A mutation in NPS1/STH1, an essential gene encoding a component of a novel chromatin-remodeling complex RSC, alters the chromatin structure of Saccharomyces cerevisiae centromeres

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ABSTRACT

The NPS1/STH1 gene encodes a nuclear protein essential for the progression of G2/M phase in Saccharomyces cerevisiae. Nps1p shares homology to Snf2/Swi2p, a subunit of a protein complex known as the SNF/SWI complex. Recently, Nps1p was found to be a component of a protein complex termed RSC (1) essential for mitotic growth, whereas its function is unknown. We isolated a temperature-sensitive mutant allele of NPS1, nps1-105, and found that the mutation increases the sensitivity to thiabendazole (TBZ). At the restrictive temperature, nps1-105 arrested at the G2/M phase in MAD1-dependent manner and missegregated the mini-chromosome with higher frequency than the wild type cells. The nuclease digestion of the chromatin of the mutant cells revealed that the mutation causes the alteration of the chromatin structure around centromeres at the restrictive temperature. The results suggested that, in the nps1-105 mutant, impaired chromatin structure surrounding centromeres may lead to an impairment of kinetochore function and the cells arrest at G2/M phase through the spindle-assembly checkpoint system.

INTRODUCTION

The DNA of eukaryotic genome is packed into chromatin basically consists of ~150 base pair (bp) DNA wrapped around an octamer of four histone proteins (H2A, H2B, H3 and H4). This nucleoprotein structure contributes to store huge DNA molecules within a nucleus but, at the same time, it confers a barrier for DNA metabolism, i.e., transcription, replication, repair or recombination. Current studies on the regulation of eukaryotic gene expression revealed the presence of a protein complex, a SNF/SWI complex, which antagonizes chromatin-mediated repression of transcription (reviewed in 4–8). Purified yeast SNF/SWI complex contained at least 11 proteins (9–12). Biochemical analysis of the purified SNF/SWI complex revealed that this complex possesses a DNA-stimulated ATPase activity and can destabilize histone–DNA interactions in reconstituted nucleosomes in an ATP-dependent manner. The SNF/SWI complex is highly conserved through evolution: homologs of several Snf/Swi proteins have been identified in eukaryotes, and SNF/SWI complexes with related biochemical functions have been purified from human cells (13–16) and Drosophila melanogaster (17–19).

The Snf2/Swi2 subunit of the SNF/SWI complex contains a domain found in several DNA and RNA helicases and has been shown to harbor DNA-dependent ATPase activity (20). This protein is likely to play a key role in the chromatin remodeling activity of the complex. In Saccharomyces cerevisiae, a novel SNF/SWI-like complex termed RSC (remodel the structure of chromatin) was recently purified and characterized (3). RSC is an ~1 Mda complex that contains at least 15 polypeptides. The largest subunit of RSC that corresponds to Snf2/Swi2p of the SNF/SWI complex was proved to be the product of NPS1/STH1 previously isolated by us and others (1,2). In addition to Nps1p/Sth1p, RSC contains at least three of which are related to components of the SNF/SWI complex: Rsc6p (related to Swp73p), Rsc8p (related to Swi3p) and Sfh1p (related to Snf5p) (3,21). Like the SNF/SWI complex, RSC showed a DNA-stimulated ATPase activity and was able to alter histone–DNA interactions in reconstituted mononucleosomes in an ATP-dependent manner (3). Despite the fact that the constituents of the SNF/SWI complex are encoded by non-essential genes, Nps1p, Rsc6p, Rsc8p and Sfh1p are encoded by genes essential for mitotic growth. Moreover, a LexA-Sth1p fusion protein did not activate transcription under conditions where an analogous LexA-Snf2 protein functions as a transcriptional activator (2), indicating that the RSC complex may play a wider role than the SNF/SWI complex. However, neither the physiological function nor the downstream targets of RSC have yet been identified. As we have previously reported, depletion of Nps1p arrests the cells at the G2/M phase (1). In addition, the recent finding that the temperature-sensitive sfh1-1 mutation also causes accumulation of cells with 2C-DNA content at restrictive temperature, indicates the requirement of RSC for the progression through G2/M phase (21).

