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SMC1 coordinates DNA double-strand break repair pathways

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ABSTRACT

The SMC1/SMC3 heterodimer acts in sister chromatid cohesion, and recent data indicate a function in DNA double-strand break repair (DSBR). Since this role of SMC proteins has remained largely elusive, we explored interactions between SMC1 and the homologous recombination (HR) or non-homologous end-joining (NHEJ) pathways for DSBR in *Saccharomyces cerevisiae*. Analysis of conditional single- and double mutants of *smc1-2* with *rad52Δ*, *rad54Δ*, *rad50Δ* or *dnl4Δ* illustrates a significant contribution of SMC1 to the overall capacity of cells to repair DSBs. *smc1* but not *smc2* mutants show increased hypersensitivity of HR mutants to ionizing irradiation and to the DNA crosslinking agent *cis*-platin. Haploid, but not diploid *smc1-2* mutants were severely affected in repairing multiple genomic DNA breaks, suggesting a selective role of SMC1 in sister chromatid recombination. *smc1-2* mutants were also 15-fold less efficient and highly error-prone in plasmid end-joining through the NHEJ pathway. Strikingly, inactivation of *RAD52* or *RAD54* fully rescued efficiency and accuracy of NHEJ in the *smc1* background. Therefore, we propose coordination of HR and NHEJ processes by Smc1p through interaction with the *RAD52* pathway.

INTRODUCTION

Structural Maintenance of Chromosomes (SMCs) proteins are highly conserved eukaryotic proteins that form six distinct groups named SMC1 to SMC6 [reviewed in (1–5)]. They are essential for sister chromatid cohesion, and act in chromosome condensation, gene dosage compensation and DNA repair. Eukaryotic SMC proteins form heterodimers (SMC1/SMC3, SMC2/SMC4, SMC5/SMC6) that are embedded in large multiprotein complexes.

The SMC1/SMC3 heterodimer is the core component of the tetrameric complex cohesin, which is required for the establishment of sister chromatid cohesion during S phase, maintenance of cohesion and proper segregation of chromosomes in mitosis (6–8). More recently, evidence emerged for an additional function of these proteins in DNA double-strand break

repair (DSBR). Early indications originated from the molecular analysis of the mammalian RC-1 complex, which catalyzes cell-free strand transfer and the repair of DNA double-strand gaps in a manner dependent on DNA sequence homology (9). The SMC1/SMC3 heterodimer was identified in a complex with DNA polymerase ϵ and DNA ligase III (9–11). Another line of investigation revealed a central role of the *Saccharomyces cerevisiae* Smc3 protein in sister chromatid cohesion and genetic recombination during meiosis (12). Consistent with a function in meiotic recombination, the mammalian SMC1 and SMC3 proteins were found to associate with sites of chiasmata of spermatocyte meiotic prophase I cells (13–15). Haploid *S.cerevisiae* cells were shown to require proper establishment of sister chromatid cohesion during DNA replication for efficient postreplicative repair of DNA double-strand breaks induced by ionizing radiation (IR) (16). Cohesin proteins were also found at sites of DSBs (17), and mammalian SMC1 was shown to be a target for ATM kinase-dependent phosphorylation in response to DNA damage (18,19). Cells carrying mutations in the phosphorylation sites of SMC1 show increased sensitivity to ionizing irradiation and reduced DNA damage response.

The particular role of the SMC1/SMC3 heterodimer in DNA DSBR, however, remains largely unknown. Therefore, we set out to analyze genetically the DSBR capacity in the *S.cerevisiae smc1* mutant. For comparison, we included an *smc2* mutant. SMC2 represents a key component of a related SMC protein complex, condensin, which contributes to chromosome condensation and chromatin architecture (3–5). The phenotypes of various combinations of a hypomorphic *smc1* allele with gene defects that specifically inactivate either the homologous recombination (HR) or the non-homologous end-joining (NHEJ) pathways of DSBR revealed a role of Smc1p in coordinating the repair of DSBs. The data suggest that in a chromosomal context where DNA sequence homology between sister chromatids can be used, the availability of functional Smc1p facilitates DSBR by HR while preventing NHEJ.

MATERIALS AND METHODS

General genetic methods and yeast strains

Yeast media and general genetic methods are described in (20). All *S.cerevisiae* strains used in this study are described in Table 1. All strains are isogenic derivatives of two closely

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Table 1. *S.cerevisiae* strains used in this study

Strain	Genotype	Source or reference
FF18734	<i>MATα leu2-3 trp1-289 ura3-52 his7-2 lys1-1</i>	F. Fabre (Institut Curie, Paris, France)
FF18743	<i>MATα leu2-3 trp1-289 ura3-52 his7-2 lys1-1 rad52::URA3</i>	F. Fabre
FF181656	<i>MATα leu2-3 trp1-289 ura3-52 his7-2 lys1-1 rad14::LEU2</i>	F. Fabre
PRSY003,1	<i>MATα leu2-3 trp1-289 ura3-52 his7 lys1-1 dnl4::kanMX4</i>	(21)
PRSY005	<i>MATα leu2 trp1 ura3 his7 lys1-1 dnl4::kanMX4 rad52::URA3</i>	(21)
PRSY105,1	<i>MATα leu2 trp1 ura3 his7 smc1-2::LEU2 lys1-1</i>	This study
PRSY107a	<i>MATα leu2 trp1 ura3 his7-2 rad52::URA3 smc1-2::LEU2</i>	This study
PRSY110b	<i>MATα leu2 trp1 ura3 his7 smc2-6 lys2</i>	This study
PRSY115	<i>MATα leu2 trp1 ura3 his7(-2) dnl4::kanMX4 smc1-2::LEU2 lys1-1</i>	This study
PRSY119	<i>MATα leu2 trp1 ura3 his7-2 lys1-1 rad54::LEU2 smc1-2::LEU2</i>	This study
PRSY121	<i>MATα leu2 trp1 ura3 his7-2 lys1-1 rad50::URA3</i>	This study
PRSY123	<i>MATα leu2 trp1 ura3 his7-2 lys1-1 rad54::LEU</i>	This study
PRSY125,1	<i>MATα leu2 trp1 ura3 his7-2 lys1-1 rad50::URA3 smc1-2::LEU2</i>	This study
PRSY126	<i>MATα leu2 trp1 ura3 his7-2 lys1-1 rad50::URA3 rad52::URA3</i>	This study
PRSY127	<i>MATα leu2 trp1 ura3 his7 lys1-1 rad54::LEU2 dnl4::kanMX4</i>	This study

For strain constructions see Material and Methods.

related, congeneric series represented by FF18734 and FF18984 [F. Fabre, personal communication, (20,21)]. Congenic *smc1-2::LEU2* and *smc2-6* strains were obtained by crossing the original *smc* mutants *smc1-2* or *smc2-6*, isolated and described in (22,23), into the FF background. Desired genotypes were isolated from dissected spore tetrads from at least three consecutive crosses using standard replica plating techniques. Due to the conditional lethality of strains carrying the *smc1-2* and *smc2-6* alleles all experiments were performed at a permissive temperature of 25°C.

