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Human SMC5/6 complex promotes sister chromatid homologous recombination by recruiting the SMC1/3 cohesin complex to double-strand breaks

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The structural maintenance of chromosomes (SMC) family of proteins has been implicated in the repair of DNA double-strand breaks (DSBs) by homologous recombination (HR). The SMC1/3 cohesin complex is thought to promote HR by maintaining the close proximity of sister chromatids at DSBs. The SMC5/6 complex is also required for DNA repair, but the mechanism by which it accomplishes this is unclear. Here, we show that RNAi-mediated knockdown of the SMC5/6 complex components in human cells increases the efficiency of gene targeting due to a specific requirement for hSMC5/6 in sister chromatid HR. Knockdown of the hSMC5/6 complex decreases sister chromatid HR, but does not reduce nonhomologous end-joining (NHEJ) or intra-chromatid, homologue, or extra-chromosomal HR. The hSMC5/6 complex is itself recruited to nuclease-induced DSBs and is required for the recruitment of cohesin to DSBs. Our results establish a mechanism by which the hSMC5/6 complex promotes DNA repair and suggest a novel strategy to improve the efficiency of gene targeting in mammalian somatic cells.

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Introduction

The structural maintenance of chromosome (SMC) proteins are essential for chromosomal architecture and organization (Hirano, 2002; Hagstrom and Meyer, 2003; Petronczki *et al*, 2003; Nasmyth and Haering, 2005). The eukaryotic SMC proteins form three heterodimers, SMC1/3, SMC2/4, and SMC5/6. The SMC1/3 heterodimer forms the cohesin complex that maintains sister chromatid cohesion during mitosis (Koshland and Guacci, 2000; Hirano, 2002; Hagstrom and Meyer, 2003; Petronczki *et al*, 2003). SMC2/4 forms the condensin complex that mediates chromosome condensation during mitosis (Swedlow and Hirano, 2003; Hirano, 2005a). The SMC5/6 complex is involved in the cellular response to DNA damage (Lehmann *et al*, 1995; Fousteri and Lehmann, 2000; Hirano, 2002; Hagstrom and Meyer, 2003; Onoda *et al*, 2004). Each SMC protein consists of an N-terminal Walker A box and a C-terminal Walker B box that are separated by a flexible linker region (Hirano, 2005b). This linker region forms an intramolecular antiparallel coiled-coil that brings the Walker A and B motifs into proximity, thus reconstituting a functional ATPase module (Haering *et al*, 2002). The SMC heterodimers of cohesin and condensin form V-shaped structures (Hagstrom and Meyer, 2003; Swedlow and Hirano, 2003). The ATPase head domains can be linked by non-SMC proteins, called kleisins, to form rings (Nasmyth and Haering, 2005).

The SMC1/3 cohesin complex is essential for maintaining cohesion between sister chromatids during mitosis to promote their equal segregation to daughter cells (Nasmyth, 2002). In addition, cohesin plays an important role in the repair of DNA double-strand breaks (DSBs) (Hirano, 2005b; Lehmann, 2005). In yeast, mutations in the cohesin subunit SCC1/RAD21/MCD1 (referred to as SCC1 hereafter) are hypersensitive to DNA-damaging agents (Birkenbihl and Subramani, 1992). Furthermore, chicken DT40 cells conditionally deficient in SCC1 show a decrease in sister chromatid exchanges (SCEs) induced by 4-nitroquinoline-1-oxide (Sonoda *et al*, 2001). It has been proposed that cohesin facilitates DNA repair by holding sister chromatids together locally at DSBs to allow strand invasion and exchange with the sister chromatid repair template during homologous recombination (HR) (Sjogren and Nasmyth, 2001; Sonoda *et al*, 2001; Schar *et al*, 2004; Strom *et al*, 2004).

Studies in yeast have established that the normal replicative loading of the SMC1/3 cohesin complex on chromatin is insufficient to hold DSBs in close proximity, suggesting that the cohesin complex must be loaded post-replicatively at the vicinity of DSBs to facilitate sister chromatid HR (Strom *et al*, 2004). The cohesin complex has been shown to be recruited to DSBs in both yeast and human cells (Kim *et al*, 2002; Strom *et al*, 2004; Unal *et al*, 2004). In yeast, the recruitment of cohesin to HO endonuclease-induced DSBs requires MRE11 and the phosphorylation of H2AX (γH2AX) (Unal *et al*, 2004). Additionally, the recruitment of cohesin to DSBs in yeast represents *de novo*, postreplicative loading of cohesin at DSBs that requires the SCC2/4 loading complex (Strom *et al*, 2004; Unal *et al*, 2004).

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The SMC5/6 complex has primarily been studied in fission and budding yeasts. Cells harboring hypomorphic alleles of genes in the SMC5/6 complex show an increased sensitivity to DNA-damaging agents (Lehmann *et al*, 1995; Fousteri and Lehmann, 2000; Fujioka *et al*, 2002; McDonald *et al*, 2003; Morikawa *et al*, 2004; Onoda *et al*, 2004; Pebernard *et al*, 2004; Hu *et al*, 2005). Genetic analysis has shown that components of the SMC5/6 complex function together with RAD51 in the repair of DSBs through HR (McDonald *et al*, 2003; Harvey *et al*, 2004; Onoda *et al*, 2004; Pebernard *et al*, 2004). Mutations in the SMC5/6 complex also exhibit defects in the maintenance of DNA damage checkpoint signals (Verkade *et al*, 1999; Harvey *et al*, 2004). In addition, plants in which the SMC6 ortholog has been disrupted show a defect in HR (Mengiste *et al*, 1999).

The yeast SMC5/6 complex contains several non-SMC elements (NSE), including NSE1, MMS21/NSE2 (hereafter referred to as MMS21 for simplicity), NSE3, NSE4, NSE5, and NSE6 (Fujioka *et al*, 2002; McDonald *et al*, 2003; Morikawa *et al*, 2004; Pebernard *et al*, 2004; Hu *et al*, 2005; Sergeant *et al*, 2005; Zhao and Blobel, 2005; Pebernard *et al*, 2006). The MMS21 subunit functions as a SUMO ligase to sumoylate multiple components of the SMC5/6 complex, including SMC5, SMC6, and NSE3 (Andrews *et al*, 2005; Potts and Yu, 2005; Zhao and Blobel, 2005). Sumoylation of SMC6 by MMS21 is enhanced upon DNA damage, suggesting that the SUMO ligase activity of MMS21 is regulated by the cellular DNA damage response (Andrews *et al*, 2005).

We have recently shown that human cells deficient in hSMC5/6 are hypersensitive to DNA-damaging agents and have a decreased capacity to repair damaged DNA, suggesting that the hSMC5/6 complex is required for DNA repair (Potts and Yu, 2005). Here, we describe a mechanism by which the SMC5/6 complex facilitates DNA repair. We show that hSMC5/6 is required for HR repair using the sister chromatid as a template (inter-chromatid), but not for non-homologous end-joining (NHEJ) or HR repair using extra-chromosomal or intra-chromosomal DNA as the template. The hSMC5/6 complex is recruited to nuclease-induced DSBs in human cells and is required for the recruitment of the hSMC1/3 cohesin complex to DSBs.

Results

The hSMC5/6 complex is not required for all forms of HR

To investigate the mechanism by which the hSMC5/6 complex promotes DNA repair, we tested whether hSMC5/6 is required for the repair of a single DSB in human cells induced by the rare-cutting I-SceI endonuclease (Pierce *et al*, 1999). A repair substrate containing a direct repeat green fluorescent protein (DR-GFP) reporter was stably integrated into the genome to create 293/DR-GFP cells. Both copies of the *GFP* genes within DR-GFP are mutated: *SceGFP* contains in-frame stop codons and the 18 bp I-SceI recognition site, whereas *truncGFP* is an internal fragment of *GFP* (Figure 1A). Without HR, neither *SceGFP* nor *truncGFP* encodes functional GFP proteins, and the cells remain GFP negative. Expression of I-SceI results in a chromosomal DSB in the *SceGFP* gene. Four mechanisms can be used to repair this DSB (Moynahan *et al*, 2001): (1) HR between the two tandem copies of the mutated GFP genes within the same chromatid (intra-chromatid recombination), (2) HR between *SceGFP* on one chromatid and *truncGFP* on the sister chromatid (unequal sister chromatid recombination), (3) HR between *SceGFP* on one chromatid and *SceGFP* on the sister chromatid (equal sister chromatid recombination), and (4) NHEJ (Figure 1A). Intra-chromatid recombination and unequal sister chromatid recombination, but not equal sister chromatid recombination or NHEJ, will reconstitute a functional *GFP* gene, resulting in GFP-positive cells. The percentage of GFP-positive cells is determined by flow cytometry.

As expected, about 2% of cells were GFP positive after the cotransfection of a plasmid encoding I-SceI and LACZ siRNA (LACZ-RNAi; Figure 1B and D). Knockdown of a critical HR component, RAD51, greatly reduced the amount of GFP-positive cells (Figure 1D). Surprisingly, the percentage of GFP-positive cells increased by two-fold in cells transfected with hMMS21 or hSMC5 siRNA (Figure 1B and D). Depletion of hMMS21 was confirmed by Western blotting (Figure 1C). The increase in GFP-positive cells in hMMS21-RNAi cells was abrogated by RAD51-RNAi (Figure 1D), suggesting that the increase caused by hMMS21-RNAi is through a RAD51-dependent HR pathway. Inactivation of proteins generally required for all forms of HR, such as RAD51, BRCA1, and BRCA2, leads to a reduction of GFP-positive cells in this type of assay (Moynahan *et al*, 1999, 2001). As the DSB generated by I-SceI can be repaired by multiple pathways, there may be active competition for the repair of the DSB by these pathways. Therefore, one explanation for the increase in GFP-positive cells in hMMS21-RNAi or hSMC5-RNAi cells is that knockdown of the hSMC5/6 complex selectively blocks HR through equal sister chromatid recombination or NHEJ (pathways that do not generate GFP-positive cells), thus shunting the repair of the DSBs down the other two HR pathways that will yield GFP-positive cells.

