Identification of a Novel Non-structural Maintenance of Chromosomes (SMC) Component of the SMC5-SMC6 Complex Involved in DNA Repair*

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Structural maintenance of chromosomes (SMC) proteins play central roles in chromosome organization and dynamics. They have been classified into six subtypes, termed SMC1 to SMC6, and function as heterodimer components of large protein complexes that also include several non-SMC proteins. The SMC2-SMC4 and SMC1-SMC3 complexes are also known as condensin and cohesin, respectively, but the recently identified SMC5 and SMC6 complex is less well characterized. Here, we report that NSE1 from Saccharomyces cerevisiae encodes a novel non-SMC component of the SMC5(Yo1034wp)-SMC6(Rhc18p) complex corresponding to the 2–3-MDa molecular mass. Nse1p is essential for cell proliferation and localizes primarily in the nucleus. nse1 mutants are highly sensitive to DNA-damaging treatments and exhibit abnormal cellular morphologies, suggesting aberrant mitosis as a terminal morphological phenotype. These results are consistent with the reported features of the Schizosaccharomyces pombe SMC6 gene, rad18, which is thought to be involved in recombinational DNA repair. We conclude that Nse1p and the SMC5-SMC6 heterodimer together form a high molecular mass complex that is conserved in eukaryotes and required for both DNA repair and proliferation.

The superfamily structural maintenance of chromosomes (SMC)1 consists of proteins with important functions in chromosome maintenance. SMC proteins share structural features and have been evolutionarily conserved from most prokaryotes to higher eukaryotes (1–8). They have globular N- and C-terminal domains and two extended α-helical coiled-coil domains that are involved in protein-protein interactions, separated by a hinge region. The N- and C-terminal domains, which contain a Walker A motif and a conserved sequence termed the DA-box (putative Walker B motif), respectively, are responsible for the ATPase activity of SMC proteins. In eukaryotes, six classes of SMC proteins, generally termed SMC1 to SMC6, have been identified (1, 9). They form specific heterodimer combinations: SMC1-SMC3, SMC2-SMC4, and SMC5-SMC6. The SMC heterodimers associate with some additional non-SMC proteins to form high molecular mass complexes.

The best documented high molecular mass complexes containing SMC proteins are cohesin and condensin, which contain SMC1-SMC3 and SMC2-SMC4 heterodimers, respectively. Cohesin is involved in mitotic sister chromatid cohesion, whereas condensin mediates mitotic chromosome condensation. Several important features of these SMC complexes have been revealed by the identification and characterization of their non-SMC components. For example, the cohesin complex from the budding yeast Saccharomyces cerevisiae contains two non-SMC proteins, Scclp (10, 11) and Scc3p (12). At the metaphase-anaphase transition, cohesin is inactivated through the cleavage of Scc1 by the separin protease Esp1p, which is activated by the anaphase-promoting complex (13, 14). The Xenopus condensin is activated by phosphorylation of two non-SMC components, XCAP-D2 and XCAP-H, resulting in chromatin condensation in cell-free oocyte lysates (15).

Recently SMC5 and SMC6 have been identified and characterized in Schizosaccharomyces pombe, Arabidopsis thaliana, and mammalian cells (9, 16–18). In vivo association between SMC5 and SMC6 has been reported in S. pombe and mammalian cells. In S. pombe, Fousteri et al. (9) reported the existence of a high molecular mass complex containing Spr18p, Rad18p, and five other unidentified proteins. From the molecular size of the unidentified polypeptides (~100, 45, 43, 37, and 35 kDa), all of them appear to be non-SMC components. Studies in S. pombe indicate that the SMC5-SMC6 heterodimer is involved in DNA repair (16, 19). rad18 mutants show increased sensitivity to UV and γ-ray irradiation, and both UV-photoproduction and double-strand break repair are delayed in these cells. In addition, Rad18p is reported to be required for the maintenance of cell cycle arrest in the presence of DNA damage (19). Furthermore, both of the genes encoding SMC5 and SMC6 are essential even in the absence of extrinsic DNA damage (9, 16). Mutations in rad18 are synthetically lethal when combined with a DNA topoisomerase II mutation (top2–191) (19), suggesting that Rad18p plays a role in chromatin organization. Moreover, cells with a deletion of rad18 or overexpressing dominant-negative rad18 exhibit a wide variety of abnormal morphologies characterized by aberrant nuclear segregation. A similar phenotype is observed in cells with a deletion of the spr18 gene (9, 16).