Sensitivity to DNA-damaging treatment

Cis-platin [*cis*-diamminedichloroplatinum (II)] sensitivities were measured by plating serial dilutions of late exponential cells onto YPD agar, freshly supplemented with indicated amounts of either of the drugs [for general procedures see (21,24)]. IR sensitivity was assessed according to the procedure previously described (21). All incubations were done at 25°C. After 2, 4 and 6 days of incubation colony-forming units were scored and percentages of survival calculated as $\% = (\text{c.f.u.}_{\text{treated}}/\text{c.f.u.}_{\text{mock}}) \times 100$. The lethal doses that killed 90% of cells (LD90) were calculated from regression analyses of survival curves obtained from at least three independent experiments with each strain. All experiments were independently repeated at least three times.

Sensitivity to EcoRI- or HO-catalyzed DNA DSBs

Yeast strains were transformed with plasmids carrying the URA3 marker gene and expressing HO (24), or EcoRI (24) endonuclease under the control of the inducible GAL1 promoter. Four individual URA⁺ transformants of each strain were grown to late exponential phase (5×10^7 cells/ml) in liquid medium lacking uracil at 25°C before dropping 5 μ l of serial dilutions (2×10^7 , 2×10^6 , 2×10^5 , 2×10^4 , 2×10^3 cells/ml) onto media containing 2% of either glucose, raffinose, or raffinose + galactose. The plates were incubated at 25°C for 3–5 days and then photographed. For quantitative analyses, colony-forming units of appropriate dilutions were counted and percentages of survival calculated from at least three independent experiments.

Plasmid rescue assay

Transformation experiments were carried out using either supercoiled or EcoRI-digested pBTM116 plasmid DNA as described in (20). For analysis of joined double-strand breaks, a 1170 bp segment of the substrate plasmid spanning the EcoRI site was amplified by PCR and the products tested for the presence of a restored restriction site by EcoRI digestion (21,24,25). Properly cleaved fragments were scored as accurate end-joining events, and uncleavable fragments or clones that did not yield any PCR product were scored as inaccurate end-joining events. The junction sequences across the site of the original DSB (EcoRI cut) were analyzed by sequencing of the PCR products as described (21).

Telomere stability

Genomic DNA was prepared from exponentially growing cells as described (21). DNA was digested with XhoI, and telomere fragments characterized by Southern blotting and probing with a poly-d(GT)₂₀ probe as described (25).

RESULTS

Sensitivity to DNA-damaging treatments

To explore the specific roles of *S.cerevisiae* *SMC1* or, for control, the condensin *SMC2* in DSBR, we examined the effects of conditional *SMC1* or *SMC2* defects on cell survival after treatment with DNA-damaging agents. We generated a series of congeneric *S.cerevisiae* strains that carry mutant alleles of *SMC1* (*smc1-2*) or *SMC2* (*smc2-6*) (22) in combination with either wild-type or disrupted *RAD52* (HR), *RAD54* (HR), *RAD50* (HR and NHEJ) or *DNL4* (NHEJ) genes (Table 1). Appropriate single- and double mutant combinations along with wild-type controls were then assayed for their sensitivity to different forms of genotoxic stress. All genotoxicity tests were done under permissive conditions for the temperature-sensitive *smc* mutants (25°C) where cell division is unaffected in the absence of DNA-damaging treatment. *Smc1p*- but not *Smc2p*-compromised cells show a mild but significant hypersensitivity to ionizing irradiation (Figure 1A), which is in agreement with previously reported data (16,17,19). The