Inactivation of the hSMC5/6 complex enhances gene targeting in human somatic cells

We next tested whether downregulation of hSMC5/6 affected the rate of HR using a gene targeting assay that measured the frequency of HR between a chromosomal locus and an episomal repair plasmid (Porteus and Baltimore, 2003). Briefly, an artificial gene target (A658), containing a mutated *GFP* gene with in-frame stop codons and an I-SceI recognition site inserted, is stably integrated at a single genomic locus of 293 cells (293/A658). The 293/A658 cells are transfected with an I-SceI/repair plasmid that contains the *I-SceI* gene and a truncated *GFP* gene. In the absence of HR, neither *SceGFP* (the mutated *GFP* gene integrated in the genome) nor *truncGFP* (the truncated *GFP* gene on the I-SceI/repair plasmid) will express functional GFP, and the cells remain GFP negative (Porteus and Baltimore, 2003). Expression of I-SceI introduces a DSB within the integrated *GFP* locus, which can be repaired by three major pathways: (1) HR between the integrated *SceGFP* chromosomal locus and the episomal repair plasmid (gene targeting), (2) HR between the two sister chromatids, and (3) NHEJ (Figure 2A). Only gene targeting, but not NHEJ or HR between sister chromatids, reconstitutes a functional *GFP* gene and results in GFP-positive cells. The number of GFP-positive cells is determined by flow cytometry (Supplementary Figure S1).

If the hSMC5/6 complex is specifically required for HR between sister chromatids, downregulation of hSMC5/6 by RNAi should increase the frequency of gene targeting. We

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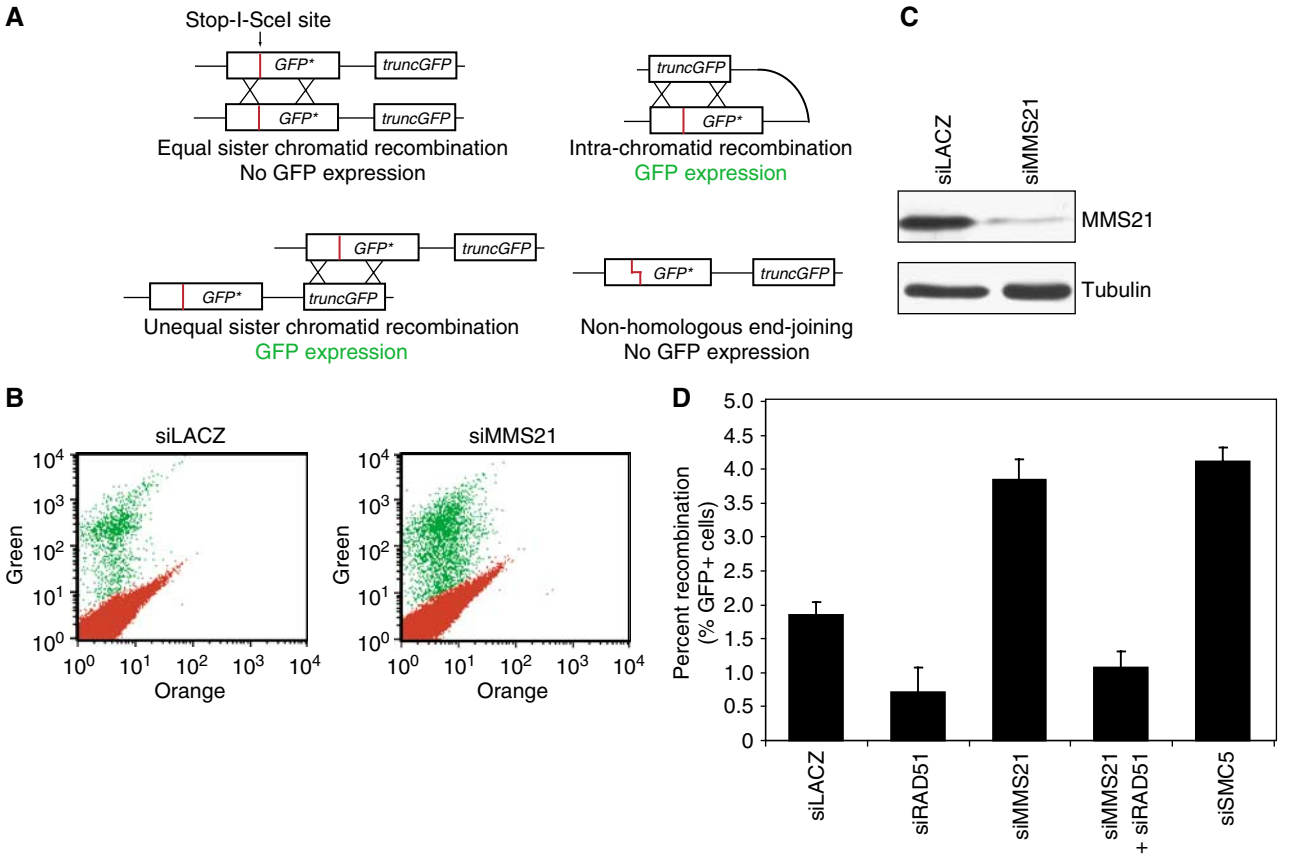

Figure 1 The hSMC5/6 complex is not required for all types of HR. (**A**) Schematic drawing of the HR assay. The direct repeat GFP (DR-GFP) repair substrate contains a mutated *GFP* gene with an in-frame stop codon and an I-SceI site inserted (shown as a red line) and a truncated *GFP* gene. The four major types of repair of the DSB produced by I-SceI that can occur in this system are shown. (**B**) 293/DR-GFP cells were transfected with an I-SceI-encoding plasmid along with the indicated siRNA oligonucleotides and analyzed 3 days later by flow cytometry. GFP-positive cells are gated and shown in green. Experiments are performed in triplicates. Only one set of representative samples is shown. (**C**) Western blotting showing the efficiency of siRNA knockdown 3 days after transfection in 293/DR-GFP cells. (**D**) Quantitation of the percentage of GFP-positive cells shown in (B). Results from three separate samples are averaged with the standard deviation indicated.

transfected 293/A658 cells with LACZ, RAD51, hMMS21, or hSMC5 siRNAs together with the I-SceI/repair plasmid. Depletion of these proteins was confirmed by Western blotting (Figure 2B and data not shown). As expected, RNAi against RAD51 decreased the frequency of gene targeting (episomal recombination) by 80% (Figure 2C). Interestingly, knockdown of either hMMS21 or hSMC5 by RNAi showed about a four-fold increase in the frequency of gene targeting (Figure 2C). RAD51-RNAi reversed the stimulatory effects of hMMS21-RNAi or hSMC5-RNAi (Figure 2C), indicating that the enhanced gene targeting is dependent on RAD51. We next restored expression of hMMS21 in hMMS21-RNAi cells by transfecting in an hMMS21 expression plasmid that contained silent mutations in the siRNA-targeting region. As expected, ectopic expression of hMMS21 in hMMS21-RNAi cells largely diminished the increase in gene targeting of hMMS21-RNAi cells (Figure 2D). This confirmed that the increase in gene targeting is due to the specific knockdown of hMMS21.

We next examined the efficiency of I-SceI to induce DSBs in control or hMMS21-RNAi cells by Southern blotting (Supplementary Figure S2) and ligation-mediated quantitative PCR (LM-QPCR; Figure 2E). Both assays suggested a slight decrease in the number of DSBs in the hMMS21-RNAi cells (Figure 2E and Supplementary Figure S2). Therefore, the increase in HR in cells depleted for hMMS21 or hSMC5 in the DR-GFP or gene targeting assays is not due to an increase in the number of DSBs in cells with defective hSMC5/6.

We next tested the effects on gene targeting by knocking down the SMC1/3 cohesin complex that had been shown to be specifically required for sister chromatid HR. Treating 293/A658 cells with hSMC1-RNAi or hSCC1-RNAi resulted in a four-fold increase in the percentage of GFP-positive cells after the expression of I-SceI (Figure 2F). This increase in gene targeting was inhibited by RAD51-RNAi (Figure 2F). As a control, RNAi against the condensin subunit, SMC2, did not significantly increase the percentage of GFP-positive cells (Figure 2F). To confirm that the increase in gene targeting by hMMS21-RNAi was not due to a decrease in cohesin protein levels, we examined the levels of hSCC1 in hMMS21-RNAi cells. As expected, hMMS21-RNAi did not affect hSCC1 or hRAD50 protein levels (Supplementary Figure S1). These results suggest that, like the SMC1/3 cohesin complex, hSMC5/6 may be specifically required for the repair of DSBs through sister chromatid HR.