Despite the observations described above, the function and regulation of the SMC5-SMC6 heterodimer are poorly understood compared with the SMC1-SMC3 and SMC2-SMC4 heterodimer. This is partly because the non-SMC proteins associating with the SMC5-SMC6 heterodimer have not been
A Non-SMC Component of the SMC5-SMC6 Complex

Yeast strains used in this study

| Strain       | Genotype                             |
|--------------|--------------------------------------|
| DFY24        | MATa/a ura3–52/ura3–52 his3/23200 leu2Δ1/leu2Δ1 lys2Δ1/lys2Δ2 202 TRP1/trp1Δ63 nse1Δ kanMX4/NSE1 |
| FWEFOO2(HE)* | MATa/a ura3–52/ura3–52 his3/23200 his3/23200 LEU2/LEU2 TRP1/trp1Δ63 nse1Δ kanMX4/NSE1 |
| FY78         | MATa his3/23200                      |
| YKW3         | MATa ura3–52 his3/23200 nse1Δ : LEU2/lys2Δ2 202 [pRS316/NSE1] |
| SKO2         | MATa ura3–52 his3/23200 leu2Δ1 trp1Δ63 nse1Δ : kanMX4 [pAML10/HSA-NSE1] |
| SKO3         | MATa ura3–52 his3/23200 leu2Δ1 trp1Δ63 nse1Δ : kanMX4 [pAML10/NSE1] |
| H202         | MATa ura3–52 smc5Δ/1 : HIS3 leu2Δ1 lys2Δ2 202 trp1Δ63 nse1Δ : kanMX4 [pAML10/HSA-NSE1] [pAML10/FLAG-SMC6] |
| YK02         | MATa ura3–52 smc5Δ/1 : HIS3 leu2Δ1 lys2Δ2 202 trp1Δ63 nse1Δ : kanMX4 [pAML10/HSA-NSE1] [pAML10/FLAG-SMC6] |
| YK05         | MATa ura3–52 his3/23200 nse1Δ : LEU2/lys2Δ2 [pRS313/NSE1] |
| YKW6         | MATa ura3–52 his3/23200 nse1Δ : LEU2/lys2Δ2 [pRS313/14] |
| YKW7         | MATa ura3–52 his3/23200 nse1Δ : LEU2/lys2Δ2 [pRS313/161] |
| SKG1         | MATa ura3–52 his3/23200 leu2Δ1 trp1Δ63 nse1Δ : kanMX4 [pGAL1/NSE1] |
| SKG2         | MATa ura3–52 his3/23200 leu2Δ1 trp1Δ63 nse1Δ : kanMX4 [pGAL1/nse1–20] |

* EUROSCARF (www.uni-frankfurt.de/fb15/mikro/euroscarf/index.html).

identified. In the present paper, we examine the function of YLR007w, a previously uncharacterized S. cerevisiae gene that was obtained from one of a collection of yeast mutants exhibiting abnormal nuclear morphology (20, 29). Our results strongly suggest that the product of YLR007w associates with Yol034wp and Rch18p, which are predicted to be the S. cerevisiae orthologues SMC5 and SMC6, respectively, to form a high molecular mass complex. Furthermore, we found that yr007tw mutant strains exhibit phenotypes resembling those of S. pombe rad18 mutants, including enhanced sensitivity to DNA-damaging treatments. We conclude that YLR007w encodes a non-SMC component of the SMC5-SMC6 high molecular mass complex, and propose that this gene be called NSE1 (non-SMC element 1).

EXPERIMENTAL PROCEDURES

Yeast Strains and Media—Yeast strains used in this paper are listed in Table I. All strains were derived from DFY24 or FWEFOO2(HE)*, both of which are diploid strains derived from S288C.

