y, Stillman DJ. FACT and Asf1 Regulate Nucleosome Dynamics and Coactivator Binding at the *HO* Promoter. *Mol Cell* 2009;34:405–415. [PubMed: 19481521]
54. Takahata S, Yu Y, Stillman DJ. The E2F functional analog SBF recruits the Rpd3(L) HDAC, via Whi5 and Stb1, and the FACT chromatin reorganizer, to yeast G1 cyclin promoters. *EMBO J*. 2009 In Press. DOI: 10.1038/emboj.2009.270.
55. Ransom M, Williams SK, Dechassa ML, Das C, Linger J, Adkins M, Liu C, Bartholomew B, Tyler JK. FACT and the proteasome promote promoter chromatin disassembly and transcriptional initiation. *J Biol Chem* 2009;284:23461–23471. [PubMed: 19574230]
56. Ross ED, Hardwidge PR, Maher LJ 3rd. HMG proteins and DNA flexibility in transcription activation. *Mol Cell Biol* 2001;21:6598–6605. [PubMed: 11533247]
57. Travers AA. Priming the nucleosome: a role for HMGB proteins? *EMBO Rep* 2003;4:131–136. [PubMed: 12612600]
58. Lu J, Kobayashi R, Brill SJ. Characterization of a high mobility group 1/2 homolog in yeast. *J Biol Chem* 1996;271:33678–33685. [PubMed: 8969238]
59. Wang W, Chi T, Xue Y, Zhou S, Kuo A, Crabtree GR. Architectural DNA binding by a high-mobility-group/kinesin-like subunit in mammalian SWI/SNF-related complexes. *Proc Natl Acad Sci U S A* 1998;95:492–498. [PubMed: 9435219]
60. Bortvin A, Winston F. Evidence that Spt6p controls chromatin structure by a direct interaction with histones. *Science* 1996;272:1473–1476. [PubMed: 8633238]
61. Labazi M, Jaafar L, Flores-Rozas H. Modulation of the DNA-binding activity of *Saccharomyces cerevisiae* MSH2-MSH6 complex by the high-mobility group protein NHP6A, in vitro. *Nucleic Acids Res*. 2009 In Press. DOI: 10.1093/nar/gkp649.
62. Krogan NJ, Cagney G, Yu H, Zhong G, Guo X, Ignatchenko A, Li J, Pu S, Datta N, Tikuisis AP, Punna T, Peregrin-Alvarez JM, Shales M, Zhang X, Davey M, Robinson MD, Paccanaro A, Bray JE, Sheung A, Beattie B, Richards DP, Canadien V, Lalev A, Mena F, Wong P, Starostine A, Canete MM, Vlasblom J, Wu S, Orsi C, Collins SR, Chandran S, Haw R, Rilstone JJ, Gandi K, Thompson NJ, Musso G, St Onge P, Ghanny S, Lam MH, Butland G, Altaf-Ul AM, Kanaya S, Shilatifard A, O'Shea E, Weissman JS, Ingles CJ, Hughes TR, Parkinson J, Gerstein M, Wodak SJ, Emili A, Greenblatt JF. Global landscape of protein complexes in the yeast *Saccharomyces cerevisiae*. *Nature* 2006;440:637–643. [PubMed: 16554755]
63. Collins SR, Kemmeren P, Zhao XC, Greenblatt JF, Spencer F, Holstege FC, Weissman JS, Krogan NJ. Toward a comprehensive atlas of the physical interactome of *Saccharomyces cerevisiae*. *Mol Cell Proteomics* 2007;6:439–450. [PubMed: 17200106]
64. Tarassov K, Messier V, Landry CR, Radinovic S, Serna Molina MM, Shames I, Malitskaya Y, Vogel J, Bussey H, Michnick SW. An in vivo map of the yeast protein interactome. *Science* 2008;320:1465–1470. [PubMed: 18467557]
65. Gavin AC, Bosche M, Krause R, Grandi P, Marzioch M, Bauer A, Schultz J, Rick JM, Michon AM, Cruciat CM, Remor M, Hofert C, Schelder M, Brajenovic M, Ruffner H, Merino A, Klein K, Hudak M, Dickson D, Rudi T, Gnau V, Bauch A, Bastuck S, Huhse B, Leutwein C, Heurtier MA, Copley RR, Edelmann A, Querfurth E, Rybin V, Drewes G, Raida M, Bouwmeester T, Bork P, Seraphin B, Kuster B, Neubauer G, Superti-Furga G. Functional organization of the yeast proteome by systematic analysis of protein complexes. *Nature* 2002;415:141–147. [PubMed: 11805826]