The hSMC5/6 complex is not required for NHEJ

An alternative explanation for the increase in gene targeting is that knockdown of hSMC5/6 blocks another DSB repair pathway, such as NHEJ, thereby resulting in a general

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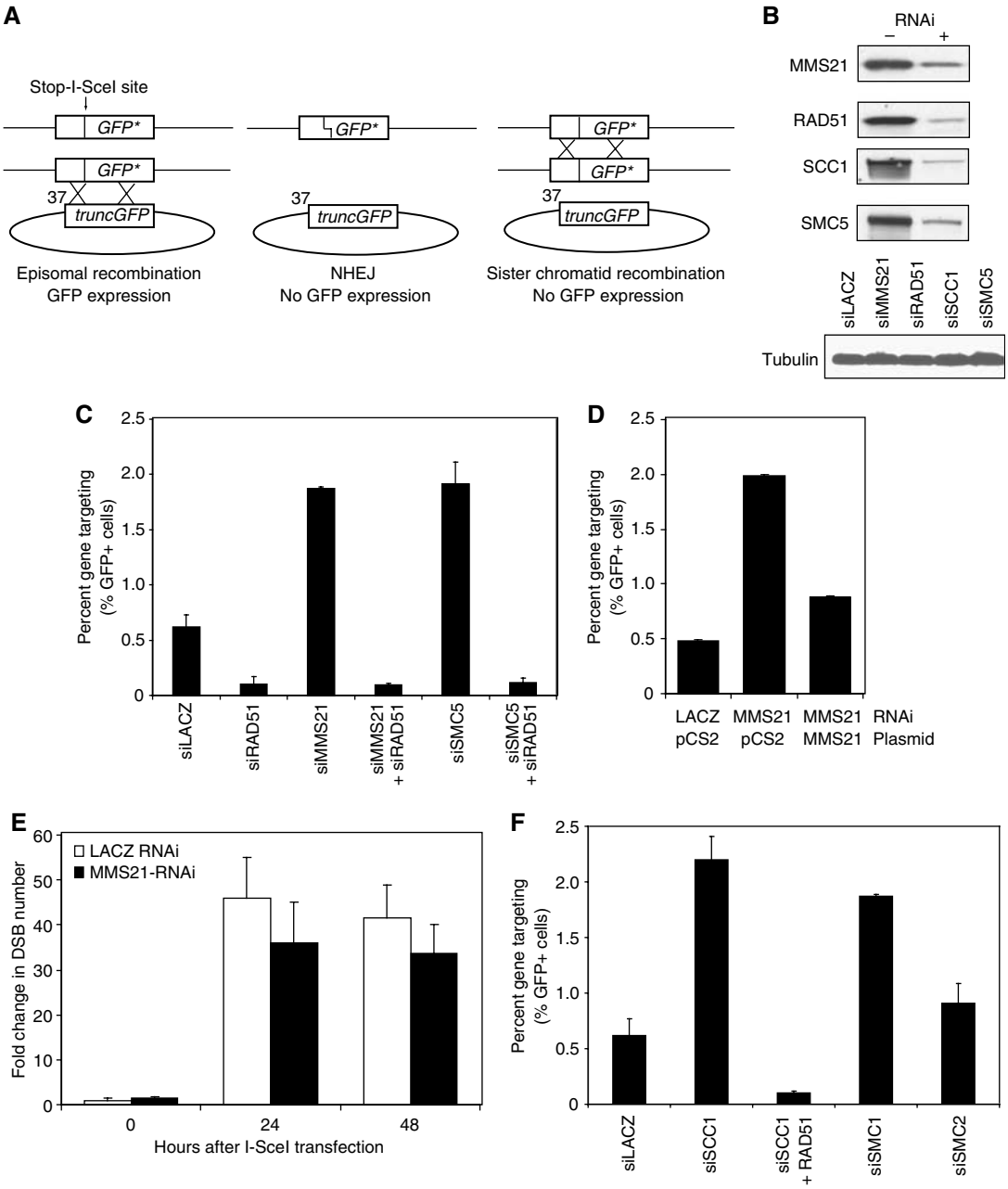

Figure 2 Downregulation of the SMC5/6 or cohesin complexes enhances gene targeting. (**A**) Schematic drawing of the *GFP* gene targeting assay. A *GFP* gene containing an insert with an in-frame stop codon and an I-SceI site is stably integrated into 293 cells. The stop-I-SceI site is indicated. Gene targeting is accomplished by expression of an I-SceI/repair plasmid that contains a truncated *GFP* (*truncGFP*) gene and an I-SceI expression cassette. Expression of I-SceI introduces a DSB at the stop-I-SceI site in the cells. The three major pathways that repair the I-SceI-induced DSB are shown. (**B**) Western blot analysis showing the efficiency of RNAi knockdown 3 days after transfection in 293/A658 cells. The siRNA and antibody used for Western blot are indicated to the left. Tubulin was used as the loading control. (**C**) 293/A658 cells were transfected with the repair plasmid along with the indicated siRNA oligonucleotides and analyzed 3 days later by flow cytometry. Quantitation of the percentage of GFP-positive cells is shown. Results from three separate samples are averaged with the standard deviations indicated. (**D**) 293/A658 cells were transfected with the indicated siRNA oligonucleotides and expression plasmids and experiments were carried out as described in (C). (**E**) 293/A658 cells were transfected with an I-SceI expression plasmid for the indicated times. Genomic DNA was collected and the number of DSBs was quantitated by ligation-mediated quantitative PCR (LM-QPCR). (**F**) 293/A658 cells were treated with the indicated siRNA oligonucleotides and experiments were carried out as described in (C).

increase in HR. To test whether hSMC5/6 is required for the repair of DSBs through NHEJ, we used an end-joining reporter cell line that measures the number of end-joining events. 293 cells (293/1040) were stably integrated with an end-joining substrate (1040) that contained a CMV/CBA promoter driving the expression of a *GFP* gene, which is flanked by I-SceI recognition sites (Figure 3A). The *CD8α* gene is downstream of the *GFP* gene and is not expressed because it lacks a promoter or an internal ribosome entry site (IRES). Therefore, cells are normally GFP positive and CD8 negative (GFP + CD8−). Upon the transfection of I-SceI, two DSBs will be generated, resulting in the deletion of *GFP*. Ligation of the two ends through NHEJ will result in CD8 expression (Figure 3A), which can be monitored with a fluorescent CD8

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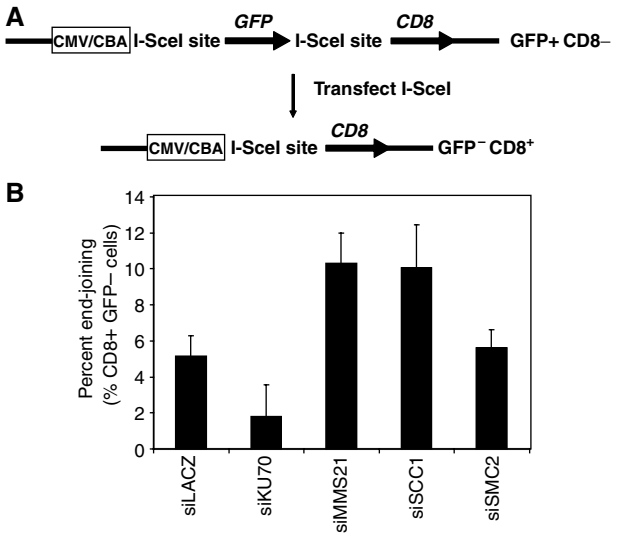

Figure 3 The hSMC5/6 complex is not required for end-joining. (**A**) Schematic drawing of the end-joining assay. 293/1040 cells containing the integrated substrate shown are GFP + CD8− before transfection. If end-joining occurs upon transfection of I-SceI, the cells will become GFP−CD8+ because the *GFP* gene will be lost and the *CD8* gene will be driven by the CMV/CBA promoter. (**B**) 293/1040 cells were transfected with the I-SceI plasmid and the indicated siRNA for 4 days. Quantitation of the percentage of CD8 + GFP− cells is shown. Results from three separate samples are averaged with the standard deviations indicated.

antibody. The rate of end-joining was determined by calculating the percentage of GFP−CD8+ cells.

Approximately 5% of the control LACZ-RNAi cells were GFP−CD8+ after I-SceI expression (Figure 3B). Knockdown of an NHEJ protein, KU70, resulted in an approximately 70% decrease in the number of GFP−CD8+ cells, validating the assay (Figure 3B). In contrast, hMMS21-RNAi increased the number of GFP−CD8+ cells by about two-fold (Figure 3B). Similarly, hSCC1-RNAi resulted in a two-fold increase in end-joining (Figure 3B). Knockdown of hSMC2 did not affect the end-joining efficiency. These findings indicate that the hSMC5/6 and hSMC1/3 cohesin complexes are not required for NHEJ. The increase in end-joining upon depletion of hSMC5/6 or cohesin complexes is consistent with a shift in the choice of DSB-repair pathway from sister chromatid HR to end-joining. Therefore, the increase in gene targeting in hSMC5- and hMMS21-RNAi cells is most likely due to a blockade of sister chromatid HR.

The hSMC5/6 complex is required for sister chromatid HR

To directly examine the function of the hSMC5/6 complex in the repair of DSBs by sister chromatid HR, we tested whether it is required for sister chromatid exchange (SCE), a form of sister chromatid HR that requires a crossing over event during the resolution of double Holliday junctions. SCE can be monitored by the incorporation of the bulkier thymidine analog, bromo-deoxyuridine (BrdU), for two cell cycles, resulting in sister chromatids with either one or both of its DNA strands containing BrdU (Wolff, 1977). This asymmetric labeling can be visualized by using a DNA-intercalating dye, acridine orange, which is excluded from chromatids with both strands incorporated with BrdU. HR events will result in sister chromatids that show a gap in staining on one chromatid with a gain of staining in the corresponding region of its sister chromatid (Figure 4A).

We first measured the number of spontaneous SCEs per metaphase in cells treated with either LACZ, hMMS21, or hSCC1 siRNAs. hMMS21- and hSCC1-RNAi cells contained about half the number of SCEs per metaphase than LACZ-RNAi cells (Supplementary Figure S3). The low level of spontaneous SCEs during a normal cell cycle can be greatly stimulated by the addition of the topoisomerase inhibitor, camptothecin, during BrdU labeling (Degrassi *et al*, 1989). Similar to spontaneous SCEs, hMMS21-RNAi and hSCC1-RNAi cells contained approximately half the number of camptothecin-induced SCEs per metaphase than LACZ-RNAi cells, 20.80±3.52 and 18.05±3.23 versus 38.66±4.17, respectively (Figure 4A and B).

