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Biochemical and Biophysical Research Communications 306 (2003) 5–9
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Genome-wide expression analysis of *NAP1* in *Saccharomyces cerevisiae*

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Received 25 April 2003

Abstract

Nap1 is a nucleosome assembly protein which is necessary to keep proper nucleosome structures in transcription and replication in vitro. In *Saccharomyces cerevisiae*, additional functions have been ascribed to Nap1, as it has been shown to interact with Clb2 (B type cyclin) and Gin4 (septum formation). In this study, we investigate genome-wide expression in the Δ*nap1* cells using DNA microarrays. About 10% of all yeast open reading frames changed the transcription level more than 2-fold in the Δ*nap1* strain, compared with wild-type strain, in one experiment. Interestingly, these genes, whose expressions are up- or down-regulated in the Δ*nap1* cells, are clustered. This result suggests that yeast *NAP1* is required for the maintenance of cumulative nucleosome formation in vivo and the loss of Nap1 leads to a change in the gene expression level in a cluster.

Keywords: NAP1; Nucleosome assembly; Gene expression; Genomic analysis; *Saccharomyces cerevisiae*

Gene expression is a dynamic process in which genes are constantly being switched on and off. Eukaryotic DNA is packaged into nucleosomes, composed of an octamer of core histones (an H3–H4 tetramer bound to two H2A–H2B dimers). DNA packed in the nucleosome structures might be hindered in both transcriptional initiation and elongation in eukaryotic cells. Thus, the remodeling of nucleosome structures is often suggested prior to the entrance of transcriptional factors and RNA polymerases in gene regulation.

Nucleosome assembly protein 1 (Nap1) was first identified in mammalian cell extracts by its intrinsic activity to facilitate nucleosome assembly in vitro [1]. The Nap1 is a highly conserved protein from yeast to human. *Drosophila* Nap1, which is also required for nucleosome assembly in vitro, was found to be associated with core histones H2A and H2B [2], similar results having been observed in HeLa cells [3], suggesting that Nap1 may act as a histone chaperone. Member of the family of nucleosome assembly protein includes TAF1/set proteins. For example, TAF1 was identified on the basis of its ability to stimulate adenovirus replication and transcription of a viral chromatin template [4]. Therefore, NAP family might play an important role of transcriptional control and DNA replication.

The study presented here is genome-wide expression analysis in yeast using DNA microarrays. The aim of this study is to investigate the genome-wide transcriptional control of *NAP1* in vivo. The GeneChip methodology developed by Affymetrix was carried out to monitor gene expression in wild-type and Δ*nap1* cells. By studying the effect of transcription in Δ*nap1* cells, we find that the open reading frames (ORFs), whose transcripts increased or decreased, are found in a cluster. The gene regulation in eukaryotic cells is sporadic in the whole chromosomes. These data have led us to propose that yeast *NAP1* is required for nucleosome maintenance in vivo.

Materials and methods

Strains and media. Saccharomyces cerevisiae strains used in this study were isogenic pairs of RAY (wild-type: *MAT*a *ura3 leu2 trp1 his3 GAL*$^+$) and YKO227 (Δ*nap1*: *MAT*a *ura3 leu2 trp1 his3 GAL*$^+$ *nap1::LEU2*). As examples of independent strains', we used YKO215

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0006-291X/03/$ - see front matter © 2003 Elsevier Science (USA). All rights reserved.
doi:10.1016/S0006-291X(03)00907-0

(*MAT*a *ura3 leu2 trp1 his3 ade2 can1-100 ssd1-d nbp2::hisG*), YKO284 (*MAT*a *ura3 leu2 trp1 his3 ade2 can1-100 ssd1-d htr1::URA3*) and their parental strain W303 (*MAT*a *ura3 leu2 trp1 his3 ade2 can1-100 ssd1-d*). Yeast cultures were grown in YPD (1% yeast extract, 2% peptone, 2% glucose, and 400 mg/L adenine) at 30 °C.