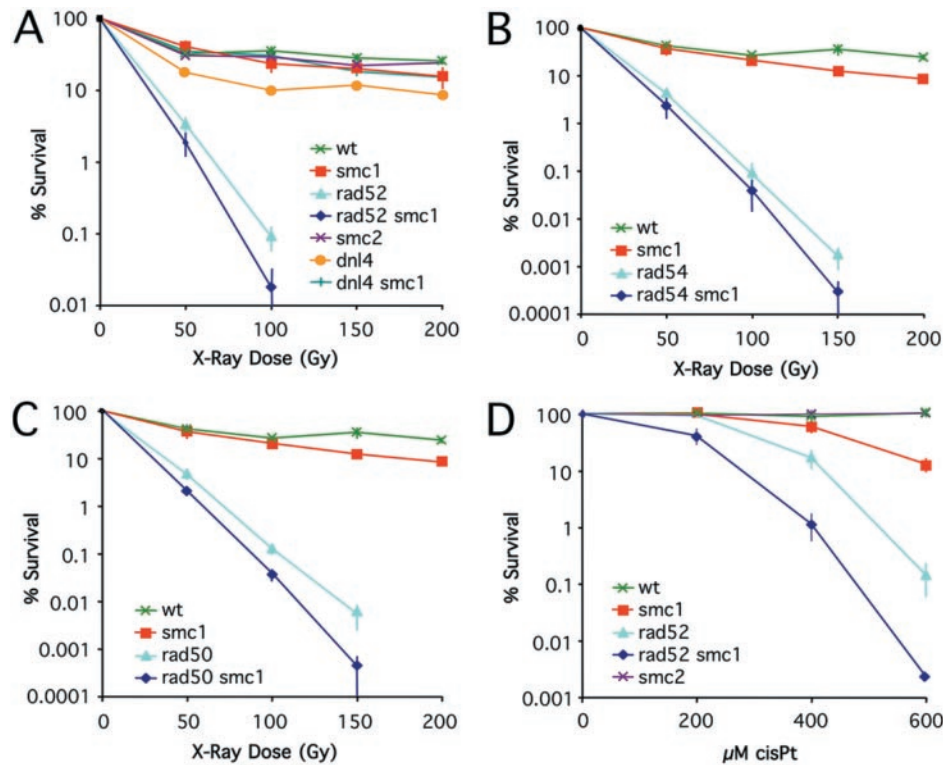


Figure 1. Sensitivity of yeast strains to DNA damaging treatment. Survival curves obtained upon treatment with (A–C) ionizing irradiation (X-ray), or (D) *cis*-platin. The strains were wild-type, FF18734; *smc1-2*, PRSY105.1; *smc2-6*, PRSY110; *rad52Δ*, FF18743; *rad52Δ smc1-2*, PRSY107a; *dnl4Δ*, PRSY003.1; *dnl4Δ smc1-2*, PRSY115; *rad54Δ*, PRSY123; *rad54Δ smc1-2*, PRSY119; *rad50Δ*, PRSY121; *rad50Δ smc1-2*, PRSY125.1; PRSY005; *dnl4Δ rad52Δ*, FF181656 *rad14Δ*.

dose–response curve translates into a statistically significant LD90 reduction factor of 1.5. IR is known to directly and indirectly induce chromosomal DNA strand-breaks [reviewed in (26)]. Repair of these lesions involves primarily HR as confirmed by the strong hypersensitivity of our HR-deficient *rad52Δ*, *rad54Δ* and *rad50Δ* mutants (Figure 1A–C; LD90 dose-reduction factors = 11.0 to 11.8). We observed that introducing the *smc1-2* allele into the *rad52Δ*, *rad54Δ* or *rad50Δ* backgrounds further sensitizes cells to IR (Figure 1A–C). The additional LD90 reduction factors are 1.2-fold in these *rad smc1-2* double mutants and are statistically significant. They translate into 5- to 10-fold differences in survival at doses between 100 and 150 Gy (Figure 1A–C). These data thus establish an additive relationship of the IR sensitivities resulting from HR and Smc1p deficiencies. The NHEJ-deficient *dnl4Δ* mutant shows mild hypersensitivity (Figure 1A; LD90 reduction factor = 2.1), which, in contrast to the HR mutants, is suppressed by the introduction of the *smc1-2* allele (Figure 1A). The limited rescue of the *dnl4Δ* (NHEJ) phenotype by the *smc1* mutation suggests that in the absence of NHEJ an Smc1p-dependent process interferes negatively with the repair of a fraction of IR-induced DSBs. These would most likely be breaks that are normally fixed by the NHEJ pathway (e.g. chromosomal breaks in G₁ phase of the cell cycle). The data thus establish the absence of additive genetic interaction between processes controlled by Smc1p and Dnl4p. The same must be concluded for the interaction between HR and NHEJ, since the *dnl4Δ rad52Δ* double mutant is only insignificantly more sensitive than the *rad52Δ* single

mutant under the experimental conditions applied (data not shown). Together, these results indicate a contribution of Smc1p to the repair of IR-induced DSB, which is functionally distinct from both, the Rad52p-mediated HR pathway and the Dnl4p-controlled NHEJ pathway.

A similar but more pronounced functional relationship between *SMC1* and *RAD52* emerged upon treatment of cells with *cis*-platin. *Cis*-platin predominantly induces intrastrand crosslinks in DNA. Frequently, these adducts are eliminated by the nucleotide excision repair (NER) system. However, if they escape correction by NER, *cis*-platin adducts will stall DNA replication and thereby generate DNA structures that require resolution by recombination processed [reviewed in (27)]. We found that *smc1-2* is hypersensitive to *cis*-platin exposure (LD90 reduction factor = 2.5), although not as pronounced as *rad52Δ* (LD90 reduction factor = 6.3) (Figure 1D). The hypersensitivity of the *smc1-2 rad52Δ* double mutant was additively enhanced (LD90 reduction factor = 9.8). Since neither the *smc1-2* single mutants nor the *smc1-2 rad52Δ* double mutants display significant hypersensitivity to UV irradiation (data not shown), we conclude that the additive effect of the *smc1-2 rad52Δ* double mutation on *cis*-platin sensitivity reflects malfunction of DNA repair pathways other than NER. Thus, as concluded for IR-induced DNA damage, the genetic interactions implicate a role for Smc1p in the repair of or the tolerance to *cis*-platin DNA adducts by a pathway that acts in addition to Rad52p-dependent HR.

The conditional *smc2-6* mutant does not display hypersensitivity to IR or to *cis*-platin. Yet, it shows markedly reduced

resistance toward treatment with hydroxyurea (HU) (LD90 reduction factor = 2.9) (data not shown), confirming that this allele behaves as a hypomorphic mutant under the experimental conditions applied.

SMC1 in the repair of defined genomic DSBs

Since treatment of cells with X-ray, *cis*-platin or other DNA reactive agents generates a large variety of DNA lesions, we next choose to analyze a potential role of Smc1p in the repair of one or multiple defined chromosomal DSBs. Therefore, we tested the resistance of wild-type or mutant strains to endogenous expression of either HO endonuclease or EcoRI restriction endonuclease. Cells carrying plasmid constructs that express the nucleases under the control of the *GAL1* promoter were cultured under repressible conditions to mid-log phase and then dropped in serial dilutions onto media containing either glucose or raffinose/galactose.