We next used a previously described reporter assay to measure long tract gene conversion sister chromatid recombination (LTGC/SCR) (Johnson and Jasin, 2000; Puget *et al*, 2005). The HR reporter in this assay is integrated into U2OS cells and contains a *GFP* gene with a stop-I-SceI site and an upstream truncated *GFP* gene as the recombination substrate upon I-SceI-induced DSB (Figure 4C). In addition, the LTGC/SCR reporter contains two halves of the *BsdR* gene that confers resistance to blasticidin. Cells that are not transfected with I-SceI are sensitive to blasticidin, as the two halves of the *BsdR* gene are in the wrong orientation and do not produce a functional protein. Upon I-SceI-induced unequal LTGC/SCR, the *BsdR* gene is duplicated. The presence of splice donor and acceptor sites then allows splicing to reconstitute a functional *BsdR* gene. The frequency of LTGC/SCR can be measured by counting the number of blasticidin-resistant clones.

We observed a 75% reduction in the number of blasticidin-resistant cells after treatment with hMMS21-RNAi as compared to LACZ-RNAi, 0.25%±0.04 and 1.02%±0.08, respectively (Figure 4D, red bars, and Supplementary Figure S3). The percentage of blasticidin-resistant cells was normalized to the plating and transfection efficiencies of these cells in the absence of blasticidin. This decrease in LTGC/SCR is similar to that observed for H2AX−/− ES cells (Xie *et al*, 2004). We also measured the frequency of intra-chromatid HR and short tract, unequal HR by examining the percentage of GFP-positive cells after I-SceI transfection in either control or hMMS21-RNAi cells. We observed a two-fold increase in the number of GFP-positive cells in hMMS21-RNAi cells (Figure 4D, green bars). This is consistent with the two-fold increase in the number of GFP-positive cells observed in hMMS21-RNAi cells using the DR-GFP reporter in 293 cells (Figure 1). In contrast, the number of GFP-positive cells decreased in H2AX−/− ES cells (Xie *et al*, 2004). This is consistent with the notion that H2AX is required for all forms of HR whereas the SMC5/6 complex is only required for sister chromatid HR.

We observe a significant decrease in both SCE (that measures sister chromatid recombination involving crossing over) and LTGC (that measures unequal sister chromatid recombination) in hMMS21-RNAi cells. These results indicate that the hSMC5/6 complex is specifically required for sister chromatid HR, but not intra-chromatid HR. Thus, the enhanced episomal HR (gene targeting), intra-chromatid HR, and end-joining upon depletion of subunits of the hSMC5/6

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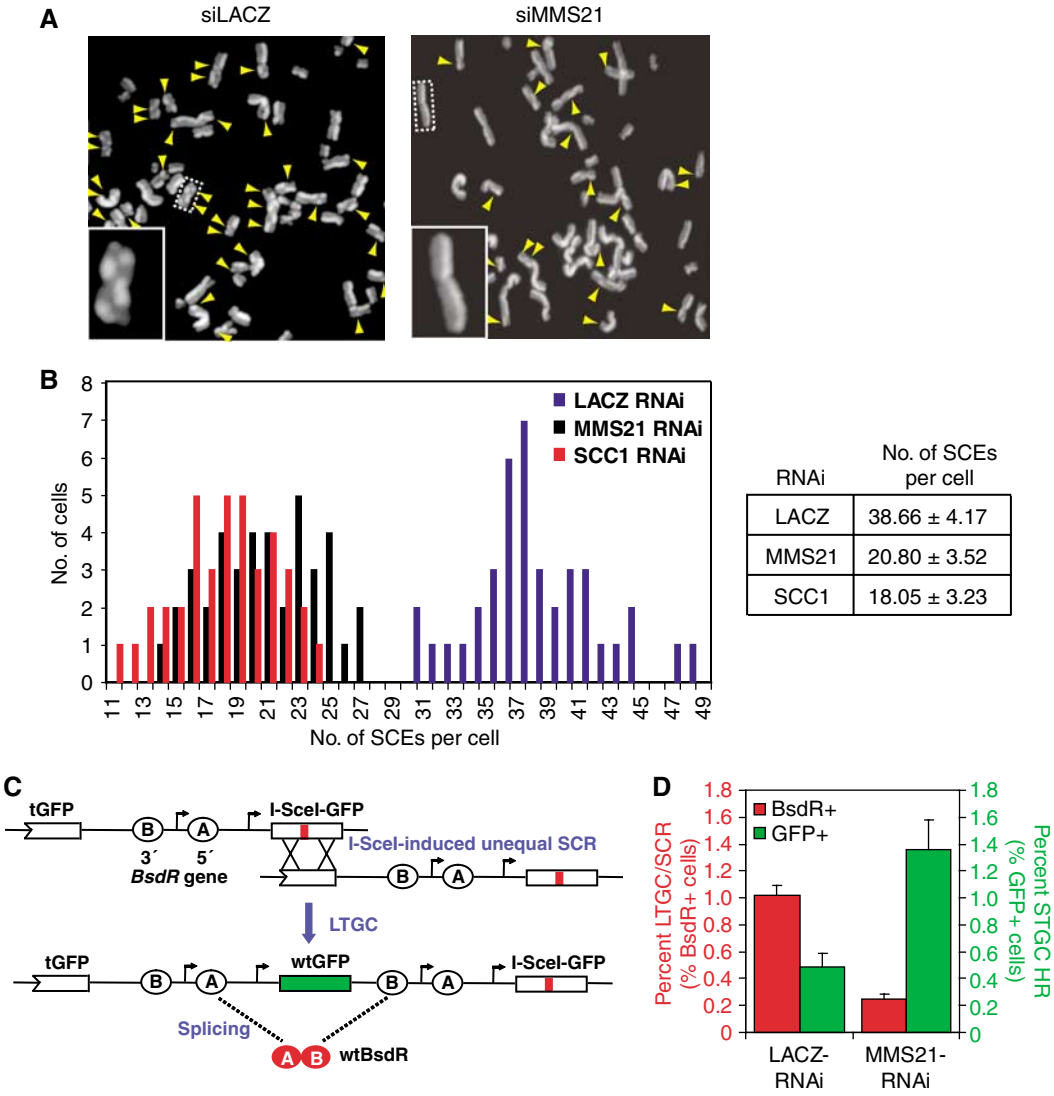

RNAi	No. of SCEs per cell
LACZ	38.66 ± 4.17
MMS21	20.80 ± 3.52
SCC1	18.05 ± 3.23

Figure 4 The hSMC5/6 complex is required for sister chromatid HR. (**A**) 293/A658 cells transfected with the indicated siRNA for 24 h were labeled with 10 μM BrdU in the presence of 2.5 μM camptothecin for 40 h (two cell divisions). Chromosome spreads were stained with acridine orange to distinguish sister chromosomes. Arrowheads indicate recombination events. (**B**) Histogram of cells in (A) with the indicated number of sister chromatid exchange events per cell. Results from three separate samples are averaged with the standard deviations indicated in the table to the right. (**C**) Schematic illustration of the HR substrate integrated into U2OS cells. A stop-I-SceI containing *GFP* gene is located downstream of a truncated *GFP*. Two halves of the *BsdR* gene (A, B) that confers blasticidin resistance is present in between the two *GFP* genes. Upon I-SceI transfection, a DSB is produced that can be repaired by unequal sister chromatid HR. If long-tract gene conversion (LTGC) occurs, the *BsdR* genes will be duplicated, therefore allowing the production of a functional *BsdR* gene by splicing, owing to the presence of splice donor and acceptor sites. Cells that have undergone LTGC/SCR are selected by blasticidin. (**D**) U2OS/SCR cells were transfected with the indicated siRNAs for 24 h followed by transfection of an I-SceI expression plasmid for an additional 24 h. For measuring LTGC/SCR (red bars), cells were replated and those that had undergone LTGC/SCR events were selected by 5 μg/ml blasticidin for 14 days. Colonies were fixed and counted. For measuring short tract gene conversion (STGC; green bars), cells were analyzed by flow cytometry for GFP-positive cells 4 days after I-SceI transfection. Results from two separate experiments are averaged with the standard deviations indicated.

complex is due to the impaired ability of these cells to repair I-SceI-induced DSBs by sister chromatid HR.

The hSMC5/6 complex is only required for DNA damage repair in cells after DNA replication

One prediction of the results described above is that the hSMC5/6 complex should only be required for the repair of damaged DNA in cells that contain sister chromatids. To test this hypothesis, we determined the ability of hMMS21-RNAi cells to repair damaged DNA when either arrested at the G1/S-boundary (without sister chromatids) or cells that had progressed through S phase (with sister chromatids). HeLa S3 cells treated with mock, hMMS21, or KU70 siRNAs for 36 h were arrested at G1/S-boundary by thymidine for 16 h. One group of cells was then released into S phase to allow DNA replication for 2 h. Both groups of cells were then treated with 0.015% methyl-methane sulfate (MMS) for 1 h to induce DNA damage. The cells were then allowed to recover in the absence of MMS for 3 h, either still arrested at G1/S-boundary or released (Figure 5A). Cells were analyzed by flow cytometry to confirm their appropriate cell cycle stages (Supplementary Figure S4). The amount of unrepaired DNA damage was measured by the comet assay (Figure 5B) (Collins, 2004).