Fig 1.

Model for how Swi/Snf, Gcn5 and Nhp6 all contribute to formation of the TBP-TFIIB-TFIIA-DNA complex. Swi/Snf, Gcn5 and Nhp6 all function in the same process. A single mutation affecting Swi/Snf, Gcn5 or Nhp6 is tolerated, but mutations affecting more than one pathway results in synthetic lethality. Similarly, certain mutations in TBP or TFIIA that modestly affect complex formation can be tolerated, but these mutations are lethal in when Swi/Snf, Gcn5 or Nhp6 are also mutated.

Fig 2.

Two step model for nucleosome reorganization by FACT. In the first step a molar excess of Nhp6 is required to effect changes in nucleosome structure. This subtly altered nucleosome can then be recognized by Spt16-Pob3, and FACT then makes more substantial changes in nucleosome structure.

Table 1

Genetic and Biochemical Interactions with Nhp6.

Genetic Interactions			
<u>Gene</u>	<u>Function</u>	Genetic Interaction	Reference
<i>ARP7</i>	Swi/Snf and RSC chromatin remodelers	Synthetic growth defect with <i>nhp6ab</i>	[28]
<i>ARP7</i>	Swi/Snf and RSC chromatin remodelers	<i>NHP6A</i> overexpression suppresses growth defect	[28]
<i>ARP9</i>	Swi/Snf and RSC chromatin remodelers	Synthetic growth defect with <i>nhp6ab</i>	[28]
<i>ARP9</i>	Swi/Snf and RSC chromatin remodelers	<i>NHP6A</i> overexpression suppresses growth defect	[28]
<i>BCK1 (SLK1)</i>	MAPKKK in the protein kinase C signaling pathway	<i>NHP6A</i> overexpression suppresses <i>slk1-1 spa2Δ</i> synthetic growth defect	[16]
<i>CDC73</i>	Paf1 elongation complex	Synthetic growth defect with <i>nhp6ab</i>	[29]
<i>EAF1</i>	NuA4 HAT complex	Synthetic growth defect with <i>nhp6ab</i>	[29]
<i>GCN5</i>	histone acetyl transferase	Synthetic growth defect with <i>nhp6ab</i>	[24]
<i>HHF2</i>	deletion of histone H3 tail	Synthetic growth defect with <i>nhp6ab</i>	[33]
<i>HHT2</i>	deletion of histone H3 tail	Synthetic growth defect with <i>nhp6ab</i>	[33]
<i>IES1</i>	Ino80 chromatin remodeler	Synthetic growth defect with <i>nhp6ab</i>	[29]
<i>IES3</i>	Ino80 chromatin remodeler	Synthetic growth defect with <i>nhp6ab</i>	[29]
<i>MOT1</i>	TBP interacting factor	Synthetic growth defect with <i>nhp6ab</i>	[32]
<i>NHP10</i>	HMGB factor	Synthetic growth defect with <i>nhp6ab</i>	[29]
<i>NOT4</i>	Ccr/Not transcription / polyadenylation complex	Synthetic growth defect with <i>nhp6ab</i>	[32]
<i>POB3</i>	FACT chromatin reorganizing complex	Synthetic growth defect with <i>nhp6ab</i>	[25,26]
<i>REG1</i>	transcriptional repressor	Synthetic growth defect with <i>nhp6ab</i>	[31]
<i>RPB4</i>	RNA pol II subunit	Multicopy <i>RPB4</i> suppresses the 6-AU sensitivity of <i>nhp6ab</i> mutants	[49]
<i>RPC40</i>	subunit of pol I and pol III	Synthetic growth defect with <i>nhp6ab</i>	[29]
<i>RSC9</i>	RSC chromatin remodeler	Synthetic growth defect with <i>nhp6ab</i>	[28,29]
<i>SET2</i>	histone methyltransferase	Synthetic growth defect with <i>nhp6ab</i>	[30]
<i>SNF5</i>	Swi/Snf chromatin remodeler	Synthetic growth defect with <i>nhp6ab</i>	[26]
<i>SNR6</i>	U6 spliceosomal RNA	Synthetic growth defect with <i>nhp6ab</i>	[36]
<i>SNR6</i>	U6 spliceosomal RNA	Multicopy <i>SNR6</i> suppresses the 6-AU sensitivity of <i>nhp6ab</i> mutants	[35]
<i>SPA2</i>	scaffold component for cell wall integrity signaling pathway	<i>NHP6A</i> overexpression suppresses <i>slk1-1 spa2Δ</i> synthetic growth defect	[16]
<i>SPT15</i>	TATA binding protein	Synthetic growth defect	[34,35]
<i>SPT15</i>	TATA binding protein	<i>NHP6A</i> overexpression suppresses <i>spt15 swi2</i> synthetic growth defect	[27]
<i>SPT16</i>	FACT chromatin reorganizing complex	Synthetic growth defect with <i>nhp6ab</i>	[25,26]
<i>SPT16</i>	FACT chromatin reorganizing complex	<i>NHP6A</i> overexpression suppresses growth defect	[25]

