Sae2, Exo1 and Sgs1 collaborate in DNA double-strand break processing

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Abstract

DNA ends exposed after introduction of double-strand breaks (DSBs) undergo 5 -3 nucleolytic degradation to generate single-stranded DNA (ssDNA), the substrate for binding by the Rad51 protein to initiate homologous recombination. This process is poorly understood in eukaryotes, but several factors have been implicated, including the Mre11 complex (Mre11-Rad50-Xrs2/NBS1), Sae2/CtIP/Ctp1 and Exo1. Here we demonstrate that yeast Exo1 nuclease and Sgs1 helicase function in alternate pathways for DSB processing. Novel, partially resected intermediates accumulate in a double mutant lacking Exo1 and Sgs1 that are poor substrates for homologous recombination. The early processing step that generates partially resected intermediates is dependent on Sae2. When Sae2 is absent, in addition to Exo1 and Sgs1, unprocessed DSBs accumulate and homology-dependent repair fails. These results suggest a two-step mechanism for DSB processing during homologous recombination. First, the Mre11 complex and Sae2 remove a small oligonucleotide(s) from the DNA ends to form an early intermediate. Second, Exo1 and/or Sgs1 rapidly process this intermediate to generate extensive tracts of ssDNA that serve as substrate for Rad51.

DSBs are potentially lethal lesions that can occur spontaneously during normal cell metabolism or by treatment of cells with DNA-damaging agents. If unrepaired or repaired inappropriately, DSBs can lead to mutagenic events such as chromosome loss, deletions, duplications or translocations. The Mre11 complex (Mre11-Rad50-Xrs2/NBS1) is rapidly recruited to DSBs, signals checkpoint activation via the Tel1/ATM kinase and regulates 5 -3 resection of the DNA ends[1–3]. The intrinsic Mre11 nuclease activity is essential for processing ends blocked by covalent adducts, such as Spo11 or hairpin-capped ends, but appears to be less important for processing HO endonuclease-induced DSBs[4–7]. Sae2/CtIP/ Ctp1 interacts with the Mre11 complex and Sae2 deficiency is phenotypically similar to loss of the Mre11 nuclease activity[6–10]. The functions of the Mre11 complex and Sae2 in processing DSBs are partially redundant with the 5 -3 double-strand exonuclease, Exo1[11]. In the absence of Exo1 and Sae2, 5 -3 resection of DSBs is reduced, but resection and homologous recombination still occur suggesting the existence of at least one other factor in DSB processing[12]. Here we demonstrate a function for the *Saccharomyces cerevisiae* RecQ helicase homolog, Sgs1, in DSB processing. As the Sgs1 homologue in human, BLM, maintains genome integrity and guards against cancer predisposition[13], our findings suggest that some of the defects observed in individuals with Bloom's syndrome could be due to altered processing of DSBs.

Sae2 and Exo1 function at different steps in resection

We developed a single-strand annealing assay (SSA), which is inherently sensitive to defects in DSB resection, to identify genes required for this process[14]. The assay employs tandem repeats of the *ade2* gene, one of which contains a recognition site for the I-*Sce*I endonuclease (Fig. 1a)[15]. The strain also harbors a *GAL1p-I-SCEI* fusion to provide regulated expression of the nuclease. Following induction of I-*Sce*I, homology-dependent repair can occur by gene conversion directed by the uncut allele, or by SSA[4]. SSA requires extensive 5 -3 degradation (~ 7-kb in this case) to expose complementary single-stranded DNA (ssDNA) followed by Rad52-dependent annealing of the repeats. After trimming residual single-stranded DNA tails and gap filling, recombinants form with loss of one of the repeats and the intervening sequence. In the *rad51* mutant gene conversion is eliminated, leaving repair of the DSB by SSA as the alternate mode[16]. The efficiency of SSA can be assessed by the plating efficiency of strains on medium containing galactose (YPRaf/Gal) to induce constitutive expression of I-*Sce*I. Changes in the kinetics of SSA can be evaluated by Southern blot analysis of *Nhe*I/*Eag*I-digested genomic DNA.

The plating efficiency of the *rad51* strain on YPRaf/Gal was the same as on medium containing glucose (YPD) and Ade+ recombinants were evident by formation of white colonies (Fig. 1b). By physical analysis, DSB fragments appeared 1-h after inducing I-*Sce*I expression and were then processed to form the SSA product visible at 2-h and accumulating to 80% of the total DNA after 24-h (Fig. 1c). 5 -3 resection past the *Eag*I and *Nhe*I sites results in loss of the cut fragments. Therefore, increased stability of the cut fragments indicates a delay or reduced rate of processing. Although the DSB fragments produced in the *rad51 exo1* mutant were processed with similar kinetics as in the *rad51* mutant, there was a delay in SSA product formation and reduced yield. In the *rad51 sae2* strain, the DSB fragments persisted for longer, but the deletion products still appeared at 2-h and accumulated to 50% of the total. The phenotypes of *sae2* and *exo1* mutants, stability of cut fragments and delay in product formation, respectively, were additive in the *rad51 exo1 sae2* mutant strain resulting in a substantial defect in SSA and a 2-fold reduction in plating efficiency on YPRaf/Gal plates (Fig. 1). To characterize the formation of ssDNA, DNA samples were applied to nylon membranes in the native or denatured state and tested for ability to hybridize to a probe adjacent to the DSB. This analysis revealed similar amounts of ssDNA adjacent to the break site in *rad51 exo1* and *rad51* strains at early time points, whereas a decrease was observed in the *rad51 sae2* mutant (Fig. 1d). ssDNA persisted in the *rad51 exo1* mutant, consistent with the observed delay in SSA product formation. These results are in line with a model in which Sae2 commences DSB processing to produce an early intermediate that is rapidly matured by the processive nucleolytic activity of Exo1. In the absence of Sae2, a smaller sub-set of DSBs is acted on directly by Exo1 yielding SSA products at the same time, but with lower yield than found in the presence of Sae2.

