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Smc5–Smc6 mediate DNA double-strand-break repair by promoting sister-chromatid recombination

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Abstract

DNA double-strand breaks (DSB) can arise during DNA replication, or after exposure to DNA-damaging agents, and their correct repair is fundamental for cell survival and genomic stability. Here, we show that the Smc5–Smc6 complex is recruited to DSBs *de novo* to support their repair by homologous recombination between sister chromatids. In addition, we demonstrate that Smc5–Smc6 is necessary to suppress gross chromosomal rearrangements. Our findings show that the Smc5–Smc6 complex is essential for genome stability as it promotes repair of DSBs by error-free sister-chromatid recombination (SCR), thereby suppressing inappropriate non-sister recombination events.

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Eukaryotic cells have evolved two main mechanisms for the repair of DSBs: non-homologous end-joining (NHEJ)[1] and homologous recombination[2]. NHEJ entails the direct rejoining of the broken ends of DNA, whereas homologous recombination involves a genomic search for similar sequences to be used as a template to repair the break. In yeast and mammalian cells, the preferred template for DSB repair by homologous recombination is an intact sister chromatid[3]. However, due to the fact that sisters are identical in sequence, SCR events are more difficult to detect by genetic or physical means. Thus, despite its physiological importance, SCR and the factors that mediate this process are poorly understood.

Mutants of the Smc5–Smc6 complex are defective in the repair of diverse types of DNA lesion[4]. On the basis of the capacity of Smc5–Smc6 proteins to interact with chromatin[5], and the function of the Smc5–Smc6 complex in DNA repair, we examined whether this complex was localized to DSBs. We investigated whether subunits of the complex were recruited to an HO-endonuclease catalysed by DSB formation at a unique site in the *MAT* locus on chromosome III (ref. 6; Fig 1a) or at a HO-recognition site inserted in chromosome VI (see Supplementary Information, Fig. S1a–c). DSBs in HO sequences are generally repaired by homologous recombination with *HML* and *HMR* loci[7]; however, to prevent repair of HO-induced DSBs, both *HM* loci were deleted in our strains[8]. Chromatin binding of Smc6 tagged with nine Myc epitopes (Smc6-9Myc) to sites around the DSBs was assayed by chromatin immunoprecipitation (ChIP; Fig. 1a and see Supplementary Information, Fig. S1c, d).

In the absence of a DSB, low Smc6 binding across these regions was observed (Fig. 1a; uncut). After DSB induction, a general increase in binding was detected around the regions flanking the break (Fig. 1a and see Supplementary Information, Fig. S1c, d; cut). The maximum DSB-induced increase was approximately 5–7-fold and localized to regions 4–5 kb away from the DNA break on either side (Fig. 1a and see Supplementary Information, Fig. S1c, d; cut). The recruitment of Smc6 to the site of DSB reached a maximum 2 h after HO induction (see Supplementary Information, Fig. S2a) and occurred specifically in G2–M cells (Fig. 1a; cut). We found no enrichment of Smc6 on other chromosomes (Fig. 1a; *met6* control), demonstrating that Smc5-Smc6 is recruited specifically to genomic sites flanking the HO break.

The recruitment of Smc5–Smc6 to HO-induced DSBs (Fig. 1a and see Supplementary Information, Fig. S1c, d) prompted us to investigate whether the complex affects pathways for DSB repair. Firstly, the effect of Smc5–Smc6 on NHEJ-mediated repair of a chromosomal DSB at *MAT* was examined in a strain that cannot repair the break by homologous recombination[9]. In *smc6-9* mutant strains, NHEJ was not affected compared with wild-type cells (Fig. 1b) demonstrating that the Smc5–Smc6 complex does not participate in NHEJ. Secondly, the role of Smc5–Smc6 in DSB repair by homologous recombination between ectopic sites was evaluated. Mating-type switching efficiency was determined by Southern blot analysis of restriction fragments[10] (Fig. 1c and see Supplementary Information, Fig. S1e). No significant differences (below twofold) were observed between *smc5–smc6* mutants and wild-type cells (Fig. 1c and see Supplementary

Information, Fig. S1e), demonstrating that Smc5–Smc6 function is not required to mediate homologous recombination between ectopic sites.