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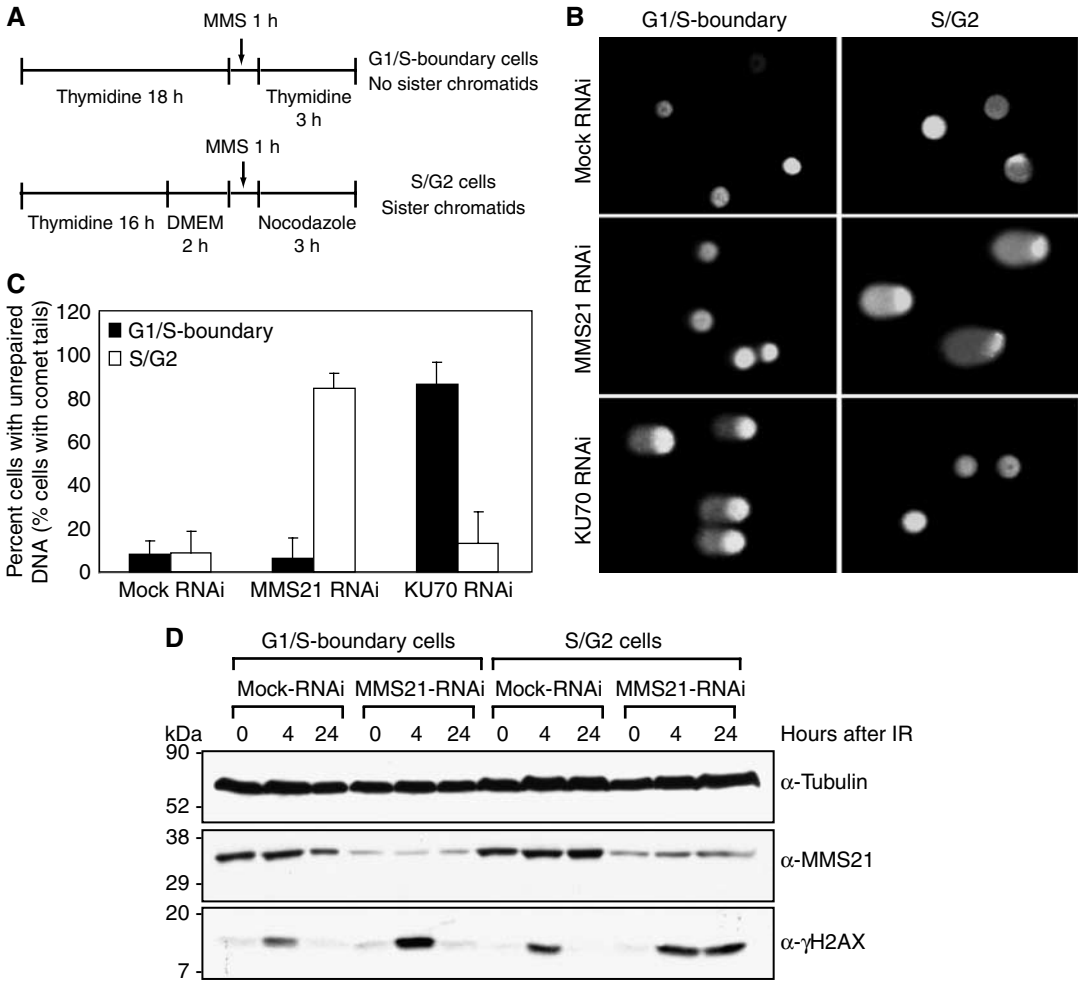

Figure 5 The hSMC5/6 complex is only required for efficient repair of damaged DNA in cells with sister chromatids. (**A**) Timeline of the experimental design. Cells were transfected with the indicated siRNA for 36 h before treatment with 2 mM thymidine as shown. One half of the cells were released from G1 and, 2 h later, both released and G1-arrested cells were treated for 1 h with 0.015% MMS. Cells were then allowed to recover for 3 h either arrested in G1 with thymidine or in S/G2/M with 0.1 μg/ml nocadozole. The presence of unrepaired damaged DNA was then measured by the comet assay. (**B**) Cells described in (A) were analyzed for the presence of unrepaired damaged DNA by the comet assay. SYBR green staining of DNA shows comet tails migrating out of the nucleus. Images are a representative sample of multiple experiments. (**C**) Quantitation of the percentage of cells shown in (B) with comet tails, representing unrepaired damaged DNA. Results from three separate samples are averaged with the standard deviations indicated. (**D**) Mock-RNAi or hMMS21-RNAi cells arrested at the G1/S-boundary or released into S phase were treated with 10 Gy IR. Cell lysates were collected at 0, 4, and 24 h after IR. Western blots are shown for Tubulin (loading control), MMS21 (RNAi efficiency), and γH2AX (unrepaired DNA damage).

Cells treated with mock siRNA were competent to repair the MMS-induced damaged DNA in the presence (S/G2) or absence (G1/S-boundary) of sister chromatids (Figure 5B and C). As predicted, hMMS21-RNAi cells were capable of efficient repair of damaged DNA in cells with no sister chromatids (G1/S-boundary), but not in cells that contained sister chromatids (S/G2), 6.6% ± 9.1 and 85.3% ± 6.3 cells with comet tails, respectively (Figure 5B and C). To ensure that both G1/S-boundary and S/G2 cells were equally susceptible to MMS-induced DNA damage, we treated cells with MMS for 1 h without allowing additional time for repair of the damaged DNA. Approximately 95% of both G1/S-boundary and S/G2 cells displayed comet tails after MMS treatment (data not shown), confirming that both G1/S-boundary and S/G2 cells are susceptible to MMS-induced DNA damage. Contrary to hMMS21-RNAi cells, KU70-RNAi cells were competent to repair MMS-induced damaged DNA in S/G2 cells, but were incapable of repairing the damaged DNA in G1/S-boundary cells (Figure 5B and C). These results suggest that hMMS21 is specifically required for the repair of MMS-induced DNA damage in S/G2 cells, but not in G1/S-boundary cells.

We next determined whether hMMS21 is required for the repair of IR-induced DSBs in S/G2 cells. Mock-RNAi or hMMS21-RNAi cells arrested at G1/S-boundary or released into S phase were treated with 10 Gy of IR to induce DSBs. Cell lysates were collected at 0, 4, and 24 h after irradiation and the repair efficiency of the cells was measured. Because H2AX phosphorylation is dramatically induced by IR-induced DSBs and its dephosphorylation is coupled with repair of those DSBs (Rothkamm *et al*, 2003), the amount of γH2AX (phosphorylated H2AX) provides a measure of unrepaired DSBs. hMMS21-RNAi cells were competent to repair IR-induced DSBs in G1/S-boundary cells, but not in S/G2 cells (Figure 5D). These results suggest that hMMS21 is specifically required for the repair of damaged DNA in cells that utilize

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sister chromatid HR (S/G2 cells), but not in cells that primarily rely on NHEJ for repair (G1/S-boundary cells).

These results demonstrate the preference for the use of NHEJ in G1/S-boundary cells and the preference for sister chromatid HR in S/G2 cells as reported previously (Rothkamm *et al*, 2003). We have shown that gene targeting is increased in cells arrested at S/G2, but decreased in cells treated with aphidicolin (Supplementary Figure S4). To confirm that the increase in gene targeting in the hMMS21-RNAi cells (Figure 2) is not merely a result of cell cycle arrest at S/G2, we determined the cell cycle profile of hMMS21-RNAi cells by flow cytometry. We observed no significant differences in the cell cycle profile of asynchronous cultures of HeLa cells treated with either hMMS21 siRNA or mock siRNA for 48 h (Supplementary Figure S4). Additionally, we determined the long-term viability and proliferative capacity of hMMS21-RNAi cells by colony formation assays. We observed no significant differences in the ability of hMMS21-RNAi cells to form colonies as compared to LACZ-RNAi cells, 3.4 ± 1.4 and $3.1 \pm 1.1\%$, respectively (data not shown). Therefore, the increased gene targeting efficiency in hMMS21-RNAi cells and their decreased ability to repair DNA damage in S/G2 is due to a defect in sister chromatid HR in these cells, rather than a secondary effect of an arrest at S/G2.

The hSMC5/6 complex is recruited to I-SceI-induced DSBs

Many proteins involved in the repair of DSBs, such as ATM, RAD51, MRE11/RAD50/NBS1 (MRN), and cohesin complexes, are recruited to DSBs to facilitate repair in functionally distinct ways (Lisby and Rothstein, 2005). To investigate whether the hSMC5/6 complex is also recruited to DSBs, we used our gene targeting cell line (293/A658) that contained an I-SceI endonuclease recognition site in the *GFP* gene. Upon transfecting I-SceI, we monitored the recruitment of proteins to a single DSB by performing chromatin immunoprecipitation (ChIP) using primers directed toward *GFP*. The hSMC5/6 complex was recruited to I-SceI-induced DSBs in cells transfected with I-SceI (Figure 6A). In cells transfected with a mock expression plasmid, no hSMC5/6 was present at the *GFP* locus, showing that these proteins were not constantly associated with the locus (Figure 6A). Expectedly, the cohesin complex (hSCC1) and γH2AX were present at the I-SceI-induced DSBs (Figure 6A). Importantly, this recruitment was specific to the DSB locus (*GFP*), as they were not recruited to other genomic loci, such as *GAPDH* (Figure 6A). The *GFP* PCR product was specific to the DSB, as 293 cells not integrated with the *GFP* construct did not produce a PCR product (Supplementary Figure S5). Our PCR analysis of the DSB was in the linear range and the chromatin from these cells was sheared to a proper size of 300–1000 bp (Supplementary Figure S5). To examine the fold-enrichment of the hSMC5/6 complex at I-SceI-induced DSBs, we performed quantitative, real-time PCR (QPCR). Using two different validated primer sets, we observed a 15–20-fold enrichment of hMMS21, hSMC5, hSCC1, and γH2AX at the DSB (Figure 6B and Supplementary Figure S5), comparable to the up to 16-fold enrichment of RAD51 at I-SceI-induced DSBs (Rodrigue *et al*, 2006). These data show that the hSMC5/6 complex is recruited to DSBs.