Sgs1 is required for DSB processing in the absence of Exo1

The presence of SSA products in the *exo1 sae2* mutant suggests at least one other activity is operative in DSB processing. The missing factor is not Mre11 because SSA products still form in *mre11* , *mre11 exo1* , and *mre11 exo1 sae2* mutants (Supplementary Fig. 1). The nuclease-defective *mre11-H125N* mutant was slightly less defective than *sae2* , by itself or with *exo1* (data not shown). One possible mechanism for DSB processing would involve a helicase in conjunction with a single-strand specific endo- or exonuclease. In *Escherichia coli*, the RecQ 3 -5 helicase and RecJ 5 -3 exonuclease function in DSB resection in the absence of the dominant RecBCD processing activity[17]. Therefore, we considered the possibility that the RecQ homologue of *S. cerevisiae*, Sgs1[18,19], might fulfill

a similar redundant function. The *rad51 sgs1* mutant showed a delay in SSA (Fig. 2 and Supplementary Fig. 2), however, the *rad51 exo1 sgs1* strain was completely deficient as evidenced by the >1000-fold decrease in plating efficiency on YPRaf/Gal medium (Fig. 2a). Furthermore, by Southern blot analysis there was no detectable deletion product, even 24-h after I-*Sce*I induction (Fig. 2b). Notably, in the *rad51 exo1 sgs1* mutant novel intermediates were observed with slightly faster mobility than the DSB cut fragments and these became more smeared over time suggesting very slow processing. We were unable to analyze the *sae2 sgs1* double mutant because of a synthetic growth defect [20].

The SSA assay requires more extensive resection than is likely to be required for Rad51-dependent strand invasion. To determine whether the partially processed intermediates that accumulate in *exo1 sgs1* strains are proficient for gene conversion, we analyzed DSB processing in *RAD51* derivatives by a mating type (*MAT*) switching assay. In this assay, DNA samples are digested with *Sty*I to detect HO endonuclease cleavage and gene conversion products by Southern blot analysis (Fig. 2c)[21]. The *exo1* and *sae2* mutants exhibited slight defects in the efficiency of repair, whereas the *sgs1* single mutant appeared normal (Supplementary Fig. 3 and Fig. 2d). In the *exo1 sgs1* double mutant the DSB fragment was processed to yield an intermediate with faster mobility than the cut fragment and this persisted for several hours, as we had observed in the SSA assay. Despite the defect in DSB processing, the 1.8-kb gene conversion product was still detected though with reduced efficiency (20%) compared with wild type and the single mutants. This suggests very short ssDNA tails are sufficient to initiate Rad51-dependent gene conversion. To determine whether the helicase activity of Sgs1 is important for DSB processing, plasmids expressing wild type or helicase- defective alleles of *SGS1* were used to transform the *exo1 sgs1* double mutant and the resulting strains tested for *MAT* switching or SSA proficiency[22]. As anticipated the *SGS1* plasmid rescued the SSA and gene conversion defects of the *sgs1 exo1* mutant, but the helicase-defective *sgs1-K706A* allele failed to complement *sgs1* in these assays (Fig. 2). Previous studies have shown the steady state level of the Sgs1-K706A protein to be similar to wild type[22]. Therefore, the helicase activity is required for DSB processing by Sgs1. The lack of full complementation by *SGS1* in the *MAT*-switching assay could be due to plasmid loss in some of the cells in the population.

Resection is severely attenuated in the *exo1Δ sgs1Δ* strain

To measure resection of 5 strands directly we analyzed HO-induced DSB fragments by restriction digestion and alkaline gel electrophoresis (Fig. 3a). Processing of the HO-induced break renders the DNA single-stranded and resistant to digestion with restriction enzymes resulting in a ladder of higher molecular weight bands[21]. By using RNA probes specific for the 3 and 5 strands to the right of the HO cut site, resection of the strands can be monitored. A *rad51* background was used for this analysis to prevent engagement of the intermediates in repair.

The probe specific for 3 strands detected extensive resection of the HO-induced break in the *rad51* strain (Fig. 3b). In the *rad51 exo1* mutant the first two ssDNA intermediates were formed with normal kinetics, but the products indicative of extensive resection were delayed resulting in accumulation of the r2 intemediate, suggesting Sgs1-dependent resection is less processive (Supplementary Fig. 4)[5]. The extent and timing of resection in the *rad51 sgs1* mutant were similar to the *rad51* strain. In contrast, only a faint band corresponding to resection beyond the first *Sty*I site was detected in the *rad51 exo1 sgs1* strain. Detection of a discrete band corresponding to degradation beyond the first *Sty*I site and failure to detect products smaller than the cut fragment suggest the 3 ends are intact in the *rad51 exo1 sgs1* mutant. This was confirmed by using a probe specific for the 5 strand. No intermediates were detected in the *rad51 exo1* or *rad51 sgs1* mutants, presumably due

to the rapid degradation of 5′ ends. However, a smeared product was detected with faster migration than the 700-bp cut fragment in the *rad51 exo1 sgs1* mutant, consistent with the severe resection defect. Interestingly, the smear does not occur immediately below the cut fragment suggesting there is an initial endonuclease cleavage step that removes about 50–100 nucleotides from the 5′ end.