As Smc5–Smc6 subunits are recruited to DSBs during the G2 and M phases (when sister chromatids are present; Fig. 1a), we tested whether the complex is required for SCR. A physical assay for the detection of DSB-induced SCR was used[11]. The assay allows the study of SCR events between two *leu2* repeats located on a single-copy plasmid[11] (see Supplementary Information, Fig. S2a and Methods). One repeat contains a mutant HO site, *HOr*, which is very inefficiently cut by the HO endonuclease (<10% of cells), thus cutting only one sister chromatid in over 90% of these cases[11]. Recombination intermediates can be detected and quantified using Southern blot analysis[11].

smc6-9 and *nse5-1* mutants were then tested in the SCR assay. Following inactivation of Smc5–Smc6 function and *HOr* cleavage, samples were collected at different times and evaluated by Southern blot analysis (see Supplementary Information, Fig. S2b). The levels of DSB repair in the *smc5–smc6* mutants were similar to wild-type cells after 4 h (Fig. 2a). At this time, over 8% of DSBs in wild-type cells had been repaired by SCR (Fig. 2a), whereas less than 2% (a fourfold reduction) were repaired by SCR in the *nse5-1* and *smc6-9* mutants (Fig. 2a). Therefore, inactivation of Smc5–Smc6 function significantly reduced the repair of *leu2–HOr* DSB by SCR.

The assay used reports on frequency of SCR by detection of unequal sister-chromatid exchange (USCE) events. Direct detection of equal sister-chromatid exchange (ESCE) events can also be monitored by the formation of dimers in undigested plasmids that contain a single *leu2–HOr* gene[11] (see Supplementary Information, Fig. S2c). ESCE-induced plasmid dimers accumulated in wild-type cells, reaching 1% after 6 h (Fig. 2b). In contrast, *nse5-1* and *smc6-9* cells showed significantly reduced levels of plasmid dimer formation (Fig. 2b and see Supplementary Information, Fig. S2d). These results confirm that the inactivation of the Smc5–Smc6 complex prevents SCR and this mechanism is important for preventing genomic instability.

The rates of spontaneous gross chromosomal rearrangements (GCRs) were examined in *smc6-9* cells using a previously described method[12]. *smc6-9* mutants showed an increase of approximately 100-fold in GCR rate, which was dependent on the homologous recombination machinery (Fig. 2c and see Supplementary Information, Fig. S2e). Therefore, the GCR results are consistent with Smc5–Smc6 normally having a role in mediating error-free recombinational repair between sister chromatids.