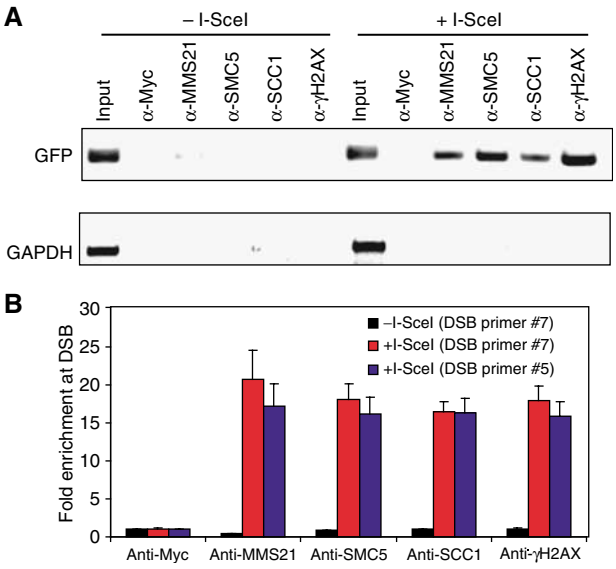

Figure 6 The hSMC5/6 complex is recruited to DSBs. (**A**) 293/A658 cells with integrated *GFP* gene containing a stop-I-SceI site were transfected with either control or I-SceI expression plasmids. ChIP were performed 24 h later with the indicated antibodies. The immunoprecipitated DNA was then analyzed by PCR using control primers (*GAPDH*) and primers near the DSB (*GFP*). Images are a representative sample of multiple experiments. (**B**) QPCR analysis of the ChIP samples described in (A). The average fold enrichment of proteins at DSBs (*GFP* normalized to the control locus *GAPDH*) represents enrichment compared to the anti-MYC control ChIP. Results from two separate samples using *GFP* primers #7 or #5 (+I-SceI only) performed in triplicates are averaged with the standard deviations indicated.

The hSMC5/6 complex is required for the recruitment of the cohesin complex to DSBs

We tested whether depletion of both the hSMC5/6 and SMC1/3 complexes had a synergistic effect in the SCE assay. Western blotting confirmed efficient depletion of hMMS21 and hSCC1 together or alone (data not shown). Knockdown of both complexes by siRNAs towards hMMS21 and hSCC1 did not further decrease the number of SCEs compared to knockdown of either one alone (Figure 7A). This suggested that the hSMC5/6 and cohesin complexes may function in a common pathway to promote sister chromatid HR.

As both complexes are recruited to DSBs, we tested whether one complex is required for the recruitment of the other to DSBs by ChIP. Although hSCC1 was significantly depleted from the DSBs, the accumulation of hMMS21 at DSBs was only slightly reduced (Figure 7B). Thus, recruitment of the hSMC5/6 complex to DSBs appears to be independent of cohesin. Remarkably, knockdown of the hSMC5/6 complex blocked the recruitment of hSCC1 to DSBs, although the phosphorylation of γH2AX was not altered (Figure 7C). As revealed by QPCR analysis, there was a severe defect in the recruitment of either hSMC1 or hSCC1 to the DSB in both hMMS21- or hSMC5-RNAi cells (Figure 7D). To determine whether this requirement for hSMC5/6 in cohesin recruitment was specific for the DSB, we examined the effects of hSMC5/6 depletion on cohesin loading at another genomic locus, AluSx on the X-chromosome (Hakimi *et al*, 2002). We measured the fold-enrichment of both hSCC1 and hSMC1 at this locus in LACZ-, hMMS21-,

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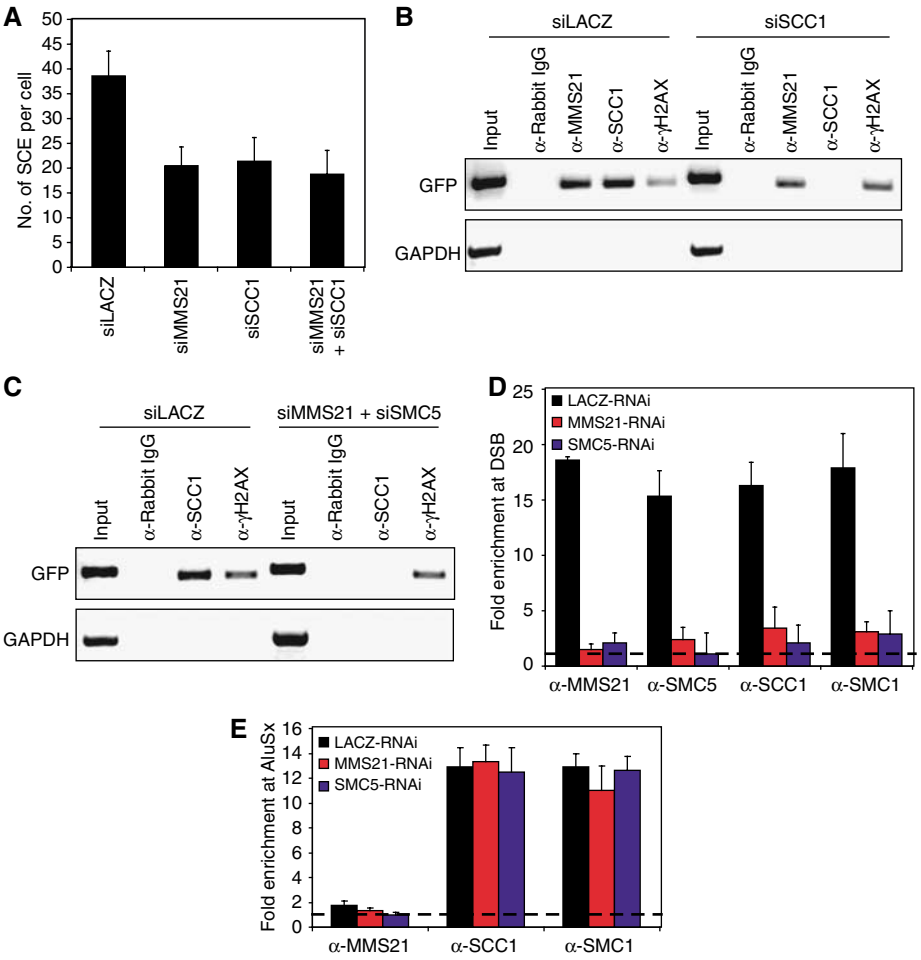

Figure 7 Recruitment of cohesin to DSBs requires the hSMC5/6 complex. (**A**) Knockdown of both hMMS21 and hSCC1 does not further decrease the number of SCEs per cell as compared to knocking down either one alone. The number of SCEs per cell was determined as described in Figure 4A. Results from three separate samples are averaged with the standard deviations indicated. (**B**) ChIP was performed as described in Figure 6A except that 293/A658 cells had been transfected with control LACZ siRNA or SCC1 siRNA for 24 h before transfection of an I-SceI expression plasmid. Images are a representative sample of multiple experiments. (**C**) ChIP was performed on 293/A658 cells that had been transfected with control LACZ siRNA or MMS21 and SMC5 siRNA for 24 h before transfection of an I-SceI expression plasmid. Images are a representative sample of multiple experiments. (**D**) ChIP was performed as described in (C) with the indicated siRNAs and antibodies. QPCR was performed using primers toward the DSB. The dashed line indicates the background from rabbit IgG ChIP. Results are shown as the average fold enrichment with the s.d. indicated. (**E**) ChIP was performed as described in (C) with the indicated siRNAs and antibodies. QPCR was performed using primers toward the AluSx genomic locus. The dashed line indicates the background from rabbit IgG ChIP. Results are shown as the average fold enrichment with the s.d. indicated.

or hSMC5-RNAi cells (Figure 7E). Depletion of either hSMC5 or hMMS21 did not affect the loading of either hSMC1 or hSCC1 at this genomic locus. These results suggest that the hSMC5/6 complex is not essential for cohesin loading throughout the genome. Instead, it is specifically required at DSBs. Thus, one mechanism by which the hSMC5/6 complex facilitates sister chromatid HR is to promote the recruitment of cohesin to DSBs.

Discussion

Role of SMC5/6 in sister chromatid HR

Studies in yeast, plants, and humans (this study) support a role of the SMC5/6 complex in DNA repair by promoting HR between sister chromatids. Both cohesin and condensin are also required for efficient DNA repair (Hagstrom and Meyer, 2003). It has been suggested that cohesin promotes DNA repair by maintaining the close proximity of sister chromatids at damage sites, thus facilitating homology-directed DNA repair using the opposing sister chromatid as template (Jessberger, 2002; Hagstrom and Meyer, 2003; Lehmann, 2005).

Cohesin loaded during S phase is insufficient to maintain sister chromatid cohesion at DSBs to facilitate sister chromatid HR (Strom *et al*, 2004). It is well established that cohesin is recruited *de novo* to DSBs, which requires the MRN complex, γH2AX, and the SCC2/4 complex (Kim *et al*, 2002; Strom *et al*, 2004; Unal *et al*, 2004). In this study, we show that an additional level of regulation exists, at least in human cells, in the recruitment of cohesin to DSBs. The hSMC5/6 complex is necessary for cohesin recruitment to DSBs, whereas cohesin is not required for the recruitment of the hSMC5/6 complex to DSBs. These results are consistent with the following model (Figure 8). In this model, the SMC5/6 complex localizes to sites of DNA damage and promotes the recruitment of cohesin to the DSB. This

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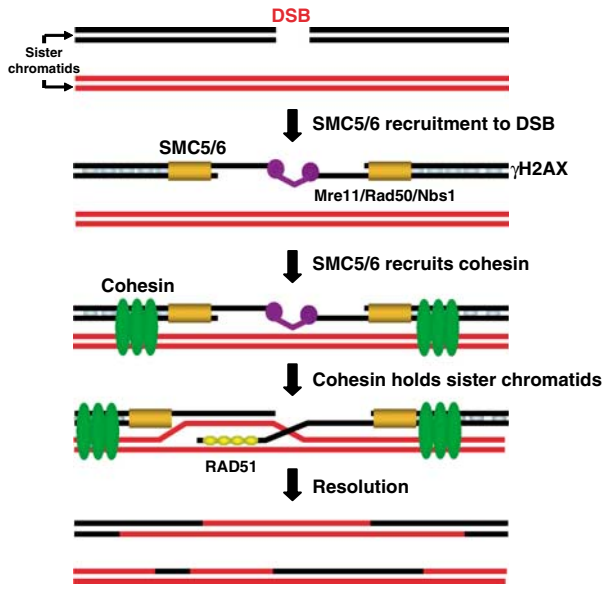

Figure 8 Model for hSMC5/6 function in sister chromatid HR. hSMC5/6 is recruited to DSBs where it functions to recruit the cohesin complex. The postreplicative loading of cohesin at the DSB brings sister chromatids in close proximity to allow strand invasion and exchange during sister chromatid HR. The MRN complex has been speculated to hold the free ends of the DSB together to facilitate repair.