Unless otherwise described, yeast strains were cultured in rich (YPD) or synthetic (SD) glucose media (for composition, see Ref. 21). For GAL1 promoter induction, we used YPG or SG, which contain 2% galactose instead of glucose. SD and SG were supplemented with the appropriate amino acids. For solid media, 2% agar was added. Genetic manipulation of yeast cells was performed as described previously (21).

Plasmids—Yeast genomic DNA containing the NSE1 gene (nucleotides 554 to 1519) was amplified by PCR, and the resulting product inserted into the yeast centromeric vectors pRS316 and pRS313 to obtain, respectively, pRS316/NSE1 and pRS313/NSE1. A fusion of S65T and S147P green fluorescent protein (GFP) cDNA (22) and the 5′ and 3′ untranslated regions of the GAL1 gene (open reading frame minus start codon) was inserted into the GAL1 promoter-driven expression vector pGMIH10 (23) to obtain pGFP-NSE1. Double-stranded oligonucleotides encoding three tandem repeats of the influenza virus hemagglutinin (HA) epitope were fused with the NSE1 gene (open reading frame minus start codon) and inserted into the ADH1 promoter-driven expression vector pA20/20 (23) to obtain pAML10/HSA-NSE1. An oligonucleotide encoding three tandem repeats of the FLAG epitope was inserted into the RBC18 gene (nucleotides 534 to 3564) and the YOL034w gene (nucleotides 893 to 3540) at positions 1 and 3279, respectively. The modified RBC18 and YOL034w genes were inserted into the centromeric vector pRS314 to generate pRS314/FLAG-RBC18 and pRS314/FLAG-YOL034w, respectively. The NSE1 gene was inserted into pGHT11 (23) to generate pGAL1/NSE1. By introduction of the nse1–20 mutation (see Fig. 1A) into pGAL1/NSE1, pGAL1/nse1–20 was obtained.

Isolation of nse1–14 and nse1–16 Mutants—A library of randomly mutagenized NSE1 genes carried on the HIS3 centromeric vector pRS313 was generated by the same procedure used to construct pRS313/NSE1, except that PCR amplification of the NSE1 gene was performed in the presence of 0.5 mM MnCl2 (error-prone PCR) (24). YKW3 cells, already containing the URAG8 vector pRS316/NSE1, were transformed with this plasmid library, and 2000 independent transformants were obtained. The wild-type pRS316/NSE1 plasmid was segregated by 5-fluoroorotic acid (5-FOA) selection, so that remaining NSE1 function was provided by pRS313/NSE1. pRS313/NSE1 plasmids that conferred slightly slower growth than the wild-type pRS316/NSE1 were taken as NSE1 mutants.

Cell Lysate Preparation, Immunoprecipitation, and Analysis of the Immunoprecipitates—Large scale immunoprecipitation was performed for mass spectrometric analysis of the immunoprecipitates. Yeast cells (2500 optical density equivalents) were suspended in 100 ml of L1 buffer (50 mM HEPES, pH 7.6, 1 mM EDTA, 1 mM diethiothreitol, 10% (v/v) glycerol, 50 mM NaCl, 0.02% Nonidet P-40, 1 mM phenethylsulfonyl fluoride, 10 μg/ml each leupeptin, pepstatin, and aprotinin) and lysed using glass beads. After clarification by ultracentrifugation at 100,000 × g for 60 min in an SW40T rotor (Beckman), the lysates were precipitated with 70% (w/v) ammonium sulfate. The pellets were dissolved with 12 buffer (50 mM HEPES, pH 7.6, 1 mM EDTA, 1 mM diethiothreitol, 10% glycerol, 150 mM NaCl, 0.02% Nonidet P-40), and incubated with 12CA5 anti-HA monoclonal antibody (Roche Molecular Biochemicals) and protein A-Sepharose 4F beads (Amersham Biosciences) at 4°C for 2 h. Immunoprecipitates were resolved by SD-SAGE and identified by in-gel digestion using lysyl endopeptidase (Wako, EC 3.4.21.50) followed by peptide mass fingerprinting on a MALDI-TOF mass spectrometer (JEOl) (refer to Refs. 25 and 26). The measured masses of peptides were used to search for protein candidates in the nonredundant protein sequence data base with program ProFound (prowl.rockefeller.edu).