Sae2 and Exo1 or Sgs1 function sequentially in DSB processing

The results presented above suggest that after formation of a DSB, the ends are trimmed by an endonuclease to an intermediate form, which is subsequently processed by Exo1 or Sgs1. The Mre11 complex and Sae2 are likely to be involved in the initial processing step because DSB fragments are stabilized in *mre11* and *sae2* mutants, and recent studies have shown Mre11-dependent processing of DSBs to form short ssDNA oligonucleotides[23]. Furthermore, Sae2 has an endonuclease activity that is stimulated by the Mre11 complex[24]. We attempted to generate *exo1 sgs1 mre11* or *exo1 sgs1 sae2* triple mutants, but these were inviable (Supplementary Fig. 5a, b); the *exo1 sgs1 mre11-H125N* triple mutant was also inviable. A conditional *sae2* allele was created in the *exo1 sgs1* background by expressing endogenous *SAE2* from the P_{tet} promoter in a strain expressing the tetracycline-controlled trans-activator (tTA) to repress transcription by growth in the presence of doxycycline[25]. The *exo1 sgs1* P_{tet}-*SAE2* strain was viable in the absence of doxycycline indicating normal function. By western blot analysis the Sae2 protein was depleted 4-h after addition of doxycycline to the growth medium (Supplementary Fig. 5c). In the absence of doxycycline, the DSB fragment generated by HO cleavage at the *MAT***a** locus was still processed to the faster mobility form, but in the presence of doxycycline the unprocessed 0.7-kb cut fragment persisted for more than 6-h and no *MAT* repaired products were detected (Fig. 4a). The cut fragments were also stabilized in the SSA assay in the absence of Sae2, even 24-h after I-*Sce*I induction, confirming the block to resection (Fig. 4b). These results show the initial processing step is Sae2 dependent. Gene conversion occurs in the *sgs1* P_{tet}-*SAE2* strain in the presence of doxycycline confirming Exo1 is sufficient for DSB processing in the absence of Sgs1 (Supplementary Fig. 5d).

Conclusions

We have identified the key factors that are essential for generating 3′ single-stranded tails in DSB processing. In agreement with previous studies, the Mre11 complex and Sae2/CtIP play a critical role, but our findings suggest this function is limited to an early step in the reaction (Fig. 5). It is possible that the Mre11 complex and Sae2 fail to bind efficiently to the processed ends preventing repeated cycles of cleavage. This initial cleavage event could be important to prevent binding by the Ku complex and subsequent repair by end joining, and also to provide a substrate for Sgs1, which shows higher affinity for 3′ tailed substrates than blunt ends[26]. The intermediate thus formed is rapidly processed by either the 5′-3′ exonuclease activity of Exo1 or by Sgs1 helicase (Fig. 5). We assume Sgs1 functions with a single-strand specific nuclease to remove the 5′ strand. The essential nuclease, Dna2, is a candidate to function in resection because conditional mutations exhibit synthetic growth defects with *exo1* and *sgs1*[27]. That Sgs1 acts at this early step is unexpected because genetic studies have implied a role for it after Rad51 action, and biochemical studies have shown the human BLM protein to dissociate D-loops and double Holliday junction intermediates[28–30]. However, BLM localizes rapidly to sites of laser light-induced DSBs, suggesting an early role in repair in addition to dissolution of recombination intermediates[31]. As all of the factors involved in resection in yeast are evolutionarily conserved, these findings suggest a general mechanism for DSB processing in eukaryotes.

METHODS

Yeast strains and plasmids

The strains used were derived from W303[32] and are listed in Supplementary Table 1. Strain LSY1092 was made by one-step gene replacement of W1588-4A with *Eco*RI/*Sph*I-digested pMJ536 (gift of M. Lichten) to replace the *SAE2* coding region with the kanMX6 cassette[33]. Strain LSY1975 was constructed by transformation of W1588-4C with a PCR fragment to generate a deletion allele of *SGS1*. PCR was performed on an *hph*MX4 cassette plasmid, pAG32 (gift of J. McCusker)[34]. Details of the primers used for gene disruption and confirmation are available on request. To create strain LSY2041, a PCR fragment amplifying the tet-*SAE2* cassette from YAR1006 (gift of A. Rattray) was transformed into W1588-4C. A PCR fragment amplifying a 13-Myc tag from plasmid pFA6a-13Myc-*TRP1*[35] was transformed into LSY2041 to generate strain LSY2077. Strains containing the *ade2* direct repeat were generated by crossing LSY1430[15] to strains within the lab collection to produce haploid progeny of the indicated genotypes.

Plasmids containing the wild type or helicase defective *SGS1* were kindly provided by R. Rothstein. pEM-*SGS1* and pEM-*sgs1-K706A* were created by moving the *SGS1* containing *Kpn*I/*Sac*I fragment from plasmids pSM100 and pRS415-*sgs1-K706A,* respectively, into the multiple cloning site of pRS316.

Media, growth conditions, and genetic methods

Media, growth conditions and genetic methods are as described previously[36]. Hygromycin B (Sigma) to 300 μg/ml was used for selection of the *hph*MX4 cassette. Doxycycline (Sigma) was used to 20 μg/ml final concentration in the conditional Sae2 experiments. G418 (Sigma) to 200 μg/ml was used for selection of the *kan*MX cassettes.