SMC5/6-facilitated recruitment of cohesin to the DSB holds the sister chromatids in close proximity to permit RAD51-dependent strand invasion and exchange using the sister chromatid as the repair template.

The exact mechanism by which the SMC5/6 complex recruits the cohesin complex to DSBs is unclear. We have so far failed to detect a direct physical interaction between the SMC5/6 and cohesin complexes (data not shown). However, hMMS21 stimulates the sumoylation of two cohesin complex subunits, hSCC1 and hSA2, in cells (Supplementary Figure S6). The functional significance of hMMS21-induced sumoylation of cohesin remains to be determined. It will be interesting to test whether and how sumoylation of the cohesin complex regulates its recruitment and loading onto chromatin around a DSB.

The cohesin and condensin complexes form a ring-shaped structure that has been proposed to trap chromatin inside (Nasmyth and Haering, 2005). Based on the homology of SMC5/6 complex with the cohesin and condensin complexes, it is plausible that the SMC5/6 complex also forms a ring structure and holds DNA (Nasmyth and Haering, 2005). An alternative and not mutually exclusive mechanism by which the SMC5/6 complex promotes sister chromatid HR is that both the SMC5/6 and SMC1/3 complexes directly hold sister chromatids together in a similar manner as the cohesin complex in a ring-shaped structure. Additionally, a third related complex, the MRN complex, exists at DSBs and is proposed to hold the broken ends of the DSB together. It will be interesting to examine the interplay between these three complexes.

Sister chromatid HR and gene targeting

Gene targeting can be used to correct genetic mutations in human somatic cells. However, its experimental and therapeutic applications have been hindered by the low rate of spontaneous gene targeting in these cells (Porteus and Carroll, 2005). Intense effort has been focused on developing ways to increase the rate of gene targeting. It has been shown that gene targeting can be greatly enhanced by introducing DSBs in the target gene, therefore promoting HR (Jasin, 1996). To create sequence-specific DSBs within the human genome, we and others have developed zinc-finger nucleases that contain both zinc-finger DNA-binding domains and endonuclease domains (Porteus and Baltimore, 2003). Another obstacle to achieving efficient gene targeting is the existence of multiple pathways of HR. Even after the introduction of DSBs in the target gene, these DSBs can be readily repaired through HR with the opposing sister chromatid as the template, instead of the episomal repair plasmid, thus minimizing the rate of gene targeting. This effect may be quite substantial, as it is likely that most DSBs repaired by HR use the sister chromatid as a template.

We show that inactivation of the human SMC5/6 complex increases the efficiency of gene targeting by about four-fold. This increase in episomal recombination is due to a specific decrease in the ability of the cells to undergo sister chromatid HR, as shown directly by the SCE and LTGC/SCR assays. In addition, we show that the increase in gene targeting and the decrease in SCE in cells with a compromised hSMC5/6 function are similarly observed in cells depleted of the SMC1/3 cohesin complex that is implicated in sister chromatid HR. Furthermore, the increase in gene targeting upon inhibition of the SMC5/6 complex is not due to a defect in NHEJ. These results support the model that inhibition of the SMC5/6 or cohesin complexes reduces sister chromatid HR, therefore shifting the DSB repair pathway to NHEJ or HR with an episomal template (gene targeting). Our results further suggest that other strategies aimed at blocking HR between sister chromatids are expected to improve the efficiency of gene targeting. The challenge is to inhibit sister chromatid HR transiently without disturbing overall sister chromatid cohesion that can lead to genomic instability. This may ultimately lead to new strategies for genomic manipulations in mammalian somatic cells for both research and therapeutic purposes.

In summary, our results suggest that the SMC5/6 complex promotes DSB repair specifically through sister chromatid HR by facilitating the recruitment of cohesin to DSBs. Our study also identifies a novel strategy to improve the rate of gene targeting by blocking sister chromatid HR.

Materials and methods

Cell culture, transfections, and siRNAs

HeLa S3, HeLa Tet-On, 293/DR-GFP, 293/A658, U20S/LTGC, and 293/1040 cells were grown in DMEM (Invitrogen) supplemented with 10% fetal bovine serum (Invitrogen), and 100 μg/ml penicillin and streptomycin (Invitrogen). At 40–50% confluency, plasmid or siRNA transfection was performed using the Effectene reagent (Qiagen) or the Oligofectamine reagent (Invitrogen), respectively, as per the manufacturer's protocols. 293 cells were transfected at 80–90% confluency with Lipofectamine 2000 (Invitrogen) when introducing both siRNA and plasmids for gene targeting, DR-GFP HR, and end-joining assays. The siRNA oligonucleotides against hMMS21, hSMC5, hSMC1, hSMC2, and hSCC1 were chemically synthesized at an in-house facility or ordered from Dharmacon. The sequences of these oligonucleotides are listed in Supplementary Table S1. The siRNA oligonucleotides against RAD51, KU70, and LACZ were obtained from the Dharmacon SMARTpool service. The

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annealing and transfection of the siRNAs were performed as previously described (Elbashir *et al*, 2001).

Immunoblotting

For determining efficiency of RNAi knockdown, 293 cells were lysed in SDS sample buffer, sonicated, boiled, separated by SDS–PAGE, and blotted with the indicated antibodies 2–3 days post-transfection of the desired siRNAs. Horseradish peroxidase-conjugated goat anti-rabbit or goat anti-mouse IgG (Amersham Biosciences) were used as secondary antibodies, and immunoblots were developed using the ECL reagent (Amersham Biosciences) as per the manufacturer's protocols. The commercial antibodies used in this study are as follows: anti-Myc (Roche, 11667203001, 1 μg/ml), anti-RAD51 (Santa Cruz Biotechnology, sc-8349, 1:500), anti-SCC1 (Oncogene, NA-80, 1:250; Bethyl Laboratory, A300, 1:500), anti-CD8-PE (Diatec, 3032, 1:15), anti-γH2AX (Upstate, 07–164 and 05–636, 1:1000). The production of polyclonal anti-hMMS21 has been described previously (Potts and Yu, 2005). Anti-SMC5 was kindly provided by A Lehmann (University of Sussex, UK).

DR-GFP and gene targeting HR assays

HR assays were performed as described previously (Pierce *et al*, 1999). Gene targeting assays were performed as described previously (Porteus and Baltimore, 2003). See Supplementary data for details.

End-joining assay

We used a 293 cell line (293/1040) stably expressing an end-joining reporter (Figure 3A) that contained a *GFP* gene flanked by I-SceI recognition sites driven by a CMV/CBA promoter for assaying end-joining. A *CD8*α gene was located downstream of the *GFP* gene and was not constitutively expressed due to the lack of an IRES. 293/1040 cells were transfected with the indicated siRNAs and either an I-SceI expression plasmid or an RFP expression plasmid as a transfection efficiency control. If I-SceI cuts both sites flanking *GFP* and end-joining occurs, GFP expression would be lost and CD8 expression would be gained. CD8 expression was measured by staining with phycoerytherin-conjugated anti-CD8 monoclonal antibody (Ditech). Cells were analyzed for the loss of GFP expression and gain of CD8 expression by FACS 3–5 days after transfection. The end-joining rate was determined by counting the percentage of GFP$-$CD8$+$ cells and normalizing to the transfection efficiency.

LTGC/SCR and SCE assays

LTGC/SCR assays using the U2OS/SCR cells (kindly provided by Dr Ralph Scully, Boston, MA) were essentially described previously (Puget *et al*, 2005). See Supplementary data for details. The SCE assay is also described in Supplementary data.

Comet and ChIP assays

Comet assays were performed according to the manufacturer's protocol (Trevigen) and as previously described (Potts and Yu, 2005). ChIP was performed as described previously (Aparicio *et al*, 2005). See Supplementary data for details of the comet and ChIP assays.

Supplementary data

Supplementary data are available at *The EMBO Journal* Online.