To explore the function of Nps1p, we isolated a temperature-sensitive nps1 mutant allele, nps1-105. In this report we describe the characterization of this mutant. We found that the nps1-105

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mutation causes the alteration of the chromatin structure on the centromeric DNA at the restrictive temperature. Our results provide a first clue in understanding the essential role of Nps1p and RSC in the cell division cycle.

MATERIALS AND METHODS

Media, yeast strains and manipulation

Both rich (YPD) and minimal (SD) media and basic yeast manipulations were as described (22). YP-galactose medium was prepared as for YPD, except that glucose (2%) was replaced by D-galactose (2%). Experiments were performed in the yeast W303 (23) strain background (ade2-1 leu2-3, 112 his3-11, 15 trp1-1 ura3-1).

Generation of temperature-sensitive NPS1 alleles

Hydroxylamine mutagenesis of pOS31NPS1 (NPS1, LEU2, CEN3, ARSI) was carried out according to Rose et al. (22). We selected 6 × 10^3 transformants in WET-1D (aα nps1Δ::HIS3/nps1Δ::HIS3 YCp50NPS1) on SC-leucine plates and transferred to SC-leucine+5FOA plates. Colonies formed on SC-leucine+5FOA plates were screened for temperature sensitivity at 37 and 38°C by replica plating onto YPD plates. Plasmids were recovered in *Escherichia coli* and retransformed into WET-1D to confirm the temperature-sensitive phenotype. One plasmid pTH105-1 (nps1-105) which showed temperature sensitivity at 38°C was sequenced to identify the mutation.

The 5.8 kb XhoI fragment containing nps1-105 was excised from pTH105-1 and transformed to WET-1 (aα nps1Δ::HIS3 YCp50NPS1) to replace the genomic NPS1-disruption allele (nps1Δ::HIS3) with the nps1-105 mutant allele. Colonies grown on a SC+5FOA plate were checked for histidine auxotrophy and temperature sensitivity. Three candidate clones of the nps1-105 mutant were transformed with pOS31NPS1 to check for the suppression of temperature sensitivity and crossed to wild type strain to check for the segregation of the temperature-sensitive phenotype. The temperature-sensitive growth phenotype of all the temperature-sensitive progeny of these crosses was selected and named WTH-1 (nps1-105).

Plasmids, primers, plasmid constructions and strain constructions

The *MAD1* gene was cloned by PCR using primers A (tttttacg-gatccacaggggttac) and B (tagatactatgtaggtgcacgg) with the genomic DNA as a template. The resulting fragment was cut with BamHI and SalI, and cloned into pUC119 (pUCMAD1). The *mad1Δ::TRP1* allele was created by replacing the internal *BglII–SalI* fragment of MAD1 in pUCMAD1 coding for amino acids 28–580 with the *BamHI–SalI* fragment of YRp7 containing the *TRP1* gene (pUC mad1Δ::TRP1). Plasmid pUC mad1Δ::TRP1 was digested with SmaI and SalI and introduced to W303-1A and WTH-1 to effect a one-step gene replacement of the chromosomal *MAD1* locus. The resultant strains were termed W-DM1 and WTH-DM1, respectively.

Flow cytometry

Flow cytometry analysis was performed on a Coulter Epics Elite ESP. Cells were prepared as described previously (1). Each FACS scan was performed on 2 × 10^5 cells.