Small scale immunoprecipitation was performed for Western blot detection. Preparation of cell lysates, immunoprecipitation, and Western analysis were performed described previously (27, 28), except that, in addition to 12CA5 antibody, M2 anti-FLAG monoclonal antibody (Sigma) and mouse IgG (Cappel) were used.

Fast Protein Liquid Gel Filtration Chromatography—With the same procedure as the large scale immunoprecipitation described above, cells were lysed by glass beads, and the cell lysates were clarified by ultracentrifugation. The clarified cell lysates were loaded on a Superose 6 FPLC column (Amersham Biosciences) equilibrated with fast protein liquid chromatography buffer (50 mM HEPES, pH 7.6, 1 mM EDTA, 1 mM diethiothreitol, 50 mM NaCl, 0.02% Nonidet P-40, and 0.05% Tween 20). The column was calibrated with a gel filtration calibration kit (Amersham Biosciences).

RESULTS

NSE1 Encodes an Essential Nuclear Protein Conserved in Eukaryotes—The predicted NSE1 (YOL034w) encodes a 336-amino acid protein with 38,320 Da, which well coincides with the predicted molecular mass of the NSE1 protein sequence (22% identity). Cysteine- and histidine-rich regions, which are conserved among the mammalian orthologues are related to Nse1p over their entire sequence (22% identity). Cysteine- and histidine-rich regions, including a conserved CXXCH motif, are located near the C terminus, suggesting that it might function as a zinc finger. These putative mammalian orthologues have so far not been characterized.

As shown in Fig. 1B, ura3 nse1Δ cells carrying the URA3 NSE1 plasmid pRS316/NSE1 were sensitive to 5-FOA even in the presence of uracil. This shows that the nse1Δ cells do not
grow when pRS316/NSE1 is lost, indicating that NSE1 is an essential gene for vegetative cell growth. Tetrad analysis of the heterozygous yeast strain disrupted for one copy of the NSE1 gene also showed that the NSE1 gene is essential for cell viability (data not shown). To visualize the intracellular localization of Nse1p, we expressed a GFP-tagged Nse1p (GFP-Nse1p). GFP-NSE1 was able to complement the growth defect of the nse1 deletion mutant, indicating that GFP-Nse1p was functional. As shown in Fig. 1C, this fusion protein was primarily localized in compartments strongly stained by 4’,6-diamino-2-phenylindole (DAPI), indicating that Nse1p is a nuclear protein.

Nse1p Forms a Large Complex with Rhe18p and Yol034wp—To identify proteins associating with Nse1p in budding yeast cells, we attempted to immunopurify complexes containing Nse1p. For this purpose, we generated the yeast strain SKO2 having a nse1/ background and expressing N-terminal HA-tagged Nse1p under the control of the ADH1 promoter. SKO2 cells grew normally, indicating that the HA-tagged version of Nse1p is functional in budding yeast cells. As described under “Experimental Procedures,” lysates of SKO2 cells and control cells producing untagged Nse1p were prepared and subjected to 70% (w/v) ammonium sulfate fractionation. The pellet fractions were resuspended and subjected to anti-HA-mediated immunoprecipitation. SDS-PAGE analysis followed by silver staining of the gel showed that only immunoprecipitates from the SKO2 cell lysate contained HA-tagged Nse1p (41 kDa) along with two other proteins (both are ~130 kDa).
The bands corresponding to the unidentified proteins were cut out from the gel and identified through lysyl endopeptidase cleavage and mass fingerprinting using matrix-assisted laser desorption ionization-time of flight (MALDI-TOF). As shown in Fig. 2 (B and C), these proteins were identified as the products of the YOL034w and RHCI8 genes, which are predicted to encode SMC5 and SMC6 of *S. cerevisiae*, respectively.