GeneChip analysis. Cells were grown in YPD medium to an OD_{600} of 0.5 at 30 °C. Total RNA was prepared as described previously [5] and poly(A)$^+$ RNA was purified from total RNA using the Oligotex(dT)30 mRNA purification kit (Takara). Poly(A)$^+$ RNA was amplified, biotin-labeled, and hybridized to oligonucleotide arrays (GeneChip Yeast Genome S98 Arrays (YG-S98), Affymetrix) by standard methods. The experiments were independently carried out three times.

Table 1
Genome-wide analysis of Δ*nap1* cells

Experiment[a]	Percentage of genes changed in their expression[b]		
	2-fold down	2-fold up	Total
I	3.2	8.7	12.0
II	1.4	8.8	10.2
III	6.3	2.1	8.4

[a] Three independent experiments (I–III) were done.

[b] The proportion of the ORFs whose transcript levels were changed (decreased, increased, and total) by more than 2-fold in the Δ*nap1* cells, compared with the wild-type cells.

Genes, whose expression level was increased or decreased in comparison with parental strain, were listed, based on the following criteria: the average change of three experiments was more than 2.5-fold, the change in each experiment was greater than 1.5-fold, and the change is the same direction in three experiments. The raw data can be retrieved from server: ftp://gscarray.gsc.riken.go.jp/Ohkunietal/ (username: anonymous, password: your e-mail address).

Cluster analyses were carried out as described below. In case of Δ*nap1* strain, the ORFs whose expression level changed by more than 2-fold are counted (*T*). When the ORFs counted as (*T*) were clustered or consecutive, their number was given in (*C*). Percentage of cluster is guided by expression $(C)/(T) \times 100$. To compare the result of YKO227 with other strains, such as YKO215 or YKO284, it is important to count the equal number of ORFs, whose expression level was changed. Thus, we used different cut off values for YKO215 or YKO284.

Results and discussion

To investigate the genes whose transcripts are regulated by *NAP1*, we analyzed transcription profile of Δ*nap1* cells using DNA microarrays, manufactured by Affymetrix. As shown in Table 1, about 10% of all yeast ORFs were up- or down-regulated by over 2-fold in the Δ*nap1* strain, compared with wild type, in each

Table 2
Changes in transcription level caused by deletion of *NAP1*

ORF	Gene	Fold changes[a]				Function
		I	II	III	Average	
Increased genes						
YDL156W		26.0	19.7	8.8	18.2	Unknown
YGR221C	*TOS2*	15.3	17.5	1.8	11.5	Target of SBF
YBR156C	*SLI15*	12.6	8.8	6.1	9.2	Mitotic spindle protein involved in chromosome segregation
YDR467C		8.8	9.9	2.6	7.1	Unknown
YML027W	*YOX1*	6.0	11.9	2.2	6.7	Homeobox-domain containing protein
YHL005C		4.8	9.2	2.3	5.4	Unknown
YMR199W	*CLN1*	11.0	2.0	1.6	4.9	G1 cyclin
YDR480W	*DIG2*	5.6	3.9	1.7	3.7	MAP kinase-associated protein
YNL289W	*PCL1*	2.1	6.0	2.4	3.5	G1 cyclin associates with *PHO85*
YAL067C	*SEO1*	4.0	4.0	2.3	3.4	Permease
YGL260W		4.1	4.6	1.5	3.4	Unknown
YJR079W		5.2	2.1	2.4	3.2	Unknown
YOR192C		3.6	4.0	1.6	3.1	Transporter activity
YGL258W	*VEL1*	4.3	3.2	1.8	3.1	Unknown
YPR077C		5.0	2.0	1.9	3.0	Unknown
YNL333W	*SNZ2*	3.3	2.8	2.6	2.9	Snooze: stationary phase-induced gene family
YOR152C		1.7	4.3	2.2	2.7	Unknown
YIL037C	*PRM2*	4.2	2.0	1.8	2.7	Pheromone-regulated membrane protein
YNL279W	*PRM1*	4.7	1.5	1.8	2.7	Pheromone-regulated membrane protein
Decreased genes						
YLR327C		−1.6	−2.8	−3.1	−2.5	Unknown
YFR015C	*GSY1*	−2.0	−3.4	−2.5	−2.6	Glycogen synthase (UDP–glucose–starch glucosyltransferase)
YGR088W	*CTT1*	−1.6	−4.2	−2.3	−2.7	Cytoplasmic catalase T
YGR242W		−2.7	−3.5	−3.6	−3.3	Unknown
YEL011W	*GLC3*	−1.6	−4.3	−4.2	−3.4	1,4-glucan-6-(1,4-glucano)-transferase
YMR172C		−6.1	−3.5	−2.0	−3.9	Unknown
YKR048C	*NAP1*	−27.6	−33.4	−30.7	−30.6	Nucleosome assembly protein 1