Physical analysis of mating type switching

Strains to be tested were transformed to Trp$^+$ with HO expressing plasmid, pFH800[37]. Trp$^+$ transformants were grown in 5 ml of SC medium lacking tryptophan (SC-Trp) for 18 h. Cells were harvested, washed with water and used to inoculate 200 ml of SCRaf-Trp. Cultures were grown to an OD_{600} of 0.3 to 0.4, a 50ml sample was removed (t=0h) and galactose was added to the medium to a final concentration of 2%. One hour later, the cultures were harvested, washed and resuspended in 200 ml of SCRaff-Trp + 2% glucose. Samples (40 ml) were removed at 1-h intervals after induction for DNA analysis. Cells were harvested by centrifugation and the cell pellets were stored at −20°. DNA was extracted, digested with *Sty*I, and DNA fragments were separated by electrophoresis through 1% agarose gels. DNA fragments were transferred to nylon membranes and hybridized with a PCR fragment generated by amplification of *MAT* sequences distal to the HO-cut site (coordinates 201176 to 201580 on the ChrIII sequence). For the conditional Sae2 experiments, the same protocol was followed with the exception that before addition of galactose, the cells were pre-treated with 20 μg/ml doxycycline for 6h to ensure full repression of Sae2 expression.

To quantitate the repair efficiency we compared the intensity of the gene conversion product to the total intensity of the lane, using ImageJ (NIH, USA). This ratio was then corrected for the HO cutting efficiency for each experiment. The cutting efficiency was quantitated using a set of primers that specifically anneal to Ya and Z2 sequences and amplify only the uncut substrate. PCR conditions for log-linear region amplification were used and PCR performed using as templates genomic DNA from t=0h and t=2h. In the same PCR reaction a second set of primers with the same annealing temperature but unrelated target locus were used as loading control. The PCR products were analyzed on agarose gels and the intensity of the

bands was quantitated using ImageJ. For each time point we calculated the ratio of uncut MAT/control, designated as x. The ratio of x_{2h}/x_{0h} provides the percentage of uncut MATa that when subtracted from 1 gives the cutting efficiency.

Physical analysis of single strand annealing

Strains to be tested were grown in 5 ml of SC-Trp. Cells were harvested, washed with water and used to inoculate 200 ml of YPLactate (3%) medium. Cultures were grown to an OD_{600} of 0.3 to 0.4, a 50ml sample was removed (t=0h) and 16.8ml of 20% galactose were added to the medium. One hour later, the cultures were harvested, washed and resuspended in 200 ml of YPLactate + 2% glucose. Samples were removed at 1-h intervals after induction for DNA analysis as described above. DNA was digested with *Nhe*I/*Eag*I, and DNA fragments were separated by electrophoresis through 0.8% agarose gels. DNA fragments were transferred to nylon membranes and hybridized with a 3.7kb *Bgl*II fragment of the *ADE2* locus. The final SSA product was quantitated by the ratio of the intensity of the SSA product to the whole intensity of the lane. For the conditional Sae2 experiments, the same protocol was followed with the exception that before addition of galactose, the cells were pre-treated with 20 μg/ml doxycycline for 6h.

Dot blot analysis

DNA samples were applied to nylon membranes in the native or denatured state. 1 μg versus 0.1 μg of total genomic DNA was used for the native and denatured samples, respectively, after adjusting the concentration to 10xSSC. Samples were transferred to nylon membranes using a dot blot apparatus. The membrane was UV cross-linked and the blots were hybridized with the *ADE2* probe. The signal of the dots was quantified by scanning the optic density of each dot using ImageJ.

Alkaline electrophoresis

ssDNA intermediates were analyzed by alkaline gel electrophoresis as described[21] and the blots hybridized with single stranded probes complementary to the 5 or 3 strand. The probes were obtained by in vitro transcription using Epicentre Riboscribe T7 synthesis system and plasmids pEM-MAT or pEM-TAM as templates. The plasmids were obtained by cloning a *MAT* locus PCR fragment (coordinates 201176 to 201580 on the ChrIII sequence) into pGEM-T Easy (Promega) in either orientation. To quantitate the r2 band over time in different strain backgrounds we plotted the ratio of intensity of the r2 band to the intensity of an unrelated locus as a loading control.

Western blot analysis

Whole cells extracts were analyzed by SDS-PAGE electrophoresis and Sae-Myc was detected using anti-c-myc antibody (Sigma).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

References

1. Lee SE, et al. Saccharomyces Ku70, mre11/rad50 and RPA proteins regulate adaptation to G2/M arrest after DNA damage. Cell. 1998; 94:399–409. [PubMed: 9708741]

2. Lisby M, Barlow JH, Burgess RC, Rothstein R. Choreography of the DNA damage response: spatiotemporal relationships among checkpoint and repair proteins. Cell. 2004; 118:699–713. [PubMed: 15369670]

3. Nelms BE, Maser RS, MacKay JF, Lagally MG, Petrini JH. In situ visualization of DNA double-strand break repair in human fibroblasts. Science. 1998; 280:590–2. [PubMed: 9554850]

4. Krogh BO, Symington LS. Recombination proteins in yeast. Annu Rev Genet. 2004; 38:233–71. [PubMed: 15568977]

5. Llorente B, Symington LS. The Mre11 nuclease is not required for 5 to 3 resection at multiple HO-induced double-strand breaks. Mol Cell Biol. 2004; 24:9682–94. [PubMed: 15485933]

6. Lobachev KS, Gordenin DA, Resnick MA. The Mre11 complex is required for repair of hairpin-capped double-strand breaks and prevention of chromosome rearrangements. Cell. 2002; 108:183–93. [PubMed: 11832209]

7. Rattray AJ, McGill CB, Shafer BK, Strathern JN. Fidelity of mitotic double-strand-break repair in *Saccharomyces cerevisiae*: a role for *SAE2/COM1*. Genetics. 2001; 158:109–22. [PubMed: 11333222]