To confirm the intracellular association between Nse1p and Rhc18p, we produced the yeast strain RKO2, having an nse1Δ/h9004 strain producing plasmid-borne HA-tagged Nse1p and FLAG-tagged Rhc18p, and subjected to immunoprecipitation with anti-HA antibody. As shown in Fig. 2 (D and E), FLAG-tagged Rhc18p, as well as HA-tagged Nse1p, was detected in the anti-HA immunoprecipitate. Similarly, in the anti-FLAG immunoprecipitate, both HA-tagged Nse1p and FLAG-tagged Rhc18p were detected. In contrast, immunoprecipitation using nonspecific mouse IgG did not pull down either HA-tagged Nse1p or FLAG-tagged Rhc18p. A similar result indicating an intracellular association between Nse1p and Yol034w was observed in the RKO2 strain expressing both plasmid-borne HA-tagged Nse1p and FLAG-tagged Yol034w, as shown in Fig. 2 (F and G).
obtained using the strain YKO2, an nse1Δ yol034wΔ strain carrying HA-tagged Nse1p and FLAG-tagged Yol034wp (Fig. 2E).

When the RKO2 cell lysate was subjected to gel filtration chromatography, both HA-tagged Nse1p and FLAG-tagged Rhc18p were detected in fractions corresponding to a molecular mass of 2–3 MDa (Fig. 2F). Similarly, gel filtration of the YKO2 cell lysate showed that both HA-tagged Nse1p and FLAG-tagged Yol034wp were present in fractions corresponding to a molecular mass of 2–3 MDa (Fig. 2G). These observations strongly suggest that Nse1p, together with Rhc18p and Yol034wp, forms a large complex with a molecular mass of 2–3 MDa.

Mutations in the NSE1 Gene Cause High Sensitivity to DNA-damaging Treatments—To investigate the physiological role of Nse1p, we attempted to isolate and characterize budding yeast cells carrying mutations in the NSE1 gene. As NSE1 is an essential gene, we attempted to isolate conditional lethal nse1 mutants. For this purpose, the NSE1 gene was amplified by error-prone PCR, inserted into the centromeric HIS3 vector pRS313, and introduced into YKW3, a ura3 nse1Δ strain bearing the mid-borne NSE1, nse1–14, and nse1–16 genes, respectively. B, quantitative UVC sensitivity assay. YKW3, YKW5, and YKW6 cells were plated onto YPD media plates and irradiated with a UVC irradiator at the indicated doses. After incubation of the plates at 30 °C for 2–3 days, colonies were counted and a survival percentage was calculated using the nonirradiated plates as the standard of 100% growth.

As the fission yeast Rad18p, which is the orthologue of budding yeast Rrc18p, is reported to be involved in DNA repair, we expected that nse1 mutants would show defects in DNA repair. We tested whether nse1–14 and nse1–16 cells are hypersensitive to treatments which induce DNA damage. As shown in Fig. 3A, growth of nse1–14 cells on solid media was significantly retarded by irradiation with UVC light or γ-ray or exposure to the alkylating agent methyl methanesulfonate (MMS). To a lesser extent, growth retardation by these treatments was also observed in nse1–16 cells treated with UVC light and MMS. As shown in Fig. 3B, the higher sensitivities of nse1–14 and nse1–16 cells to UVC light were confirmed by the quantitative UVC sensitivity assay. In contrast to the survival rate of wild-type cells (14%), nse1–16 and nse1–14 showed survival rates of 1.7 and 1%, respectively, at 100 J/m².

**Morphological Aberration Is a Terminal Phenotype Observed in a Conditional Lethal nse1 Mutant**—As described above, we failed to select conditional lethal nse1 mutants from a stock of strains carrying randomly mutagenized NSE1 genes. However, we were able to obtain a conditional mutant as follows. We tested whether nse1–14 and nse1–16 cells were treated with UVC light and MMS. As shown in Fig. 3B, the higher sensitivities of nse1–14 and nse1–16 cells to UVC light were confirmed by the quantitative UVC sensitivity assay. In contrast to the survival rate of wild-type cells (14%), nse1–16 and nse1–14 showed survival rates of 1.7 and 1%, respectively, at 100 J/m².