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References

Andrews EA, Palecek J, Sergeant J, Taylor E, Lehmann AR, Watts FZ (2005) Nse2, a component of the Smc5–6 complex, is a SUMO ligase required for the response to DNA damage. *Mol Cell Biol* **25:** 185–196

Aparicio O, Geisbert JV, Sekinger E, Yang A, Moqtaderi Z, Struhl K (2005) *Current Protocols in Molecular Biology*. Wiley, New York, NY, pp. 21.3.1–21.3.33

Birkenbihl RP, Subramani S (1992) Cloning and characterization of rad21 an essential gene of *Schizosaccharomyces pombe* involved in DNA double-strand-break repair. *Nucleic Acids Res* **20:** 6605–6611

Collins AR (2004) The comet assay for DNA damage and repair: principles, applications, and limitations. *Mol Biotechnol* **26:** 249–261

Degrassi F, De Salvia R, Tanzarella C, Palitti F (1989) Induction of chromosomal aberrations and SCE by camptothecin, an inhibitor of mammalian topoisomerase I. *Mutat Res* **211:** 125–130

Elbashir SM, Harborth J, Lendeckel W, Yalcin A, Weber K, Tuschl T (2001) Duplexes of 21-nucleotide RNAs mediate RNA interference in cultured mammalian cells. *Nature* **411:** 494–498

Fousteri MI, Lehmann AR (2000) A novel SMC protein complex in *Schizosaccharomyces pombe* contains the Rad18 DNA repair protein. *EMBO J* **19:** 1691–1702

Fujioka Y, Kimata Y, Nomaguchi K, Watanabe K, Kohno K (2002) Identification of a novel non-structural maintenance of chromosomes (SMC) component of the SMC5–SMC6 complex involved in DNA repair. *J Biol Chem* **277:** 21585–21591

Haering CH, Lowe J, Hochwagen A, Nasmyth K (2002) Molecular architecture of SMC proteins and the yeast cohesin complex. *Mol Cell* **9:** 773–788

Hagstrom KA, Meyer BJ (2003) Condensin and cohesin: more than chromosome compactor and glue. *Nat Rev Genet* **4:** 520–534

Hakimi MA, Bochar DA, Schmiesing JA, Dong Y, Barak OG, Speicher DW, Yokomori K, Shiekhattar R (2002) A chromatin remodelling complex that loads cohesin onto human chromosomes. *Nature* **418:** 994–998

Harvey SH, Sheedy DM, Cuddihy AR, O'Connell MJ (2004) Coordination of DNA damage responses via the Smc5/Smc6 complex. *Mol Cell Biol* **24:** 662–674

Hirano T (2002) The ABCs of SMC proteins: two-armed ATPases for chromosome condensation, cohesion, and repair. *Genes Dev* **16:** 399–414

Hirano T (2005a) Condensins: organizing and segregating the genome. *Curr Biol* **15:** R265–275

Hirano T (2005b) SMC proteins and chromosome mechanics: from bacteria to humans. *Philos Trans R Soc Lond B Biol Sci* **360:** 507–514

Hu B, Liao C, Millson SH, Mollapour M, Prodromou C, Pearl LH, Piper PW, Panaretou B (2005) Qri2/Nse4, a component of the essential Smc5/6 DNA repair complex. *Mol Microbiol* **55:** 1735–1750

Jasin M (1996) Genetic manipulation of genomes with rare-cutting endonucleases. *Trends Genet* **12:** 224–228

Jessberger R (2002) The many functions of SMC proteins in chromosome dynamics. *Nat Rev Mol Cell Biol* **3:** 767–778

Johnson RD, Jasin M (2000) Sister chromatid gene conversion is a prominent double-strand break repair pathway in mammalian cells. *EMBO J* **19:** 3398–3407

Kim JS, Krasieva TB, LaMorte V, Taylor AM, Yokomori K (2002) Specific recruitment of human cohesin to laser-induced DNA damage. *J Biol Chem* **277:** 45149–45153

Koshland DE, Guacci V (2000) Sister chromatid cohesion: the beginning of a long and beautiful relationship. *Curr Opin Cell Biol* **12:** 297–301

Lehmann AR (2005) The role of SMC proteins in the responses to DNA damage. *DNA Repair (Amst)* **4:** 309–314

Lehmann AR, Walicka M, Griffiths DJ, Murray JM, Watts FZ, McCready S, Carr AM (1995) The rad18 gene of

©2006 European Molecular Biology Organization

Schizosaccharomyces pombe defines a new subgroup of the SMC superfamily involved in DNA repair. *Mol Cell Biol* **15:** 7067–7080

Lisby M, Rothstein R (2005) Localization of checkpoint and repair proteins in eukaryotes. *Biochimie* **87:** 579–589

McDonald WH, Pavlova Y, Yates III JR, Boddy MN (2003) Novel essential DNA repair proteins Nse1 and Nse2 are subunits of the fission yeast Smc5–Smc6 complex. *J Biol Chem* **278:** 45460–45467

Mengiste T, Revenkova E, Bechtold N, Paszkowski J (1999) An SMC-like protein is required for efficient homologous recombination in Arabidopsis. *EMBO J* **18:** 4505–4512

Morikawa H, Morishita T, Kawane S, Iwasaki H, Carr AM, Shinagawa H (2004) Rad62 protein functionally and physically associates with the smc5/smc6 protein complex and is required for chromosome integrity and recombination repair in fission yeast. *Mol Cell Biol* **24:** 9401–9413

Moynahan ME, Chiu JW, Koller BH, Jasin M (1999) Brca1 controls homology-directed DNA repair. *Mol Cell* **4:** 511–518

Moynahan ME, Pierce AJ, Jasin M (2001) BRCA2 is required for homology-directed repair of chromosomal breaks. *Mol Cell* **7:** 263–272

Nasmyth K (2002) Segregating sister genomes: the molecular biology of chromosome separation. *Science* **297:** 559–565

Nasmyth K, Haering CH (2005) The structure and function of SMC and kleisin complexes. *Annu Rev Biochem* **74:** 595–648

Onoda F, Takeda M, Seki M, Maeda D, Tajima J, Ui A, Yagi H, Enomoto T (2004) SMC6 is required for MMS-induced interchromosomal and sister chromatid recombinations in *Saccharomyces cerevisiae*. *DNA Repair (Amst)* **3:** 429–439

Pebernard S, McDonald WH, Pavlova Y, Yates III JR, Boddy MN (2004) Nse1, Nse2, and a novel subunit of the Smc5–Smc6 complex, Nse3, play a crucial role in meiosis. *Mol Biol Cell* **15:** 4866–4876

Pebernard S, Wohlschlegel J, McDonald WH, Yates III JR, Boddy MN (2006) The Nse5–Nse6 dimer mediates DNA repair roles of the Smc5–Smc6 complex. *Mol Cell Biol* **26:** 1617–1630

Petronczki M, Siomos MF, Nasmyth K (2003) Un menage a quatre: the molecular biology of chromosome segregation in meiosis. *Cell* **112:** 423–440

Pierce AJ, Johnson RD, Thompson LH, Jasin M (1999) XRCC3 promotes homology-directed repair of DNA damage in mammalian cells. *Genes Dev* **13:** 2633–2638

Porteus MH, Baltimore D (2003) Chimeric nucleases stimulate gene targeting in human cells. *Science* **300:** 763

Porteus MH, Carroll D (2005) Gene targeting using zinc finger nucleases. *Nat Biotechnol* **23:** 967–973

Potts PR, Yu H (2005) Human MMS21/NSE2 is a SUMO ligase required for DNA repair. *Mol Cell Biol* **25:** 7021–7032

Puget N, Knowlton M, Scully R (2005) Molecular analysis of sister chromatid recombination in mammalian cells. *DNA Repair (Amst)* **4:** 149–161

Rodrigue A, Lafrance M, Gauthier MC, McDonald D, Hendzel M, West SC, Jasin M, Masson JY (2006) Interplay between human DNA repair proteins at a unique double-strand break *in vivo*. *EMBO J* **25:** 222–231

Rothkamm K, Kruger I, Thompson LH, Lobrich M (2003) Pathways of DNA double-strand break repair during the mammalian cell cycle. *Mol Cell Biol* **23:** 5706–5715

Schar P, Fasi M, Jessberger R (2004) SMC1 coordinates DNA double-strand break repair pathways. *Nucleic Acids Res* **32:** 3921–3929

Sergeant J, Taylor E, Palecek J, Fousteri M, Andrews EA, Sweeney S, Shinagawa H, Watts FZ, Lehmann AR (2005) Composition and architecture of the *Schizosaccharomyces pombe* Rad18 (Smc5–6) complex. *Mol Cell Biol* **25:** 172–184

Sjogren C, Nasmyth K (2001) Sister chromatid cohesion is required for postreplicative double-strand break repair in *Saccharomyces cerevisiae*. *Curr Biol* **11:** 991–995

Sonoda E, Matsusaka T, Morrison C, Vagnarelli P, Hoshi O, Ushiki T, Nojima K, Fukagawa T, Waizenegger IC, Peters JM, Earnshaw WC, Takeda S (2001) Scc1/Rad21/Mcd1 is required for sister chromatid cohesion and kinetochore function in vertebrate cells. *Dev Cell* **1:** 759–770

Strom L, Lindroos HB, Shirahige K, Sjogren C (2004) Postreplicative recruitment of cohesin to double-strand breaks is required for DNA repair. *Mol Cell* **16:** 1003–1015

Swedlow JR, Hirano T (2003) The making of the mitotic chromosome: modern insights into classical questions. *Mol Cell* **11:** 557–569

Unal E, Arbel-Eden A, Sattler U, Shroff R, Lichten M, Haber JE, Koshland D (2004) DNA damage response pathway uses histone modification to assemble a double-strand break-specific cohesin domain. *Mol Cell* **16:** 991–1002

Verkade HM, Bugg SJ, Lindsay HD, Carr AM, O'Connell MJ (1999) Rad18 is required for DNA repair and checkpoint responses in fission yeast. *Mol Biol Cell* **10:** 2905–2918

Wolff S (1977) Sister chromatid exchange. *Annu Rev Genet* **11:** 183–201

Xie A, Puget N, Shim I, Odate S, Jarzyna I, Bassing CH, Alt FW, Scully R (2004) Control of sister chromatid recombination by histone H2AX. *Mol Cell* **16:** 1017–1025

Zhao X, Blobel G (2005) A SUMO ligase is part of a nuclear multiprotein complex that affects DNA repair and chromosomal organization. *Proc Natl Acad Sci USA* **102:** 4777–4782

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