Here, we have shown that the Smc5–Smc6 complex is recruited to DSB sites to mediate repair with the aligned, identical sister chromatid. Interestingly, the binding pattern of the Smc5–Smc6 complex to DSBs is similar to that of the related complex, cohesin[13,14], which is known to hold sister chromatids together and is required for DSB repair[15]. However, the precise mechanism by which cohesin promotes SCR, and whether the role of these two complexes in DSB repair is related, has yet to be determined. Our results reveal that the enigmatic Smc5–Smc6 complex is an important factor in ensuring error-free repair and minimising genome instability across eukaryotes.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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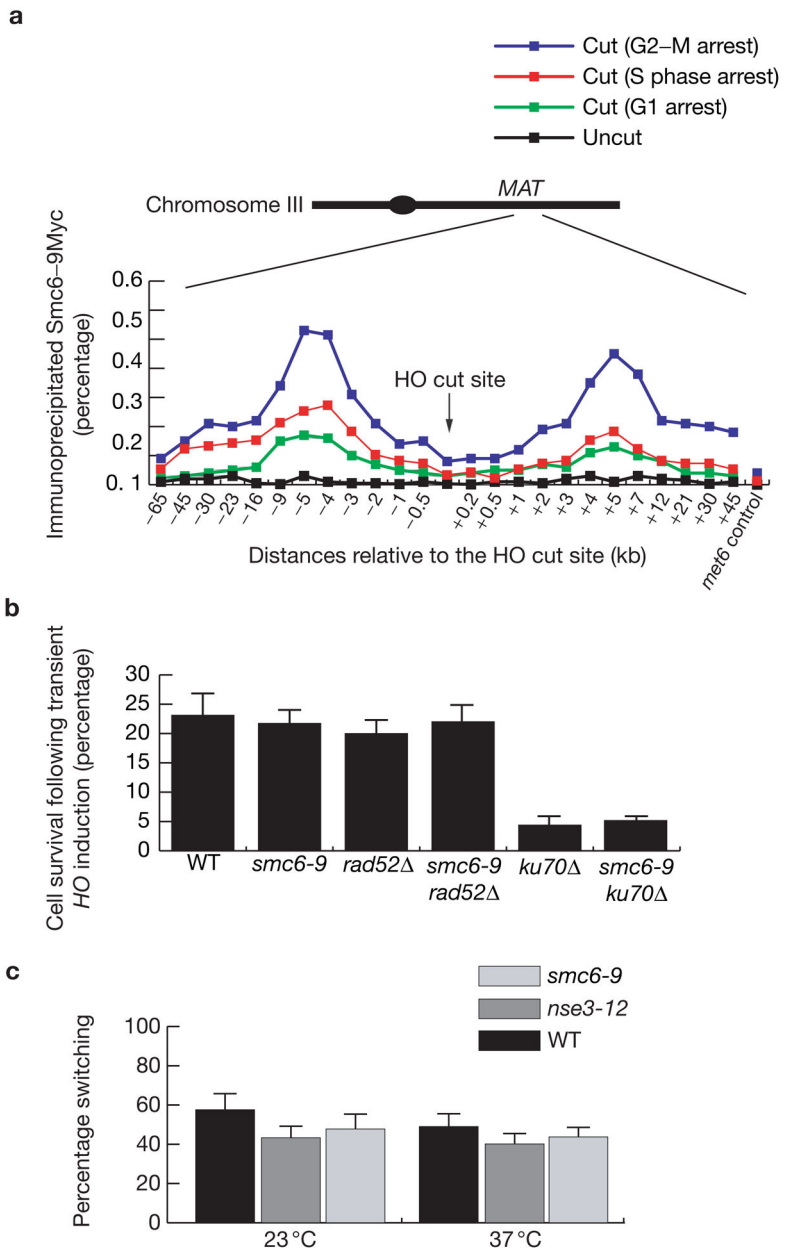
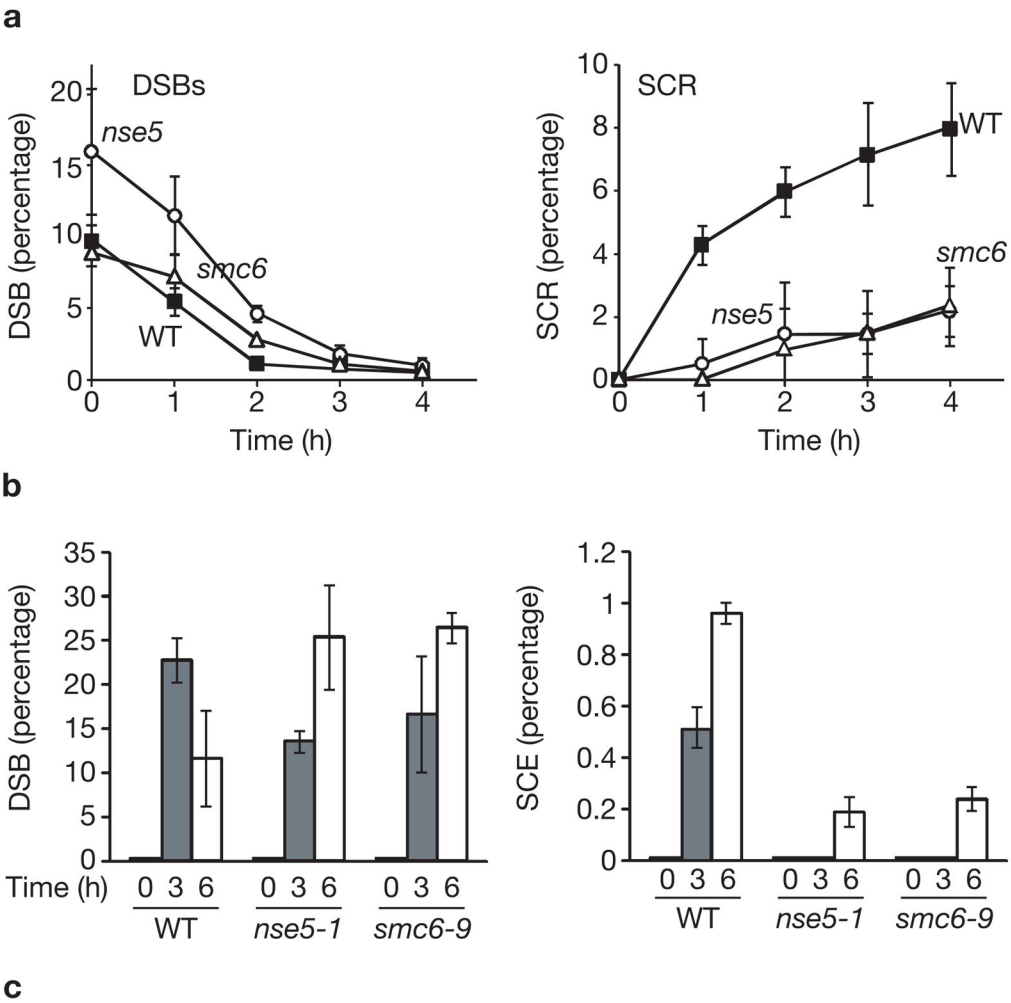