Expression of HO in *S.cerevisiae* induces cleavage of the chromosomal DNA at one site, the *MAT* locus, and the resulting DSB is repaired by HR (gene conversion) using the intact donor sequence from either of two silent mating type loci, *HML* or *HMR*, located on the same chromosome. Naturally, this is an intramolecular recombination process occurring in G₁ phase of the cell cycle and, thus is an intramolecular event (28). If, however, HO is continuously expressed from the *GAL1* promoter, DSBs are also generated in S and/or G₂ phase of the cell cycle and their repair will also involve intermolecular sister chromatid recombination. DSB repair of HO-induced breaks depends on the *RAD52* pathway. Consequently, we found the *rad54Δ* mutant to be highly sensitive (Figure 2A). The *smc1-2* and *smc2-6* (data not shown) mutants were not sensitive and the *rad54Δ smc1-2* double mutant showed no elevated sensitivity compared to the *rad54Δ* single mutant. Consistent with the view that cells proficient in HR do rarely employ NHEJ to repair HO induced DSBs at the *MAT* locus, the *dnl4Δ* mutant was not sensitive to HO expression. Interestingly, disruption of *DNL4* in the *smc1-2* background synergistically enhances sensitivity, indicating a contribution of both, Smc1p and Dnl4p to the repair HO-induced DSBs under specific conditions. Apparently, when Smc1p function is compromised, NHEJ gains a more prominent role in the repair of HO-induced breaks. However, the phenotype of the *smc1-2 dnl4Δ* double mutant was not nearly as dramatic as the disruption of *RAD54* in the *dnl4Δ* background, which revealed a clear synergistic relationship between NHEJ and HR in the repair of HO breaks. We also examined the efficiency of mating type switching following HO expression in these strains. As expected, the strains carrying the *rad54Δ* disruption showed a dramatically reduced efficiency of switching whereas neither the *smc1-2* nor the *dnl4Δ* mutations had any notable effect (data not shown).

The results obtained after introduction of multiple genomic DSBs by expression of EcoRI were remarkably different. Unlike HO, EcoRI more often generates breaks at homologous positions in sister chromatids that require repair by NHEJ rather than by HR. Indeed, we measured pronounced hypersensitivities for both *rad54Δ* and *dnl4Δ* single mutant cells upon induction of EcoRI, indicating that the breaks generated are dealt with in part by HR and in part by NHEJ. Remarkably, the *smc1-2* mutant was nearly as sensitive as the *rad54Δ* and

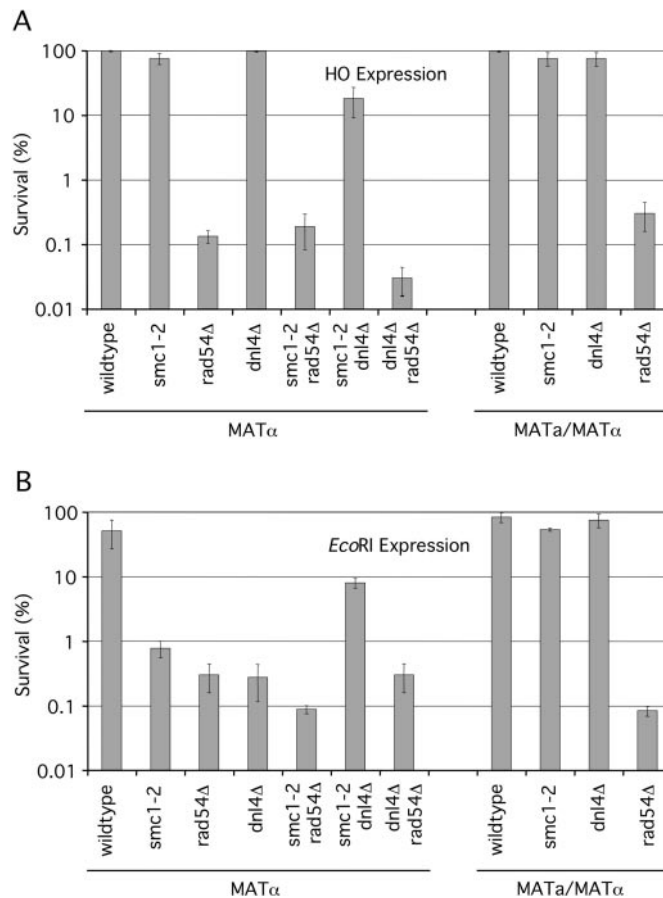


Figure 2. Sensitivity of yeast strains to genomic DSBs introduced by expression of HO or EcoRI endonuclease. The indicated strains were transformed with plasmid constructs for *GAL1*-controlled expression of HO (A) or EcoRI (B). Exponentially growing cultures were plated in serial dilutions onto selective media containing galactose for induction of endonuclease expression. After 3–5 days of incubation, c.f.u.s were counted and percentages of survival calculated relative to isogenic strains carrying the expression vector without endonuclease insert. The strains were wild-type, FF18734; *smc1-2*, PRSY105.1; *rad54Δ*, PRSY123; *dnl4Δ*, PRSY003.1; *rad54Δ smc1-2*, PRSY119; PRSY005; *dnl4Δ smc1-2*, PRSY115.1; *dnl4Δ rad54Δ*, PRSY127; *MATα* haploid strains, *MATα/MATα* diploid strains homozygous for the relevant genotype.

the *dnl4Δ* mutants (Figure 2B), and the double mutants *rad54Δ smc1-2* showed an even enhanced hypersensitivity, demonstrating that Smc1p plays an important role in the repair of the DSBs generated in this assay. The *dnl4Δ smc1-2* double mutant, however, is significantly less sensitive than either of the single mutants. Thus, in the absence of a fully functional Smc1p, NHEJ interferes negatively with the productive repair of a substantial fraction of the EcoRI breaks, or, vice versa, intact Smc1p conveys a negative effect on productive repair of EcoRI cuts in the absence of Dnl4p. These data suggest that an Smc1p-dependent process acts to avoid mis-engagement of NHEJ at DSBs that need to be repaired by HR and, at the same time, mediates non-productive repair of DSBs that need to be repaired by NHEJ.

Interestingly, there seems to be no involvement of Smc1p in the repair of EcoRI or HO breaks in diploid cells (*MATα/MATα*), since a homozygous *smc1-2/smcl-2* condition does not affect cell survival upon induction of either of the

nucleases. Under identical conditions a homozygous *rad54Δ/rad54Δ* diploid is hypersensitive to both HO and EcoRI expression, documenting that the DSBs generated are predominantly repaired by the HR pathway.