**Fig. 3. Sensitivity of nse1 mutants to DNA-damaging treatments.** A, cells were grown to logarithmic phase at 30 °C, diluted serially 5-fold, and spotted onto YPD media plates. For UVC light (254 nm) and γ-ray (137Cs) irradiation, plates were treated with irradiators just after spotting of cells. For treatment with MMS, YPD media plates containing MMS were used. These plates were incubated at 30 °C for 2–3 days and photographed. The strains used were YKW3, YKW5, and YKW6, nse1Δ strains carrying the plasmid-borne NSE1, nse1–14, and nse1–16 genes, respectively. B, quantitative UVC sensitivity assay. YKW3, YKW5, and YKW6 cells were plated onto YPD media plates and irradiated with a UVC irradiator at the indicated doses. After incubation of the plates at 30 °C for 2–3 days, colonies were counted and a survival percentage was calculated using the nonirradiated plates as the standard of 100% growth.

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and S. pombe (data not shown). Growth ceased within 24 h of being shifted to glucose media.

DAPI (Fig. 4 A, c–d) microscopically grown at 30 °C in YPG medium, or shifted to YPD medium for 24 h. A, phase-contrast (left) or DAPI-strained (right) microscopic images of SKG2 cells cultured in YPD (a–c) or YPG (d) medium. B, the proportion (in percentages) of cell shapes.

FIG. 4. Morphological aberration of a conditional lethal nse1 mutant. SKG1 and SKG2 cells (see Table I for genotype) were logarithmically grown at 30 °C in YPG medium, or shifted to YPD medium for 24 h. A, phase-contrast (left) or DAPI-strained (right) microscopic images of SKG2 cells cultured in YPD (a–c) or YPG (d) medium. B, the proportion (in percentages) of cell shapes.

tose). One change introduced were amino acid replacements of two conserved Cys residues in NSE1 to Ser (nse1–20 mutation; see Fig. 1A). This construct was used to generate SKG2, a nse1Δ strain expressing nse1–20 under control of the GAL1 promoter. SKG2 cells grew normally in galactose media, but the cells were multibudded, and the ratio of small budded and unbudded cells was reduced. Moreover, the ratio of cells having elongated buds was significantly increased. The nuclei of the SKG2 cells cultured in glucose media also showed various abnormal morphologies. Some nuclei were apparently fragmented or aggregated, and others were weakly stained with DAPI (Fig. 4A, a–c).

As shown in Fig. 4 (A and B), SKG2 cells that stopped growing in glucose media showed aberrant morphology (see also Table I). When incubated in YPD medium for 24 h, 18% of the cells were multibudded, and the ratio of small budded and unbudded cells was reduced. Moreover, the ratio of cells having elongated buds was significantly increased. The nuclei of the SKG2 cells cultured in glucose media also showed various abnormal morphologies. Some nuclei were apparently fragmented or aggregated, and others were weakly stained with DAPI (Fig. 4A, a–c).

DISCUSSION

In this paper, we have addressed the physiological role of the previously uncharacterised gene NSE1. As shown in Fig. 2 (A–E), the product of the NSE1 gene was coimmunoprecipitated with Rhc18p and Yol034wp from cell lysates. Nse1p was detected in the same fractions as Rhc18p and Yol034wp, corresponding to a size of 2–3 MDa (Fig. 2, F and G), by size exclusion chromatography. These observations indicate that Nse1p is a component of a large molecular complex containing Yol034wp and Rhc18p, which are predicted to be the S. cerevisiae counterparts of the S. pombe Spr18p (SMC5) and Rad18p (SMC6) proteins, respectively (Table II). This idea is supported by the similarity of the phenotypes between the nse1 mutants and S. pombe cells having a rad18 deletion or mutations as described below. In S. pombe, Fousteri et al. (9) have previously reported the existence of a high molecular mass complex containing at least five unidentified non-SMC proteins in addition to Rad18p and Spr18p. The complex that we observed in this study is probably the S. cerevisiae version of this complex. This complex is conserved in eukaryotes, because, in addition to the previously reported human SMC5-SMC6 heterodimer, our database search found several mammalian Nse1p orthologues (Fig. 1A). To avoid confusion in the nomenclature and to make their physiological functions more clear, we propose here that the genes encoding YOL034w and RHC18 be designated SMC5 and SMC6, respectively, as described in Table II. Unfortunately we could not find the S. pombe Nse1p orthologue with high homology to Nse1p. However, when we used mammalian NSE1 protein sequence as a probe of data base search, spec550.05 gene of S. pombe was captured as the candidate of Nse1p orthologue, if this sequence would contain the undetermined intron. To identify these candidates as real functional orthologues, further study is required.