Digestion of chromatin with nucleases and Southern blot

Cells of W303D (aα) and WTH-1D (aα nps1-105/nps1105) in mid-log phase were rapidly shifted from 27 to 38°C by the addition of an equal volume of medium pre-warmed to 45°C. After 3 h incubation, cells were harvested and converted to sphearoplasts as described by Szent-Gyorgyi and Isenberg (24) with the following modifications. Sodium azide and phenylmethylsulfonyl fluoride (PMSF) were added to 20 and 0.5 mM, respectively, immediately prior to harvesting the cells. Zymolase 100T (Seikagaku Kogyo, 50 µg/ml) was used instead of lyticase. Sphearoplasting was carried out for 30 min at 34 or 38°C for the cells without or with the heat treatment, respectively. Sphearoplast suspensions were then diluted with 4 vol of buffer A (1.4 M sorbitol, 40 mM N-2-hydroxyethylpiperazine-N’-2-ethanesulfonic acid/KOH, pH 7.5, 0.5 mM MgCl2, 20 mM NaN3, 0.5 mM PMSF) and sphearoplast concentration was estimated by measuring the optical density of the suspension at 260 nm after the lysis in 1% sodium dodecyl sulfate. Sphearoplasts corresponding to OD520 value of 150–250 were harvested by centrifugation, washed once with buffer B [1 M sorbitol, 10 mM piperazine-N-N’-bis 2-ethanesulfonic acid (PIPES), pH 7.5, 0.2 mM CaCl2, 20 mM NaN3, 0.5 mM PMSF] and resuspended in 2 ml of MNase digestion buffer (10 mM PIPES, pH 7.5, 0.2 mM CaCl2, 1 mM MnCl2, 20 mM NaCl, 1 mM dithiothreitol, 1 mM PMSF, 20 mM NaN3) (25). The suspension was prewarmed at 37°C for 2 min, and MNase (50 U/ml, Boehringer Mannheim) was added. Samples were withdrawn at various time points and EDTA was added to 25 mM to stop reaction. For Drai digestion, various amounts of Drai were added to each sphearoplast suspension (300 µl) and the reaction was carried out at 37°C for 30 min. After the digestion, DNA was deproteinized and purified as described by Ling et al. (26). For the experiments with naked DNA, sphearoplasts were prepared exactly as described for chromatin digests, but immediately before nuclease cleavage, DNA was deproteinized and purified. Southern blot analysis was done by using fluorescein-labeled probes and Gene Images labeling and detection kit (Amersham). Probes used for detecting *CEN3* and *CEN4* are the 592 bp *NheI–BamHI* fragment from pOS31 that contains *CEN3* DNA sequence and the 1018 bp *SpeI–SauI* fragment from YCP50 that contains *CEN4* DNA sequence, respectively.

Analysis of DNA topoisomers

The topoisomer distribution of the yeast 2 µm circle plasmid DNA was assayed as described previously (27). Topoisomers were separated by electrophoresis in 1% agarose gels containing 2.5 µg/ml of Ethidium bromide and detected by Southern blot as described in the above section. The *EcoRI–SauI* fragment from YEpl2 that contains the 2 µm DNA sequence was used to probe endogenous 2 µm plasmids.
In addition to temperature-sensitive growth phenotype, the nps1-105 mutant showed increased sensitivity to microtubule-destabilizing agents such as thiabendazole (TBZ), methylbenz-carbamate (MBC) and benomyl (Fig. 1, data for MBC and benomyl are not shown). The nps1-105 mutant could not grow on a YPD plate containing >110 or 70 µg/ml of TBZ at permissive (30°C) or at semipermissive temperature (35°C), respectively. The wild type strain, on the other hand, could grow on a YPD plate containing up to 160 µg/ml of TBZ at both 30 and 35°C. We also tested the sensitivity of nps1-105 to hydroxyurea, methylmethane sulphonate, cycloheximide and trifluoperazine. No difference was observed between wild type and nps1-105 in the sensitivity to these drugs, indicating that the mutant is specifically sensitive to microtubule-destabilizing agents (data not shown). Both temperature and TBZ sensitivities of nps1-105 cosegregated in the haploid progenies from NPS1/nps1-105 diploid after sporulation, and complemented by the introduction of wild type NPS1 on a low-copy-number plasmid (data not shown).

**MADI disruption causes a failure in the cell cycle arrest and loss of viability in nps1-105 at the restrictive temperature**

The above observations indicated the possibility that NPS1 affects the organization and/or stability of mitotic spindles. Spindle malformation is detected by spindle-assembly checkpoint control including MAD and BUB gene products as components (reviewed in 29). If Nps1p is involved in these processes, the cell cycle arrest of nps1-105 at elevated temperature is interfered with by the loss of spindle-assembly checkpoint function. To test this...
possibility, we disrupted the MAD1 gene in the nps1-105 mutant. The nps1-105 mad1Δ double mutant could not arrest at G2/M phase after 5 h incubation at 38°C (Fig. 2). In addition, viability of the double mutant cells decreased in dependence with the incubation period at 38°C. In contrast, no notable decrease of viability was observed with wild type, nps1-105 or mad1Δ mutants in 8 h incubation at 38°C.