Taken together, these genetic interactions implicate that Smc1p acts to support DSB repair in haploid cells by a mechanism that affects the relative efficiencies of both NHEJ or HR, but may not be a core component of either machinery itself.

smc1 mutants are deficient in non-homologous end-joining

To further dissect the relative contribution of Smc1p to either HR or NHEJ, we next addressed its role in the repair of a well-defined episomal DSB under conditions where only NHEJ is possible. In a standardized assay for non-homologous end-joining of an episomal DSB (21,25), yeast cells were transformed in parallel with equal amounts of EcoRI-linearized and circular plasmid DNA, and then selected for expression of the TRP1 marker gene encoded by the plasmid. Since stable expression of the TRP1 gene is possible only after successful ligation of the non-homologous DNA ends of the plasmid, the NHEJ efficiency can be directly calculated from the relative numbers of TRP⁺ transformants obtained with cut or uncut plasmid DNA, respectively. The results obtained for wild type, *smc1-2*, *smc2-6*, *dnl4Δ*, *rad52Δ*, *rad54Δ* and *rad50Δ* single- and double mutants are summarized in Figure 3. We found that the *smc1-2* but not the *smc2-6* mutant was affected in NHEJ. *smc1-2* mutant cells were more than 15-fold less efficient in plasmid rejoining than wild-type cells, and this phenotype co-segregated with the *smc1-2* mutation through three consecutive crosses. Compared to *dnl4Δ* mutants, this is an intermediate but significant reduction in NHEJ efficiency. Unexpectedly, the NHEJ deficiency of the *smc1-2* mutant was dependent on functional Rad52p or Rad54p, since the disruption of RAD52 or RAD54 in the *smc1-2* background

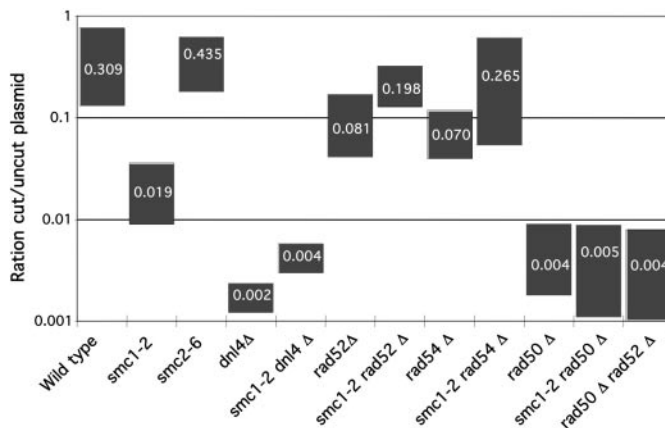


Figure 3. Episomal DNA double-strand break-rejoining assay. To show primary results, the entire ranges of relative transformation efficiencies (ratio cut/uncut plasmid) as obtained in three independent experiments are illustrated by boxes. The numbers within the boxes indicate the mean values. The strains were wildtype, FF18734; *dnl4Δ*, PRSY003,1; *smc1-2*, PRSY105,1; *dnl4Δ smc1*, PRSY115; *rad52Δ*, FF18743; *rad52Δ smc1-2*, PRSY107a; *smc2-6*, PRSY110; *rad54Δ*; PRSY123; *rad54Δ smc1-2*, PRSY119; *rad50Δ*, PRSY121; *rad50Δ smc1-2*, PRSY125; *rad50Δ rad52Δ*, PRSY126.

restored almost wild-type levels of plasmid rejoining. In contrast, no rescue was observed in the *smc1-2 rad50Δ*, the *rad50Δ rad52Δ* or *dnl4Δ rad52Δ* (data not shown) double mutants. Thus, processes dependent on Rad52p or Rad54p reduce the efficiency of NHEJ if the function of Smc1p is impaired.

End-joining is mutagenic in the absence of Smc1p

In the plasmid end-joining assay, removal of *RAD52* or *RAD54* in the absence of *SMC1* restores end-joining, which is defective in the *smc1* single mutant. These genetic interactions between *SMC1* and *RAD52* or *RAD54* therefore implicate that Smc1p prevents negative interference of Rad52p or Rad54p with the repair of non-homologous DNA ends by the NHEJ pathway. Since Rad52p was shown to act in HR as a DNA annealing factor with affinity to DNA ends (29) and is in this respect functionally similar to the Ku heterodimer, which acts in NHEJ (30), interference may arise at the level of plasmid DNA end-binding and processing. This would predict that processing of DNA ends of transformed, linearized plasmid DNA is differently affected in *smc1* mutants with or without Rad52p present. We therefore examined the nature of NHEJ events in individual TRP⁺ transformants of *smc1-2* and *rad52Δ* single- and double-mutant strains at the DNA sequence level. Consistent with previous results, we found that in a wild-type strain, joining of cohesive, EcoRI-digested ends in episomal DNA is error free in >92% of the events [Table 2; (21,25)]. The same accuracy was established for the *rad52Δ* mutant. Strikingly, in *smc1-2* deficient strains one-third of all NHEJ events scored are inaccurate (32%), but only when functional Rad52p is available; *smc1-2 rad52Δ* double mutants perform NHEJ with wild-type precision (95% precise joining).

For more detailed analysis of the underlying molecular events, we sequenced across the critical junction in EcoRI-resistant DNA fragments from eight *smc1-2*-derived clones (Figure 4). The sequences reveal a characteristic pattern of error-prone end-joining. Joining occurred either near the initial site of plasmid linearization (five clones) or at a specific location ~85 bp upstream of the former EcoRI site (three clones). The -85 bp site is next to a 5'-GAAATTCGC-3' sequence, which resembles the EcoRI recognition site GAATTC. Thus, it is likely that in these clones, the microhomology that exists

Table 2. Accuracy of plasmid end-joining

Relevant genotype	Accurate religations	Inaccurate religations	Total events	Ratio accurate/inaccurate	Percentage inaccurate	No PCR product
Wild type	51	4	55	13	7	1
<i>smc1-2</i>	27	13	40	2	32	1
<i>rad52Δ</i>	23	2	25	12	8	2
<i>smc1-2 rad52Δ</i>	38	2	40	19	5	2

A segment of the substrate plasmid spanning the site of DSB (EcoRI site) was amplified by PCR, and the products were tested for the presence of a restored EcoRI site by restriction digestion. Cleavable fragments were scored as accurate end-joining events, uncleavable fragments of clones that did not yield the PCR product were scored as inaccurate end-joining events, but are also listed separately. The strains examined were wild type, FF19734; *smc1-2*, PRSY 105.1; *rad52Δ*, FF18743; *smc1-2rad52Δ*, PRSY107a.