8. Clerici M, Mantiero D, Lucchini G, Longhese MP. The *Saccharomyces cerevisiae* Sae2 protein promotes resection and bridging of double strand break ends. J Biol Chem. 2005; 280:38631–8. [PubMed: 16162495]

9. Limbo O, et al. Ctp1 is a cell-cycle-regulated protein that functions with Mre11 complex to control double-strand break repair by homologous recombination. Mol Cell. 2007; 28:134–46. [PubMed: 17936710]

10. Sartori AA, et al. Human CtIP promotes DNA end resection. Nature. 2007; 450:509–14. [PubMed: 17965729]

11. Tran PT, Erdeniz N, Symington LS, Liskay RM. EXO1-A multi-tasking eukaryotic nuclease. DNA Repair (Amst). 2004; 3:1549–59. [PubMed: 15474417]

12. Clerici M, Mantiero D, Lucchini G, Longhese MP. The *Saccharomyces cerevisiae* Sae2 protein negatively regulates DNA damage checkpoint signalling. EMBO Rep. 2006; 7:212–8. [PubMed: 16374511]

13. Hickson ID. RecQ helicases: caretakers of the genome. Nat Rev Cancer. 2003; 3:169–78. [PubMed: 12612652]

14. Vaze MB, et al. Recovery from checkpoint-mediated arrest after repair of a double-strand break requires Srs2 helicase. Mol Cell. 2002; 10:373–85. [PubMed: 12191482]

15. Mozlin AM, Fung CW, Symington LS. Role of the *Saccharomyces cerevisiae* Rad51 paralogs in sister chromatid recombination. Genetics. 2008; 178:113–26. [PubMed: 18202362]

16. Ivanov EL, Sugawara N, Fishman-Lobell J, Haber JE. Genetic requirements for the single-strand annealing pathway of double-strand break repair in Saccharomyces cerevisiae. Genetics. 1996; 142:693–704. [PubMed: 8849880]

17. Amundsen SK, Smith GR. Interchangeable parts of the *Escherichia coli* recombination machinery. Cell. 2003; 112:741–4. [PubMed: 12654241]

18. Gangloff S, McDonald JP, Bendixen C, Arthur L, Rothstein R. The yeast type I topoisomerase Top3 interacts with Sgs1, a DNA helicase homolog: a potential eukaryotic reverse gyrase. Mol Cell Biol. 1994; 14:8391–8. [PubMed: 7969174]

19. Watt PM, Louis EJ, Borts RH, Hickson ID. Sgs1: a eukaryotic homolog of *E. coli* RecQ that interacts with topoisomerase II in vivo and is required for faithful chromosome segregation. Cell. 1995; 81:253–60. [PubMed: 7736577]

20. Pan X, et al. A DNA integrity network in the yeast *Saccharomyces cerevisiae*. Cell. 2006; 124:1069–81. [PubMed: 16487579]

21. White CI, Haber JE. Intermediates of recombination during mating type switching in Saccharomyces cerevisiae. EMBO J. 1990; 9:663–73. [PubMed: 2178924]

22. Mullen JR, Kaliraman V, Brill SJ. Bipartite structure of the SGS1 DNA helicase in Saccharomyces cerevisiae. Genetics. 2000; 154:1101–14. [PubMed: 10757756]

23. Jazayeri A, Balestrini A, Garner E, Haber JE, Costanzo V. Mre11-Rad50-Nbs1-dependent processing of DNA breaks generates oligonucleotides that stimulate ATM activity. EMBO J. 2008

24. Lengsfeld BM, Rattray AJ, Bhaskara V, Ghirlando R, Paull TT. Sae2 is an endonuclease that processes hairpin DNA cooperatively with the Mre11/Rad50/Xrs2 complex. Mol Cell. 2007; 28:638–51. [PubMed: 18042458]

25. Baron U, Bujard H. Tet repressor-based system for regulated gene expression in eukaryotic cells: principles and advances. Methods Enzymol. 2000; 327:401–21. [PubMed: 11044999]

26. Bennett RJ, Keck JL, Wang JC. Binding specificity determines polarity of DNA unwinding by the Sgs1 protein of *S. cerevisiae*. J Mol Biol. 1999; 289:235–48. [PubMed: 10366502]

27. Budd ME, et al. A network of multi-tasking proteins at the DNA replication fork preserves genome stability. PLoS Genet. 2005; 1:e61. [PubMed: 16327883]

28. Gangloff S, Soustelle C, Fabre F. Homologous recombination is responsible for cell death in the absence of the Sgs1 and Srs2 helicases. Nat Genet. 2000; 25:192–4. [PubMed: 10835635]

29. van Brabant AJ, et al. Binding and melting of D-loops by the Bloom syndrome helicase. Biochemistry. 2000; 39:14617–25. [PubMed: 11087418]

30. Wu L, Hickson ID. The Bloom's syndrome helicase suppresses crossing over during homologous recombination. Nature. 2003; 426:870–4. [PubMed: 14685245]

31. Karmakar P, et al. BLM is an early responder to DNA double-strand breaks. Biochem Biophys Res Commun. 2006; 348:62–9. [PubMed: 16876111]

32. Zou H, Rothstein R. Holliday junctions accumulate in replication mutants via a RecA homolog-independent mechanism. Cell. 1997; 90:87–96. [PubMed: 9230305]

33. Borde V, Wu TC, Lichten M. Use of a recombination reporter insert to define meiotic recombination domains on chromosome III of *Saccharomyces cerevisiae*. Mol Cell Biol. 1999; 19:4832–42. [PubMed: 10373533]

34. Goldstein AL, McCusker JH. Three new dominant drug resistance cassettes for gene disruption in *Saccharomyces cerevisiae*. Yeast. 1999; 15:1541–53. [PubMed: 10514571]

35. Longtine MS, et al. Additional modules for versatile and economical PCR-based gene deletion and modification in *Saccharomyces cerevisiae*. Yeast. 1998; 14:953–61. [PubMed: 9717241]

36. Sherman, F.; Fink, G.; Hicks, J. Methods in Yeast Genetics. Cold Spring Harbor Laboratory; Cold Spring Harbor, N.Y: 1986.