The nps1-105 mutation alters the chromatin structure around the centromere

Recent finding that RSC exhibits an activity to perturb nucleosome structure in vitro (3) and our results described above, suggested the possibility of the relevance of Rns1p on the chromatin structure at centromeres. Bloom and Carbon showed that yeast centromeres are packaged into a unique chromatin structure composed of a nuclease-resistant core flanked by ordered arrays of nucleosomes (30). We examined the chromatin structure of CEN3 and flanking sequences in nps1-105 after 3 h incubation at 38°C. Permeabilized spheroplasts were partially digested with micrococcal nuclease (MNase) and DNA was deproteinized and digested with BamHI. Southern blot analysis of the DNA sample from wild type cells with CEN3 probe showed that a 250 bp centromere-core region of DNA occurring between 350 and 600 bp from the wild type cells with DraI accessibility of heat-treated DNA samples prepared from the cells without (lanes 1 and 2) and with the heat treatment (lanes 3 and 4) were separated on agarose gels containing 2.5 µg/ml of chloroquine and subjected to Southern blot analysis.

Figure 3. Effects of the nps1-105 mutation on chromatin structures. (A) MNase digestion pattern of the wild type and the nps1-105 chromatin. Wild type (WT) and nps1-105 cells were grown to mid-log phase at 27°C, shifted to 38°C for 3 h and converted to spheroplasts. Spheroplasts (125 OD260 U/ml) were digested with 50 U/ml of MNase at 37°C for 50 s at 37°C. After deproteinization and purification, DNA samples were subjected to electrophoresis on 1.3% agarose gel and stained with ethidium-bromide. (B) MNase-sensitive sites on the centromeric chromatin from chromosome III. Spheroplasts (75 OD260 U/ml) prepared as described in (A) were digested with 50 U/ml of MNase for 80 s at 37°C (lanes a). MNase digestion of naked DNA (lanes n) was carried out with 1 U/ml of MNase for 50 s at 37°C. After deproteinization and purification, DNA samples were subsequently digested with BamHI and subjected to Southern blot analysis with fluorescein labeled NheI-BamHI fragment of CEN3. The sizes of molecular weight markers (M) are indicated in bp. At the left side of (B), schematic map of the chromosomal region surrounding CEN3 is cited. BamHI cleavage site is indicated with a closed circle. I, II and III show the relative position of the sequence elements CDEI, CDEII and CDEIII, respectively. (C) Dral accessibility of CEN3 and CEN4 chromatin increases in nps1-105 upon shift to 38°C. Spheroplasts (90 OD260 U/ml) were prepared from wild type and nps1-105 cells treated for 3 h at 38°C and digested with Dral for 30 min at 37°C. Genomic map of DNA regions surrounding CEN3 and CEN4 is schematically indicated at the top of the panel with the positions of probes used for Southern hybridization. Relative positions of CDEI and CDEIII are denoted by black and shadowed boxes, respectively, and CDEII lies between these elements. Dral and BamHI sites are indicated with bars and a closed circle, respectively. Arrowheads 1 and 2 at the right side of CEN4 panel indicate 2400 and 2100 bp fragments, respectively. The sizes of molecular weight markers (M) are indicated in bp. (D) Superhelicities of endogenous 2 µ plasmid DNA in wild type and nps1-105 cells. Wild type (lanes 1 and 3) and nps1-105 (lanes 2 and 4) cells were grown at 27°C to early-log phase and treated at 38°C for 3 h. DNA samples prepared from the cells without (lanes 1 and 2) and with the heat treatment (lanes 3 and 4) were separated on agarose gels containing 2.5 µg/ml of chloroquine and subjected to Southern blot analysis.
Next, we asked if the structural changes of chromatin occur in other centromere regions. The perturbation in the structural integrity of centromere DNA could be examined by the increase in nuclease accessibility of chromatin for restriction enzyme sites within the internal centromere region. Saccharomyces cerevisiae centromere DNA contains three elements, CDEI, CDEII and CDEIII (31–33). CDEI and CDEIII are 8 and 25 bp DNA elements with partial dyad symmetry, respectively. CDEI is a 76–86 bp region highly rich in A and T and contains at least one DraI recognition site (TTTAAA). Both CEN3 and CEN4 contain three closely spaced DraI sites within their CDEII.

Permeabilized spheroplasts prepared from cells treated at 38°C were digested with an increasing amount of DraI and DNA was purified after deproteinization. For the analysis of CEN3, purified DNA samples were digested with BamHI. If the DraI sites within the CEN3 CDEII are accessible to the enzyme, BamHI digestion after the deproteinization will give rise to the 480 bp band. As shown in Figure 3C, the 480 bp cutting band appeared in nps1-105 chromatin lanes but not in wild type ones. The result is consistent with that of the MNase digestion in Figure 3B.