Figure 1.

The Smc5–Smc6 complex is enriched around a HO-induced DSB during G2–M. (**a**) Binding of Smc6 around the *MAT* locus on chromosome III in JKM179 cells (Smc6–9Myc), without (uncut) or with (cut) a DSB at *MAT*, in cells arrested at different stages of the cell cycle. Cells were grown at 30 °C, arrested and galactose was added to half of the cells (cut), before being processed for ChIP. Input DNA and DNA coimmunoprecipitated with anti-Myc antibody (IP) were amplified using primer sets corresponding to sequences different distances from the *MAT* cut. (**b**) NHEJ assays with transient HO induction. Percentage cell survival is shown. WT, wild type. (**c**) *MAT* switching assays in wild-type and *smc5–smc6* mutant strains. Percent switching was calculated from the ratio of the amount of HO

cleavage at 1 h compared with the amount of final product, normalized to the *MAT* distal DNA in each lane. s.d. from 3 independent experiments are shown in **b** and **c**.



c

Relevant genotype	WT Strain	WT GCR rate (Can^r 5-FOA^r)
WT	RDKY3615	3.5×10^{-10} (1)
smc6-9	YKJM3088	2.7×10^{-8} (76)

Figure 2.

Inactivation of Smc5–Smc6 reduces DSB repair by SCR. (**a**) Quantification of HO-induced DSB repair by SCR in wild-type, *nse5-1* and *smc6-9* cells. DSB disappearance (left) and SCR accumulation (right) are shown. A schematic representation and Southern blot analysis of recombination intermediates are shown in the Supplementary Information, Fig. S2a, b. (**b**) Quantification of HO-induced DSB repair by SCE in wild-type, *nse5-1* and *smc6-9* cells. DSB disappearance (left) and accumulation of SCE products (right) are shown. A schematic representation and Southern blot analysis of recombination intermediates are shown in the Supplementary Information, Fig. S2c, d. The average of 3 experiments with s.d. are shown in **a** and **b**. (**c**) Increased GCR formation in *smc6-9* mutants. The *smc6-9* strain is isogenic

with the wild-type strain, RDKY3615 [*ura3-52, leu2Δ1, trp1Δ63, his3Δ200, lys2ΔBgl, hom3-10, ade2Δ1, ade8, YEL069::URA3*]. The numbers in parenthesis indicate the fold induction of GCR rate relative to the wild-type GCR rate. Can^r, canavanine resistance; 5-FOA^r, 5-fluoracetic acid resistance.