[a] Three independent experiments (I–III) were done. Positive and negative numbers indicate that transcripts are increased and decreased, respectively.

experiment. The expression of *NAP1* was greatly reduced in three experiments to the ground level, because of deletion (Table 2). The ORFs, whose transcripts increased or decreased more than 2.5-fold (average of three experiments), are listed in Table 2. The increased and decreased groups include 19 and 7 ORFs, respectively. In both cases, around 40% of the genes encoded protein with yet undefined functions.

It is noticed that several genes required for cell cycle are up-regulated in Δ*nap1* cells. The *Saccharomyces* Genome Database (SGD) revealed that *TOS2* is a member of target of SBF (Swi4–Swi6 cell cycle box binding factor). *SLI15* encodes mitotic spindle protein [6]. *YOX1* is a member of target of SBF and acts as a repressor targeted at early cell cycle boxes (ECBs) [7,8]. *CLN1* and *PCL1* encode G1 cyclin [9]. Previous studies have shown that Nap1 binds Clb2 and Gin4, which are required for the proper control of mitotic events [10–12]. These data led us to suggest that yeast *NAP1* has an important role in cell cycle regulation.

NAP1 has been shown to be required for nucleosome assembly in vitro [13]. If *Nap1* is also required in vivo, genome-wide expression may be affected in Δ*nap1* cells. To test this hypothesis, we challenged cluster analysis in every chromosome (see Materials and methods). In comparison, we used two strains; one disrupted the *NBP2*, which is required for mitotic growth at high temperature, and the other disrupted the *HTR1*/*UTH4*, which affects life span [14,15].

For example, in the case of the longest chromosome IV, there were 13 regions whose expressions are affected in cluster in Δ*nap1* cells (Fig. 1A). In contrast, there appeared 5 and 6 regions in the same chromosome IV, where the adjacent genes were changed in Δ*nbp2* and Δ*htr1* cells, respectively (Figs. 1B and C). In the Δ*nap1* cells, about 35.4% of the genes, whose expression ratio changed more than 2-fold, were in a cluster (Table 3). This rate was larger than that of Δ*nbp2* (12.7%) and Δ*htr1* (12.5%). We next examined all chromosomes in yeast (Table 3). In most of the chromosomes, particularly in longer ones, the proportion of clustered genes in Δ*nap1* cells was larger than that of Δ*nbp2* and Δ*htr1* cells. In one experiment, proportions of genes in a cluster were 28.3% (Δ*nap1*), 15.0% (Δ*nbp2*), and 16.9% (Δ*htr1*) in whole genome. In another experiment, they were 30.7% (Δ*nap1*), 18.1% (Δ*nbp2*), and 14.8% (Δ*htr1*). If the transcription starts from a certain gene and its nucleosome structures are altered, this alteration of nucleosome may be spread to the several adjacent genes to form a cluster of expressed regions. Thus, we conclude that in the Δ*nap1* strain, proper nucleosome assembly could not be recovered immediately and transcriptions continued.