In the case of CEN4, two additional DraI sites are located both to the left and right sides flanking the core centromere elements at the distance of 626 and 611 bp, respectively. So, in this case, the accessibility of the DraI sites within CDEII and either or both sites in the flanking region, or the accessibility of the sites only in the flanking region will give rise of a cutting fragment of ~600 or 1237 bp, respectively (Fig. 3C, CEN4). Neither 600 nor 1237 bp band was observed with wild type chromatin, indicating the DraI sites in CDEII and at least one site in the flanking region are protected from the digestion. In contrast, in the mutant chromatin lanes, the appearance of 600 bp band is apparent, showing the digestion of DraI sites within CDEII and in the either site in the flanking region. In Figure 3C CEN4 panel, 2400 and 2100 bp fragment (arrowheads 1 and 2, respectively) appeared in both wild type- and nps1-105 and nps1-105 chromatin lanes, respectively.

In our analysis, the DraI site flanking the right side of CEN4 core elements is susceptible to the digestion in both wild type and the mutant chromatin (data not shown). Database searches for DraI cleavage sites in the DNA region neighboring CEN4 revealed that two additional sites are located at 2423 and 2111 bp upstream of the right-most DraI site in Figure 3C. Therefore, the proximal DraI site is protected from the digestion in the wild type but not in the mutant. The results show that in chromosome IV, at least 1.5 kb DNA region in the left side of the centromere core elements may be assembled in phased nucleosome array and the structural integrity of both centromere core and flanking region are perturbed in nps1-105 after the treatment at 38°C. With wild type or the mutant cells grown at 27°C, no increase in the sensitivity to DraI was observed at both CEN3 and CEN4 DNA (data not shown).

Saunders et al. (34) reported that nucleosome depletion alters the chromatin structure of centromeres. A loss of nucleosomes increases more positively supercoiled species in closed circular DNA. To ask if the perturbed structural integrity of centromere chromatin in the nps1-105 mutant results from the loss of nucleosomes, we analyzed the distribution of topoisomers of naturally occurring yeast 2 μm plasmid. This plasmid exists as a covalently closed circular mini-chromosome assembled into nucleosomal chromatin (reviewed in 35). No change of the topoisomer distribution was introduced by the heat treatment either in wild type or in the mutant, suggesting that the alteration of the centromere structure of the mutant is not a result of the loss of nucleosomes (Fig. 3D).

The nps1-105 mutation affects transmission fidelity of mini-chromosome

Mutations in CEN DNA or histone H4 and depletion of histones H4 or H2B affect the chromatin structure at the centromere and cause chromosome missegregation (36–41). We asked if the nps1-105 mutation affects chromosome segregation by monitoring the rate of loss (1:0 segregation) and non-disjunction (2:0 segregation) events in nps1-105 ade2-101/ade2-101 diploid carrying SUP11 on a mini-chromosome. In this assay, loss and non-disjunction events occurred on the first division after plating result in the appearance of half-pink half-red and half-white half-red colonies, respectively (42,43).

The rates of 1:0 and 2:0 segregation of the wild type cells that were grown at 25°C were both 0.1% and these rates remained unaltered after the 3 h treatment at 38°C. On the other hand, 2:0 segregation rate was noticeably higher in the mutant cells that were grown at 25°C and the rate was increased by the heat treatment (Table 1, experiment 1). The rate of 1:0 segregation in the mutant was somewhat higher than the wild type but was not notably increased by the heat treatment. The results indicate that the nps1-105 mutation affects the segregation of the plasmid.

We next carried out this assay for the cells treated with TBZ at semipermissive temperature (34°C). Although the rates of 1:0 and 2:0 segregation in the mutant cells were unaltered in the absence of TBZ, the presence of the drug during the 3 h incubation increased the rate of 2:0 segregation (Table 1, experiment 2). The same treatment induced little increase of 2:0 segregation rate in the wild type cells, suggesting that missegregation of mini-chromosome in the mutant is caused by the failure during the reassembly of microtubules.

