Biochimica et Biophysica Acta 1799 (2010) 175–180

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

journal homepage: www.elsevier.com/locate/bbagrm

Review

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, USA

ARTICLE INFO

Article history:
Received 25 September 2009
Received in revised form 28 October 2009
Accepted 3 November 2009

Keywords:
HMGB
Chromatin
Nucleosome
Pol II
Pol III
TBP
TFIIA
TFIIB
FACT
Spt16
Pob3
Swi/Snf
Rsc

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.

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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 one to two nucleosomes, which is similar to the value of 1 HMG1/2 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 3 times [6] to 10 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 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

* Tel.: +1 801 581 5429; fax: +1 801 581 4517.
E-mail address: david.stillman@path.utah.edu.

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doi:10.1016/j.bbagrm.2009.11.010

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 1 M 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

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