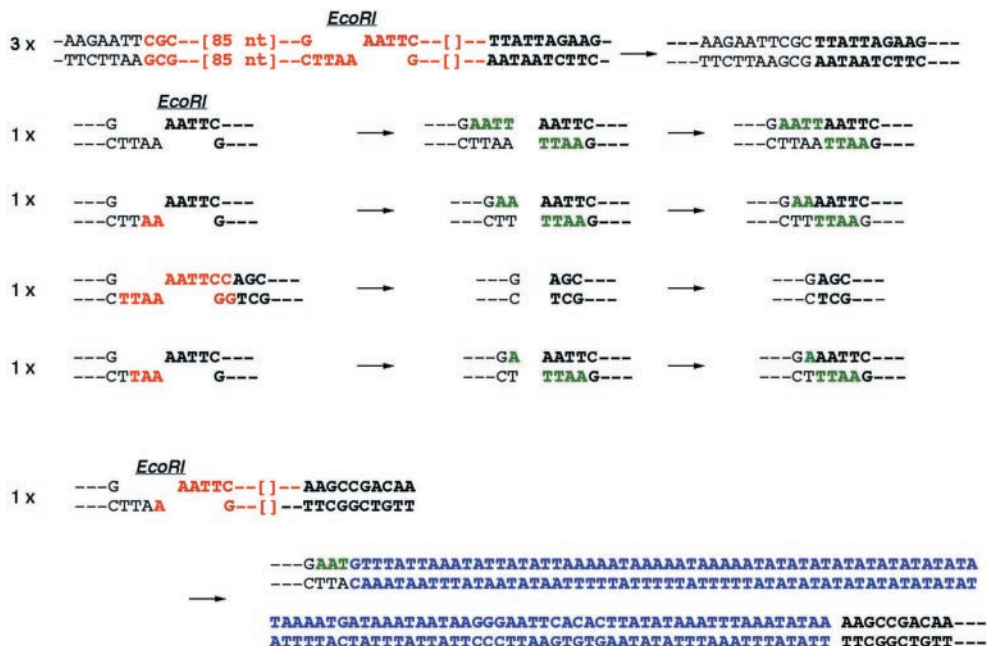


Figure 4. Products of NHEJ in *smc1* mutants. Junction sequences across the site of the original DSB (*EcoRI* cut) were analyzed. Plasmid sequence alterations found in end-joining products isolated from the transformants of the *smc1* mutant strain are shown together with the deduced DSB intermediates. Red letters highlight deleted nucleotides, green letters indicate templated nucleotides newly added by fill-in DNA synthesis and blue letters show a large insertion of unknown origin.

between the free *EcoRI* DNA end (AATT overhang) and the –85 bp site was used in a Rad52p-dependent attempt to repair the double-strand break by HR. In four of the five joints that were located close to the original *EcoRI* site, insertions of A or T nucleotides were found. These insertions reflect in part the AATT core sequence of the *EcoRI* site and can all be explained by minor DNA end-processing events involving exonucleolytic degradation and DNA synthesis before ligation. Finally, one clone carried an unusual sequence, i.e. a long A/T-rich insertion of unknown origin. This sequence was neither found in the vector sequence nor the *S.cerevisiae* genome, and may have been generated by aberrant DNA synthesis and multiplication after extensive exonucleolytic degradation of both plasmid ends.

Telomere stability is unaffected in *smc1* mutants

Mutational inactivation of some proteins required for NHEJ in *S.cerevisiae* (Ku, Mre11p, Xrs2p, Rad50p, but not Dnl4p or Lif1p) causes a defect in telomere length maintenance (24,25). Considering the episomal end-joining deficiency of *smc1-2* mutants, we examined whether Smc1p is also required for telomere stability. Genomic DNA prepared from a set of wild-type or mutant *S.cerevisiae* strains (*smc1-2*, *rad52Δ*, *dnl4Δ*, *smc1-2 rad52Δ* and *sir3Δ*) was subjected to restriction digestion by *XhoI*, which generates characteristic fragments of the sub-telomeric Y'-region that light up as 1.3 kbp bands in Southern blots after hybridization with a poly-d(GT)₂₀ probe (31) (Figure 5). The Southern blots established that the lengths of the Y'-telomeric regions were not significantly altered in the *smc1-2*, *smc1-2 rad52Δ*, *rad52Δ* and *dnl4Δ* mutants, but as expected were shortened in the *sir3Δ* mutant. Thus, the conditional *smc1-2* mutation does not affect telomere stability

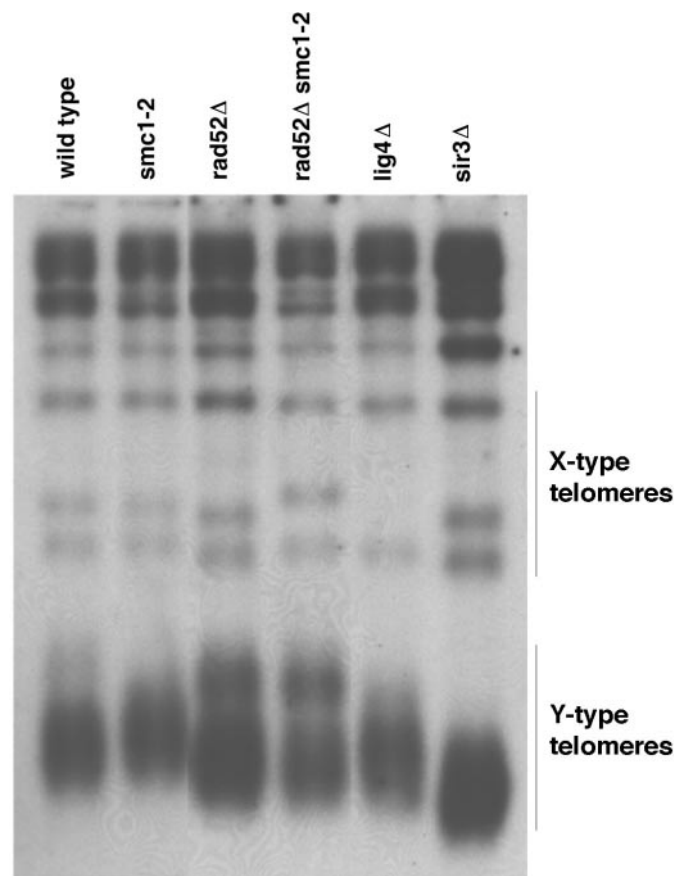


Figure 5. Telomere stability in *smc1-2* and other mutants. *XhoI*-digested DNA was of 10 μg analysed by Southern blotting and probed with a poly-d(GT)₂₀ probe for length of telomeric fragments. X- and Y-type telomers are indicated.

under conditions where the phenotypes associated with DNA recombination are observed.