DISCUSSION

We isolated a temperature-sensitive allele of NPS1, nps1-105, and found that the mutation causes the alteration of chromatin structure surrounding the centromeric DNA region at elevated temperature. As the centromere chromatin structure is known to be maintained throughout the yeast cell cycle as well (39), this alteration in nps1-105 seems not to be the result of G2/M arrest of the mutant cells. Therefore, it was suggested that in the NPS1 mutant, impaired chromatin structure at centromeres may lead to a loss of kinetochore function and the cells arrest at G2/M phase through the spindle-assembly checkpoint system. Several lines of evidence support this idea. First, the nps1-105 mutant is specifically sensitive to microtubule-destabilizing agents (Fig. 1).

It is known that the mutation in the genes encoding kinetochore components causes elevated sensitivity to benomyl (44–47). Second, the nps1-105 cell-cycle-arrest phenotype is MAD1 dependent (Fig. 2). Finally, the nps1-105 mutation causes an increase in the frequency of non-disjunction of mini-chromosome (Table 1). Non-disjunction events are resulted from the segregation of both replicated sister chromatids to the same pole at anaphase. A subset of non-disjunction events are caused by the failure of the centromeres of paired sister chromatids to interact properly with the microtubules.
Table 1. Analysis of NPS1 function in mitotic chromosome transmission by half-sectored colonies\textsuperscript{a}

<table>
<thead>
<tr>
<th>Strain</th>
<th>Heat treatment (h)</th>
<th>TBZ (µg/ml)</th>
<th>Colonies (%)</th>
<th>Total no. of colonies scored</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>P+R</td>
<td>R</td>
<td>W/P (1:0)</td>
</tr>
<tr>
<td>WT</td>
<td>38°C 60</td>
<td>0 –</td>
<td>92 7.5</td>
<td>0.1 0.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>nps1-105</td>
<td>0 –</td>
<td>84 14.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3 –</td>
<td>82 14.3</td>
<td>1.9 0.4</td>
</tr>
<tr>
<td>Experiment 1</td>
<td></td>
<td>34°C 12.6</td>
<td>0.1 0.1</td>
<td>0.1 0.5</td>
</tr>
<tr>
<td>WT</td>
<td></td>
<td>3 –</td>
<td>86 13.9</td>
<td>0.1 0.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3 +</td>
<td>87 13.8</td>
<td>0.1 0.1</td>
</tr>
<tr>
<td>nps1-105</td>
<td></td>
<td>0 –</td>
<td>86 12.6</td>
<td>0.5 0.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3 –</td>
<td>85 13.2</td>
<td>0.5 0.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3 +</td>
<td>82 13.9</td>
<td>1.9 0.3</td>
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</table>

\textsuperscript{a}nps1-105 (YTW-6D, ade2-101/ade2-101 nps1-105) and its isogenic wild type (WT) strain (YTW-30D), harboring pHM410 (CEN4 SUP11 URA3) were subjected to heat treatment. Cells were removed from the cultures at the indicated times and plated on color media plates. The cells were allowed to form colonies by the incubation at 25°C for 5 days. The percentage of each colony type was calculated and is presented in the table. P, W and R indicate pink, white and red colonies, and W/P, P/R and W/R indicate half-white half-pink, half-pink half-red and half-white half-red sectored colonies, respectively. Standard deviation was within 10%.