We were not able to deduce anything obvious concerning the functions of Nse1p based on its amino acid sequence alone, although a conserved CXXCH motif in the C-terminal Cys- and His-rich region was identified (see Fig. 1A). The importance of this CXXCH motif is supported by the observation that substitution of these Cys residues with serine in the nse1–20 mutation leads to a growth defect. We could find no obvious nuclear localization signal in Nse1p, although Nse1p was primarily localized to the nucleus when visualized as a GFP fusion (Fig. 1C). The localization of Nse1p in the nucleus may be promoted by an association with other nuclear proteins having nuclear localization signal or DNA binding activity. In S. pombe, Rad18p has a bipartite nuclear localization signal, and is localized to the nucleus (16, 19). Furthermore, human SMC5 and SMC6 have been reported to be nuclear proteins (18). Considering the physiological role of SMC complexes in modulating chromosome structure and organization, it is reasonable that their components would be localized to the nucleus.

It is apparent that Nse1p functions in the DNA repair system together with Smc5p and Smc6p. Both nse1 mutants tested so far, nse1–14 and nse1–16, have exhibited increased sensitivity to DNA-damaging treatments (Fig. 3). The involvement of SMC6 in DNA repair has been demonstrated mainly by studies on S. pombe rad18 mutants. These rad18 mutants are hypersensitive to UV and γ-ray irradiation, and exhibit delayed repair of UV-induced double-strand DNA breaks. rad18 belongs to the same epistasis group as rhp51, which is the S. pombe orthologue of the S. cerevisiae RAD51, thought to be involved in homologous recombination (16). Moreover a defect in intrachromosomal recombination, as well as in DNA repair, is observed in a Rad18 mutant of A. thaliana (17). Hence, it is likely that the Smc5p-Smc6p high molecular mass complex that contains Nse1p is involved in recombinational repair. This complex may bring together and/or hold two broken chromosomal ends produced by double-strand breaks. On the other hand, Nse1p was also shown to be essential for proliferation in the absence of genotoxic treatments. As shown in Fig. 1B, deletion of the NSE1 gene is lethal. Consistent with this result, deletion of either SMC5 or SMC6 is lethal in S. pombe and S. cerevisiae. In this report, we examined the terminal phenotype of SKG2, a conditional-lethal nse1 mutant. This mutant exhibited various abnormal cellular morphologies that suggest aberrant mitosis (Fig. 4, A and B). Similar observations have been reported in rad18 and spr18 mutations, or in strains over-

### Table II

| SMC5     | spr18 | SMC5/YOL034w | SMC6     | rad18 | SMC6/RHC18 |
|----------|-------|--------------|----------|-------|------------|
| S. pombe |       |              | S. cerevisiae |      |            |

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pressing dominant-negative rad18 mutants in \textit{S. pombe}. Furthermore, mutations in \textit{S. pombe rad18} are synthetically lethal when combined with a topoisomerase II mutant (top2–191) (19). Hence, it is likely that the Smc5p-Smc6p complex containing Nse1p plays a role in the structural maintenance of chromosomes, a function important for mitotic events.

Our results, taken together with previously reported observations, indicate that the high molecular mass complex containing Smc5p-Smc6p and Nse1p is involved in the structural maintenance of chromosomes during both DNA repair and cell proliferation. However, little is known concerning the details of the molecular mechanism of how this complex operates during these cellular events. As several important features of condensin and cohesin were revealed by studying their non-SMC components, we expect the function of the SMC5-SMC6 complex will also be uncovered through further investigation of Nse1p, the first identified non-SMC component of this complex.

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A Non-SMC Component of the SMC5-SMC6 Complex

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