The ability of *NAP1* to keep proper nucleosome assembly implies a plausible model that transcription in the Δ*nap1* cells may be disordered (Fig. 2). Fig. 2A depicts the situation in wild-type cells, while Fig. 2B shows hypothetical nucleosome arrangement in the Δ*nap1* cells. DNA in wild-type cells is packaged in regularly spaced nucleosome structure. If *NAP1* is deleted, DNA is not sufficiently packaged into regularly spaced nucleosomes, as a result, there yields tight and loose loci. In tight region of DNA, gene expression is repressed in a cluster, while in loose region, gene expression is released in a cluster.

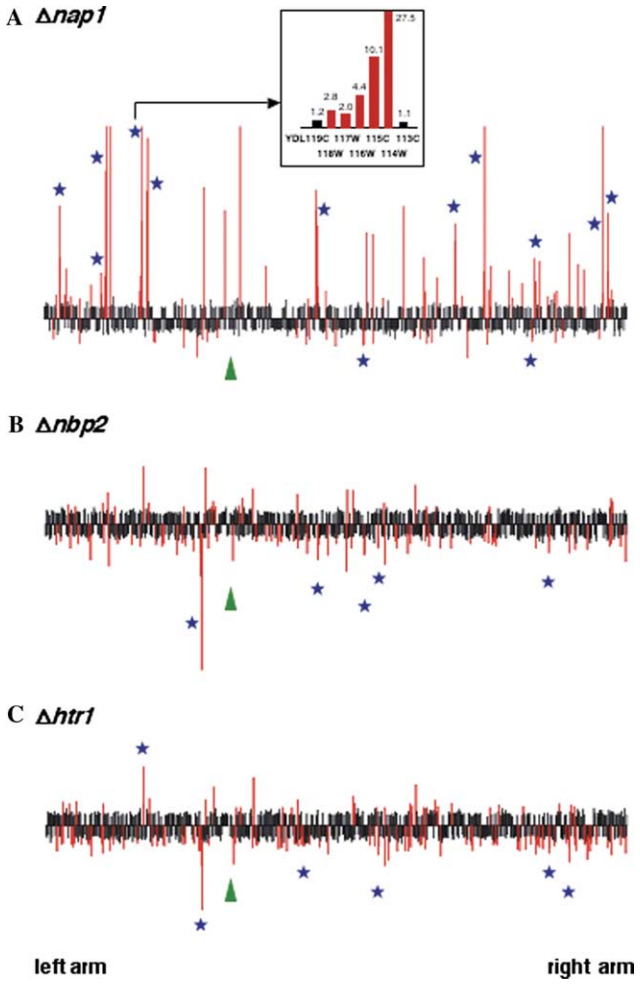

Fig. 1. Cluster analysis in chromosome IV. Horizontal line represents the chromosome on which each gene is arranged successively from left to right. Expression ratio of every gene, compared with wild-type strain, is represented in the height of perpendicular lines. Up-regulated genes are indicated as upward lines, while down-regulated genes are indicated as downward lines. Red lines indicate the expression ratio more than 2- (Δ*nap1*), 1.9- (Δ*nbp2*), and 2.1- (Δ*htr1*) fold. Cluster regions indicated by the blue star exist in 13 (Δ*nap1*), 5 (Δ*nbp2*), and 6 (Δ*htr1*) places. The green triangles indicate the centromer position of chromosome IV.

How does Nap1 affect nucleosome arrangement? It is shown that in *Drosophila*, Nap1 is required to dislocate H2A–H2B after histone acetylation in vitro [16]. The open nucleosomes might help to activate transcription. In addition, Kimura and Cook [17] demonstrated that in HeLa cells, H2B at the surface is continually exchanged