37. Nickoloff JA, Singer JD, Hoekstra MF, Heffron F. Double-strand breaks stimulate alternative mechanisms of recombination repair. J Mol Biol. 1989; 207:527–41. [PubMed: 2668534]
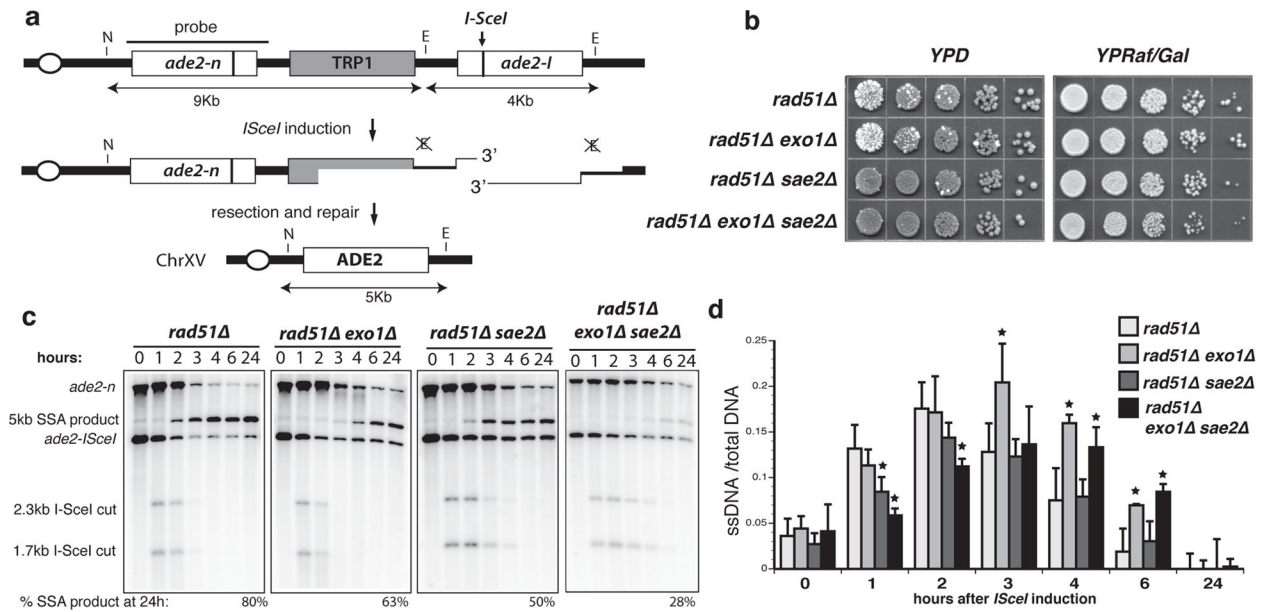
Figure 1. Sae2 and Exo1 function at different steps in DSB resection

a. Map of the chromosome *XV* region containing the *ade2* direct repeat. The *ade2-I* allele is cleaved by I-*Sce*I to yield the 2.3-kb and 1.7-kb cut fragments that disappear over time concomitant with repair to the 5-kb SSA product. b. SSA efficiency as indicated by sectoring on YPD plates and survival on YPRaf/Gal plates. Ade[+] (white) colonies arise from *ade2* (red) colonies by SSA during growth on YPD due to leaky expression of I-*Sce*I. c. SSA physical assay: Monitoring of resection and repair of the I-*Sce*I induced DSB by southern blot analysis of *Nhe*I/*Eag*I digested genomic DNA. d. Quantitation of the *ADE2* hybridization signal on native versus denatured DNA samples bound on nylon membrane. The means from multiple experiments from three independent inductions are presented, error bars indicate s.d.. * Denotes statistical significance of $P<0.02$ (unpaired t-test).
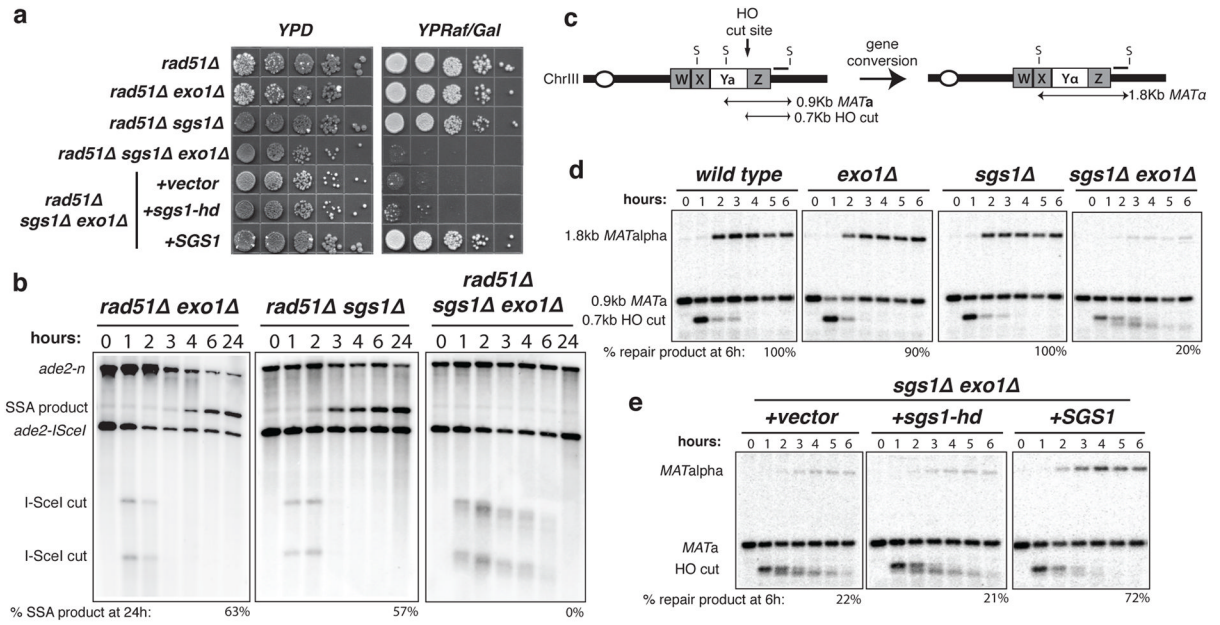
Figure 2. Sgs1 and Exo1 function redundantly in DSB resection