DISCUSSION

In this report, we present the first evidence for a role of SMC1 in the coordination of repair of DSBs. Our experiments were all performed at permissive temperature allowing for full survival of the conditional *smc* mutants without genotoxic treatment. Therefore, the effects measured are entirely DNA damage-dependent, and reflect the limited potential of hypomorphic *smc* alleles to contribute to the repair of DNA DSBs. Our data establish that SMC1 is required for efficient and accurate repair of a variety of different DSBs and DNA lesions through a mechanism that affects the engagement and the outcome of both HR and NHEJ, but is not an intrinsic component of either of these pathways. Under experimental conditions that suffice to provoke the DNA repair phenotype of the *smc1-2* mutant and HU sensitivity of both, the *smc1-2* and the *smc2-6* mutant, the latter does not show any DSB repair deficiency. More subtle effects on DNA repair of an SMC2 defect, which may be revealed only at non-permissive temperatures or with different alleles, however, cannot be excluded. The sensitivity of the *smc2-6* mutant to HU is in agreement with a report on HU sensitivity of a *Schizosaccharomyces pombe* mutant deficient in another subunit of the condensin complex, Cnd2 (32).

The requirement for SMC1 in DSB repair was observed in three independent assays: (i) the introduction of DNA damage by IR or *cis*-platinum treatment; (ii) the induction of a defined single genomic DSB or of multiple genomic DSBs by expression of DNA endonucleases; and (iii) the repair of an episomal DSB upon transformation of linearized plasmid DNA into cells. Performance in each of these repair assays was negatively affected in the *smc1-2* mutant background.

Upon treatment of cells with genotoxic doses of X-ray or *cis*-platinum, the *smc1-2* mutant shows a mild but significant hypersensitivity both in wild type and in *rad52Δ*, *rad54Δ* or *rad50Δ* backgrounds. This indicates that Smc1p contributes to the repair of induced DSBs by a pathway independent of that controlled by the RAD52 epistasis group of genes. Also, since Rad50p functions in multiple DSB repair processes, including HR, single-strand annealing SSA and NHEJ (32), the additive response of the combined *smc1-2* and *rad50Δ* mutations to ionizing irradiation argues for Smc1p acting in DSB repair independently of any of these pathways.

In the repair of the HO-induced DSB, neither Smc1p nor Dnl4p appear to be important, but in an *smc1-2* background, a contribution of Dnl4p becomes apparent. Thus, impairment of Smc1p function leads to an engagement of NHEJ to repair HO-induced DSBs that would otherwise be repaired by HR; it reduces the efficiency of HR while facilitating an involvement of NHEJ, which is not apparent in the wild-type cell. The principal possibility of an involvement of NHEJ in the repair of HO-induced breaks in our strain background is demonstrated clearly by the synergistic effect of inactivating NHEJ (*dnl4Δ*) in the background of HR deficiency (*rad54Δ*). We thus envision the role of Smc1p in the context of constitutive HO expression to facilitate homologous sister chromatid repair in the S/G₂ phase of the cell cycle, most likely through its function in sister chromatid cohesion. A

contribution of the cohesin complex to the repair of HO breaks induced in the G₁ phase of the cell cycle seems less likely because intermolecular sister chromatid interactions are not involved in the repair of these breaks that naturally lead to mating type switching. This is compatible with our observation that the *smc1-2* mutation does not affect mating type switching in any of the HR-proficient strains tested.

While the *smc1-2* strain readily repairs a single HO-induced DSB in the genome, the productive repair of multiple breaks introduced by EcoRI is impaired nearly as much as in the HR-deficient *rad54Δ* strain. This is in agreement with the hypersensitivity of the *smc1-2* mutant to DNA strand-break-inducing agents, and with data from a different allele of *smc1* that affects the establishment of sister chromatid cohesion during DNA replication (16). NHEJ (DNL4) also gains importance in this assay, most likely because EcoRI will frequently cut sister chromatids at homologous positions, making repair by HR impossible. This raises the question of whether the phenotype of the *smc1-2* mutant revealed upon EcoRI cleavage reflects a defect in HR or in NHEJ. Features of the double mutants suggest that neither seems to be the case. The combinations of *smc1-2* with *rad54Δ* enhances sensitivity, while the combination of *smc1-2* with *dnl4Δ* yields a clear suppressive effect. These genetic relationships indicate again that rather than being part of the molecular machines of HR and NHEJ, Smc1p appears to have a separate function that affects their relative contribution to DSB repair. This is illustrated most clearly by the suppression of the *smc1-2* phenotype through inactivation of DNL4, which we interpret to reflect negative interference of NHEJ with productive (non-lethal) DSB repair in the absence of fully functional Smc1p. Therefore, Smc1p may coordinate productive repair of chromosomal EcoRI breaks by suppressing NHEJ and supporting HR. Smc1p can do so through its structural function in sister chromatid cohesion, which is mediated by the Smc1p/Smc3p heterodimer and generates a chromatin organization that supports repair by HR.

Interestingly, *smc1-2* homozygous diploid cells are not affected in survival after DSB induction. NHEJ does not contribute to survival since it is non-functional in diploids (33–35), while HR is essential. Thus, the function of Smc1p in DSB repair does not show under conditions where cells can use homologous chromosomes for the repair of DSBs, indicating that it is limited to DSB repair processes involving sister chromatids, which is perfectly consistent with its essential role in sister chromatid cohesion.