Genetic Interactions			
<i>SPT3</i>	SAGA complex	<i>spt3</i> suppresses <i>nhp6ab</i> defects	[27,34]
<i>SPT4</i>	elongation factors	Synthetic growth defect with <i>nhp6ab</i>	[25]
<i>SPT5</i>	elongation factors	Synthetic growth defect with <i>nhp6ab</i>	[26]
<i>STH1</i>	RSC chromatin remodeler	Synthetic growth defect with <i>nhp6ab</i>	[28]
<i>SWI2</i>	Swi/Snf chromatin remodeler	Synthetic growth defect with <i>nhp6ab</i>	[27,28]
<i>SWI2</i>	Swi/Snf chromatin remodeler	<i>NHP6A</i> overexpression suppresses <i>spt15 swi2</i> synthetic growth defect	[27]
<i>SWI6</i>	SBF DNA-binding factor	<i>NHP6A</i> overexpression suppresses growth defect	[37]
<i>TOA1</i>	TFIIA basal factor	synthetic growth defect with <i>nhp6ab</i>	[27]

Biochemical Interactions		
Factor	Biochemical Interaction	Reference
FACT (Spt16-Pob3)	Nhp6 is required for FACT binding to nucleosomes	[25]
Msh2-Msh6	Msh2-Msh6 stimulates Nhp6 binding to DNA,	[61]
Msh2-Msh6	Nhp6 reduces binding of Msh2-Msh6 to non-mismatched DNA	[61]
RSC	RSC stimulates Nhp6 binding to nucleosomes	[28]
RSC	Nhp6 stimulates nucleosome sliding by RSC	[A]
Swi/Snf	Nhp6 stimulates nucleosome sliding by Swi/Snf	[A]
Spt6	Nhp6 promotes nucleosome binding by Spt6	[B]

Two-hybrid and purification / mass spectrometry studies describe additional protein interactions with Nhp6A or Nhp6b [49,62–65], but the significance of these potential interactions has not been determined genetically.

[A] M. Hepp, X. Steinberg, and J.L. Gutierrez, personal communication.

[B] H. Xin and T. Formosa personal communication.