a. SSA plating efficiency as described in Fig. 1b. b. SSA physical assay as described in Fig. 1c. c. Schematic representation of *MAT* switching: the 0.9-kb *MAT***a** *Sty*I fragment is cleaved by HO resulting in a 0.7-kb cut fragment that disappears over time concomitant with repair to an 1.8-kb *MAT* fragment. d. *MAT* switching assay: HO cut fragment processing and gene conversion efficiency as monitored by southern blot analysis of *Sty*I digested genomic DNA. e. *MAT* switching assay on *sgs1 exo1* cultures complemented with mutant or wild-type *SGS1*.
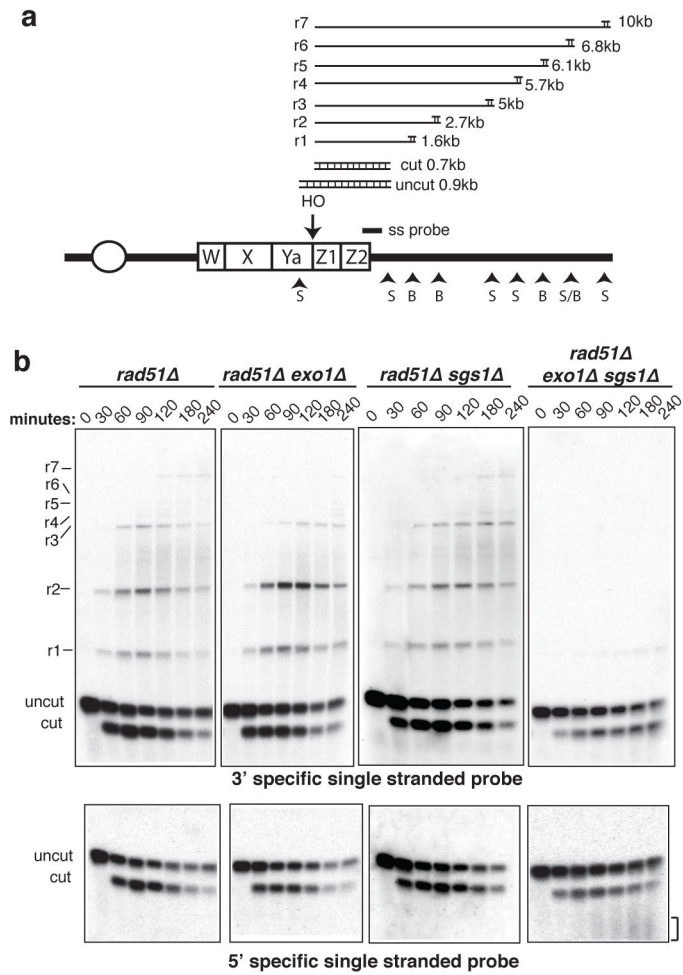
Figure 3. Single stranded intermediates fail to form in the absence of Exo1 and Sgs1
a. Schematic representation of the method used to detect single stranded intermediates after
5´-3´ resection of the *MAT* locus, showing the positions of the HO cut site, the probe and the
*Sty*I (S) and *Bst*XI (B) sites. Processing of the HO-induced break renders the DNA single-
stranded and resistant to digestion with restriction enzymes, giving rise to distinct sets of
fragments[21]. b. Alkaline electrophoresis of *Sty*I/*Bst*XI digested genomic DNA: ssDNA
intermediates formed after the resection of the HO-induced break were detected by using 3´
or 5´ specific riboprobes. The bracket indicates the smeared 5´-terminated strands observed
in the *rad51Δ exo1Δ sgs1Δ* mutant.