Table 3
Cluster analysis of experimental expression profiles

Chromosome	Total genes	*Δnap1*				YKO215				YKO284			
		Number of regulated genes C/T^a				Number of regulated genes C/T^a				Number of regulated genes C/T^a			
		X-fold	Up	Down	Total (% of cluster)	*X*-fold	Up	Down	Total (% of cluster)	*X*-fold	Up	Down	Total (% of cluster)
I	115	2.0	11/20	0/6	11/26 (42.3)	1.9	0/9	0/15	0/24 (0.0)	1.9	0/11	0/14	0/25 (0.0)
II	435	2.0	10/35	0/11	10/46 (21.7)	2.0	4/18	8/27	12/45 (26.7)	2.2	2/19	6/27	8/46 (17.4)
III	179	2.0	2/15	0/8	2/23 (8.7)	2.0	0/3	2/18	2/21 (9.5)	2.2	0/4	4/22	4/26 (15.4)
IV	815	2.0	30/66	4/30	34/96 (35.4)	1.9	0/25	13/77	13/102 (12.7)	2.1	2/24	11/80	13/104 (12.5)
V	292	2.0	8/23	2/15	10/38 (26.3)	1.9	2/10	2/28	4/38 (10.5)	2.2	2/16	5/26	7/42 (16.7)
VI	131	2.0	0/9	0/5	0/14 (0.0)	2.1	2/5	0/10	2/15 (13.3)	2.3	2/6	2/9	4/15 (26.7)
VII	574	2.0	12/34	2/22	14/56 (25.0)	2.2	0/14	9/47	9/61 (14.8)	2.3	0/13	8/41	8/54 (14.8)
VIII	282	2.0	7/29	0/6	7/35 (20.0)	2.1	0/4	6/29	6/33 (18.2)	2.2	2/11	2/22	4/33 (12.1)
IX	228	2.0	0/16	2/10	2/26 (7.7)	2.0	0/2	2/22	2/24 (8.3)	2.1	2/10	6/20	8/30 (26.7)
X	397	2.0	21/45	0/7	21/52 (40.4)	2.0	2/21	6/31	8/52 (15.4)	2.0	2/20	12/32	14/52 (26.9)
XI	348	2.0	9/26	2/13	11/39 (28.2)	2.0	0/12	3/26	3/38 (7.9)	2.1	0/11	4/31	4/42 (9.5)
XII	564	2.0	25/67	0/20	25/87 (28.7)	1.9	7/43	7/47	14/90 (15.6)	2.0	16/35	13/57	29/92 (31.5)
XIII	497	2.0	21/43	0/14	21/57 (36.8)	2.0	6/25	4/32	10/57 (17.5)	2.1	4/27	4/40	8/67 (11.9)
XIV	424	2.0	2/32	4/17	6/49 (12.2)	2.0	6/21	11/32	17/53 (32.1)	2.3	4/19	2/28	6/47 (12.8)
XV	573	2.0	21/53	2/11	23/64 (35.9)	2.0	2/20	6/40	8/60 (13.3)	2.1	2/24	6/44	8/68 (11.8)
XVI	495	2.0	16/42	2/10	18/52 (34.6)	2.0	2/23	2/25	4/48 (8.3)	2.1	8/25	2/31	10/56 (17.9)
Whole genome					215/760 (28.3)				114/761 (15.0)				135/799 (16.9)

[a] (Number of genes in a cluster)/(Total number of genes whose expression ratio changed more than *X*-fold).

Fig. 2. A speculative model for Nap1 function as nucleosome assembly protein. (A) Regularly spaced nucleosomal array is shown in wild-type cells. (B) One part of DNA is packaged tightly, another part is loose in the *Δnap1* cells. In case of tight region, genes are repressed in a cluster. In case of loose region, genes are expressed in a cluster. Arrow head indicates gene expression.

in the active nucleosomes in vivo. Our experiments suggest that yeast Nap1 is required for maintenance of nucleosome structure in vivo. Thus, Nap1 might be responsible to recruit H2A–H2B molecules to maintain ordered nucleosome arrangement and influence transcriptional switching in the adjacent genes.

Acknowledgments

We thank Yuki Katou for technical assistance of GeneChip analysis. We also thank Yoshiyuki Nakagawa, Toshio Kanbe, Tomohiro Akashi, Ikuyo Mizuguchi, Ayako Sakaguchi, Tsuya Taneda, and Tomoko Tsuruta for helpful discussions and encouragement. This study was supported in part by grants from the Ministry of Education, Science, Sports and Culture of Japan.

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