Whereas the role of Smc1p in homology-dependent repair of induced chromosomal strand breaks appears to be direct, its involvement in homology-independent end-joining is more indirect. Conditional *smc1-2* mutants display a significant defect in accurate religation of non-homologous DNA ends of an episomal plasmid substrate. In the absence of fully functional Smc1p, Rad52p-dependent processes interfere with efficient DSB repair by end-joining. Malfunctioning Smc1p complexes such as cohesin apparently lead to a non-coordinated interaction of HR proteins with the DNA ends of the non-homologous plasmid substrate. The consequence may be a direct competition of HR with NHEJ proteins for DNA ends, a scenario which is supported by the finding that human Rad52p binds to DNA ends with properties somewhat similar to the Ku heterodimer (29). Thus, attempted Rad52p-dependent homologous recombination in the *smc1-2*

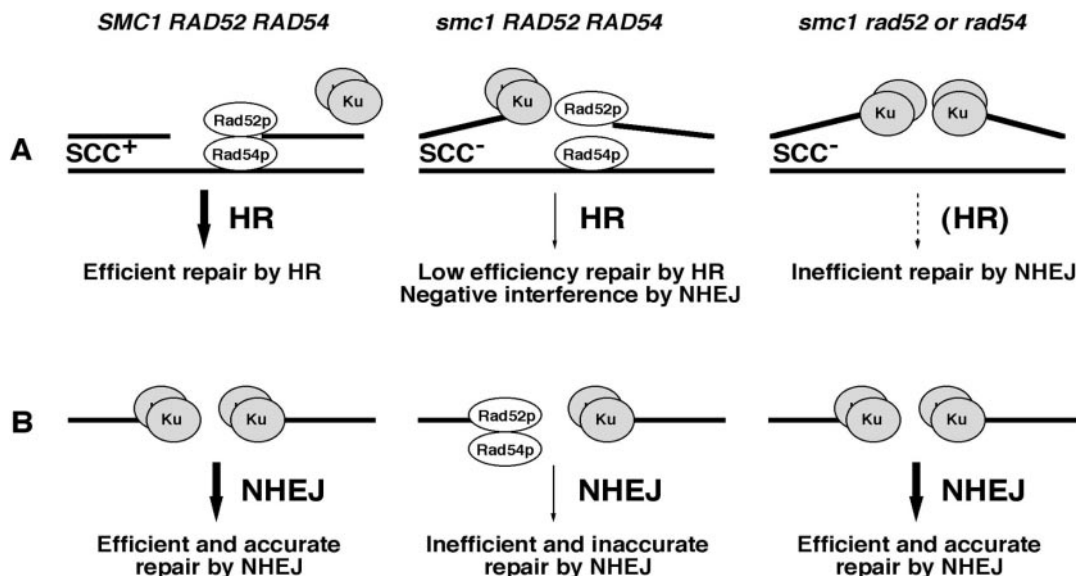


Figure 6. Hypothetical role of Smc1p in coordinating DSB repair, either in the presence (A) or absence (B) of sister chromatids. In (A), Smc1p mediated sister chromatid cohesion (SCC) supports HR between sister chromatids. NHEJ cannot compensate efficiently for an absence of either SMC1 or Rad52/Rad54, or both. In (B), the DSB is primarily repaired through NHEJ in the wild-type situation, but in the absence of Smc1, deregulated Rad52/Rad54 prevents efficient repair by NHEJ, which is rescued by eliminating the HR proteins. No function for SMC1 in inter-homolog recombination was observed. For clarity, only key representatives of the proteins involved in HR (Rad52, Rad54) or NHEJ (Ku) processes are shown.

background is unable to proceed in an orderly manner and thus reduces the overall repair efficiency and accuracy. Only additional inactivation of HR can restore a wild-type-like situation for the repair of the non-homologous substrate, allowing Ku access to DNA ends, and efficient NHEJ despite the *smc1-2* mutation. Since this rescue was observed in both the *smc1-2 rad52Δ* and the *smc1-2 rad54Δ* double mutant, it is most probably the establishment of mature Rad52p- and Rad54p-dependent intermediates of HR, rather than DNA end-binding only, that interferes with efficient NHEJ. In any case, these observations again suggest that Smc1p modulates the balance between NHEJ and HR by regulating the engagement of Rad52p or Rad54p in a substrate-dependent manner.

In contrast to the *smc1-2 rad52Δ* and *smc1-2 rad54Δ* double mutant, there is no rescue in the *smc1-2 rad50Δ* mutant nor is *rad50Δ* rescued by *rad52Δ*. These results are expected since Rad50p is a component of the Mre11/Rad50/Xrs2 complex, which is required for both efficient NHEJ and HR (36).

A coordinated engagement of HR and NHEJ in the repair of double-strand breaks is crucial for genome stability. Our data from different experimental approaches suggests that the Smc1p/Smc3p heterodimer acts as DNA substrate-dependent coordinator of recombinational activities. The precise molecular mechanisms involved in this function may now be studied. As outlined above, the most likely explanation invokes a structural contribution of cohesin or a related SMC1/SMC3 complex to HR by positioning the sister chromatid template. Sister chromatid recombination was indeed shown to be a prominent pathway for DSB repair in both yeast and mammalian cells (37–39), illustrating the link between sister chromatid cohesion and DSB repair. Additional ways in which SMC1/SMC3 may contribute to DSB repair may be by localizing to sites of DNA damage and supporting the assembly of recombination/repair protein machineries, or by promoting DNA pairing reactions (4). Initial evidence for association of cohesin with sites of

DNA damage has been reported (17). The model in Figure 6 summarizes the possible roles of Smc1p, and thus the Smc1p/Smc3p heterodimer and its higher order complexes like cohesin or RC-1, in recombinational repair of DSBs as deduced from our data. Generally, we confirm the importance of SMC1 controlled processes in DSB repair but, for the first time, are able to separate this function from the genetic pathways of HR and NHEJ. The genetic data lead us to postulate a role for the SMC1/SMC3 complex in coordination of DSB repair by favoring HR over NHEJ when homologous sister chromatids are available as templates for repair.

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