a

exo1Δ sgs1Δ
tet-SAE2

exo1Δ sgs1Δ
tet-SAE2 +20μg/ml DOX

hours: 0 1 2 3 4 6 24   0 1 2 3 4 6 24

ade2-n

ade2-ISceI

I-SceI cut

I-SceI cut

b

exo1Δ sgs1Δ
tet-SAE2

exo1Δ sgs1Δ
tet-SAE2 +20μg/ml DOX

hours: 0 1 2 3 4 5 6   0 1 2 3 4 5 6
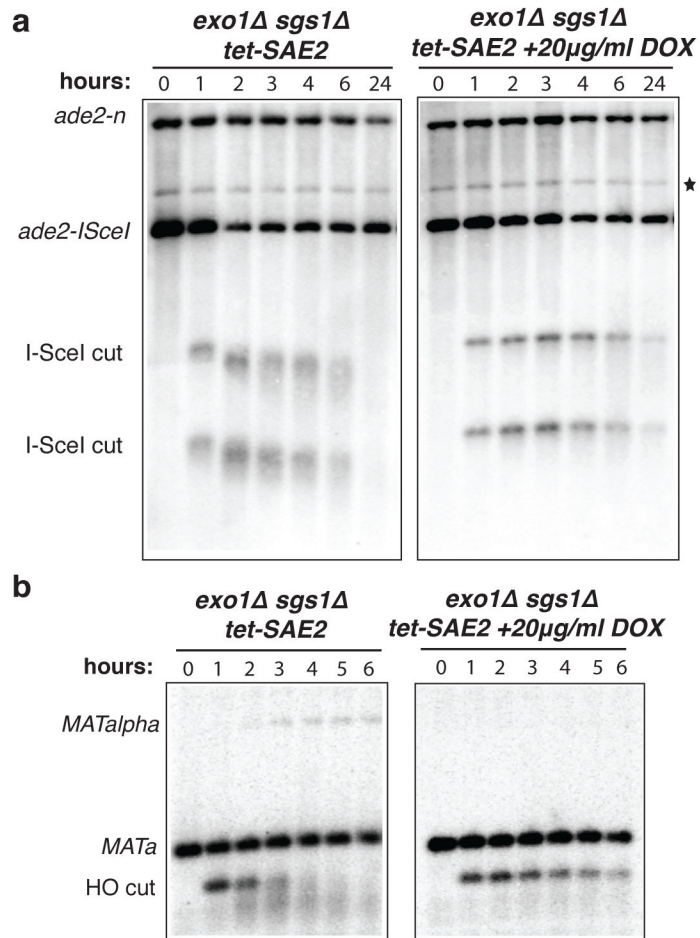
MATalpha

MATa

HO cut

Figure 4. Sae2 is required for creating the minimally resected intermediates
SSA assay (a) and mating type switching assay (b) in cells treated or untreated with doxycycline before I-*Sce*I or HO expression. *Refers to a cross-hybridizing band, which is present at time 0 h and if it were due to recombinants in the population would allow survival of cells on YPRaf/Gal medium.
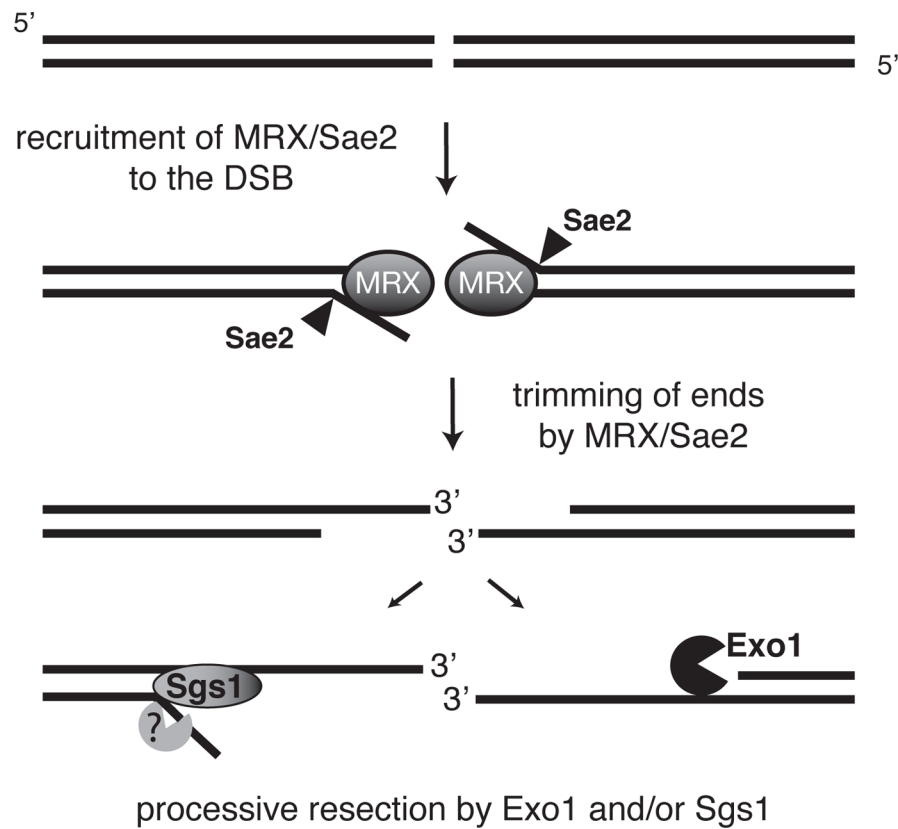
Figure 5. Two-step mechanism for DSB resection
After a DSB is formed and recognized by the MRX complex, Sae2, in collaboration with
MRX, trims the ends to create a minimally resected intermediate that is 'compatible' for
processive resection by the 5 -3 exonucleolytic activity of Exo1 or Sgs1 helicase and a
single-strand specific nuclease. Cells lacking both the nuclease and the helicase activity
accumulate the intermediates from MRX/Sae2 cleavage. Because *mre11* and *sae2* mutants
still show DSB processing, Exo1 and Sgs1 must be able to access unprocessed DNA ends,
but with reduced efficiency.