To explain the phenotypes of the nps1-105 mutant, there can be at least three possibilities. The first is that Nps1p plays a direct role on the organization and/or maintenance of the centromere chromatin structure independently from RSC complex. At present, four genes encoding RSC components are identified including NPS1 (3, 21, 48). Analysis of the conditional mutant alleles of these genes is required for understanding the role of RSC complex in chromatin structure. The second model is that Nps1p functions through RSC complex as a transcriptional regulator resembling SNF/SWI complex for genes essential for the assembly of certain chromatin structure of centromeres. Previous studies indicate that depletion of histone H2B or H4 alters chromatin structure of centromeres and causes G2 arrest (49–51). Nonetheless, phenotypes reported with the loss of either histones are different from that of nps1–105 in several points. First, depletion of either histones caused elevated sensitivity to MNase in overall chromatin, while the nps1-105 mutation did not (Fig. 3A). Second, the reduction of superhelical density in covalently closed circular plasmid was introduced by the loss of either histones but not by the defect of nps1-105 (Fig. 3D). Finally, the cell cycle arrest by the histone depletion was irreversible: the cells carrying inducible allele of either histone H2B or H4 lost viability upon shift to the repressed conditions within 2 h. On the other hand, the nps1-105 mutants remained viable at least 8 h in the restrictive condition when the MAD1 gene is functional (Fig. 2). Accordingly, Nps1p seems not to affect at least the expression of histone genes. A third possibility is that Nps1p acts as a component of RSC complex on the organization or modulation of the centromeric chromatin structure. The yeast centromeric DNA is packed into a unique chromatin structure (30). In addition, the nucleosome-protected region within the centromere is highly resistant to ionic conditions that are known to disrupt most loose DNA–protein complexes (52) and is thought to contain specialized nucleosomes. These nucleosomes are suggested to contain Cse4p, a yeast homolog of the human kinetochore protein, CENP-A, in place of histone H3 (53). CENP-A was first identified using sera from patients suffering CREST (calcinosis, Raynaud’s phenomenon, esophageal dysmotility, sclerodactyly and telangiectasia) and shown to be a centromere-specific protein which has a domain resembling histone H3 (54). CSE4 was isolated in a screen for genes that cause an increase in the rate of loss of an artificial chromosome bearing a partial loss of function mutation in CDEII and found to be essential for mitotic growth (55). The phenotypes of temperature-sensitive CSE4 allele, cse4-1, are resemble to those of nps1-105: the cse4-1 mutant arrest with a predominance of large budded cells containing single G2 nuclei and short bipolar mitotic spindles at elevated temperature and shows the increased non-disjunction frequency of a chromosome bearing a mutant centromere DNA sequence (53). Although the yeast centromeric DNA is packed into specialized nucleosomes, the question of how these specialized nucleosomes are properly localized in centromeres is not answered yet. Fundamentally, the binding of histones to DNA is not dependent on specific DNA sequence. In nuclei of growing cells, the amount of conventional nucleosomes may be much more abundant than that of specialized nucleosomes. In case of human cells, targeting of CENP-A to centromeres requires that CENP-A expression is uncoupled from histone H3 synthesis during S phase: CENP-A mRNA accumulates later in the cell cycle than histone H3, peaking in G2 (56). On the other hand, the mRNA of CSE4 is reported to present at relatively constant levels throughout the cell cycle (53). In addition, whether Cse4p possesses the DNA-binding activity with higher affinity to centromere DNA is obscure. The simplest scenario to explain the role of RSC is that the complex may be required for recruiting specialized nucleosomes, and organizing and/or maintaining the unique chromatin structure in the distinct region of the genome. In this regard, biochemical and genetic properties of SFH1, a gene encoding another component of RSC, are of interest (21). A temperature-sensitive SFH1 mutation, sfh1-1, causes G2/M arrest at elevated temperature similar to that of nps1-105. The Sfh1 protein is phosphorylated in the G1 phase and dephosphorylated upon progression to the S phase. Because Sfh1p is required for the...
progression through G2/M, the phosphorylation of Sfh1p is suggested to negatively regulate Sfh1p function: thus, the protein is likely to be activated by dephosphorylation at the S phase. The organization of newly synthesized DNA properly into highly ordered chromatin structure occurs in tight link with their synthesis during the S phase.

However, our results do not exclude the possibility of requirement of RSC for transcriptional regulation. Cairns et al. reported that RSC is at least 10-fold more abundant than SNF/SWI (3). Immunofluorescent localization of Nps1p with anti-Nps1p antibody or by expressing Nps1-GFP fusion protein revealed that this protein is not concentrated in the specific region of the nucleus but localized over the entire region stably with DAPI (1; E. Tsuchiya, unpublished result). The observation indicates that Nps1p or RSC functions not only in the organization of another chromatin region or in the transcriptional regulation. The analysis on these questions is in progress in our laboratory. It is of interest that two of the spf (suppressor of Ty) mutations, spf4-138 and spf6-140, show ctf (chromosome transmission fidelity) phenotype and are thought to cause altered chromatin structure at centromeres (57). The spf mutants were identified as extragenic suppressors of transcriptional defects caused by Ty or Ty delta element insertions in the 5′ regions of the HIS4 and LYS2 genes (58, 59). Spf4p, Spf5p and Spf6p function in a complex (60) which can repress transcription (61). There might be several protein complexes required for the organization and/or alteration of the higher order structure of chromatin concerning the progression of cell cycle and the transcriptional regulation.

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