dsDNA breaks (DSBs) are resected in a 5’→3’ direction, generating single-stranded DNA (ssDNA). This promotes DNA repair by homologous recombination and also assembly of signaling complexes that activate the DNA damage checkpoint effector kinase Chk1. In fission yeast (Schizosaccharomyces pombe), genetic screens have previously uncovered a family of three xeroderma pigmentosum G (XPG)-related nucleases (XRNs), known as Ast1, Exo1, and Rad2. Collectively, these XRNs are recruited to a euchromatic DSB and are required for ssDNA production and end resection across the genome. Here, we studied why there are three related but distinct XRN enzymes that are all conserved across a range of species, including humans, whereas all other DSB response proteins are present as single species. Using S. pombe as a model, ChiP and DSB resection analysis assays, and highly efficient 1–Ppol-induced DSBs in the 28S rDNA gene, we observed a hierarchy of recruitment for each XRN, with a progressive compensatory recruitment of the other XRN complexes as the responding enzymes are deleted. Importantly, we found that this hierarchy reflects the requirement for different XRN enzymes to effect efficient DSB resection in the rDNA, demonstrating that the presence of three XRN enzymes is not a simple division of labor. Furthermore, we uncovered a specificity of XRN function with regard to the direction of transcription. We conclude that the DSB-resection machinery is complex, is nonuniform across the genome, and has built-in failsafe mechanisms, features that are in keeping with the highly pathological nature of DSB lesions.

DNA damage is generated by multiple intrinsic and extrinsic sources. It can result in additions of chemical moieties to one or both strands (e.g. base alkylation or inter-strand cross-links), the breaking of chemical bonds leading to abasic sites, as well as single- and double-stranded DNA breaks. The double-stranded breaks (DSBs) are the most pathological, and they can arise directly from sources such as reactive oxygen or ionizing radiation or generated by DNA replication up to the point of a single-stranded break. DSBs themselves can have blunt ends, 3’- or 5’-overhangs, and need not be formed at the phosphodiester bond between the 5’-PO₄ and 3’-OH groups of adjacent dNTPs. Thus, the repair of many lesions in DNA requires the processing of the initial lesion into common substrates for eventual repair-associated DNA synthesis and ligation.

DSBs can be repaired by nonhomologous end joining, an error-prone pathway that ligates DNA ends predominantly in the G1 phase of the cell cycle. Ligation may require end processing by enzymes such as the Mre11–Rad50–Nbs1 (MRN) complex, which contains 3’→5’exonuclease activity (1). During S- and G₂-phases, the presence of an identical sister chromatid provides a template for error-free repair by homologous recombination (HR). For this to occur, and for the associated DNA damage checkpoint to be initiated, the lesion must be processed to generate single-stranded DNA (ssDNA) with an exposed 3’-OH group and a double-stranded junction with a 5’-PO₄.

The ssDNA formed at DSBs is initially coated in the high-affinity ssDNA-binding protein replication protein A (RPA), on which the DNA damage checkpoint-signaling complexes assemble to prevent entry into mitosis. These complexes include ATR/ATRIP and the 9-1-1 complex, which bind directly to the RPA-coated ssDNA, as well as BRCT-containing mediator proteins that recruit the checkpoint effector kinase Chk1. Chk1 is in turn activated at these lesions by ATR-mediated phosphorylation and arrests cells in G₂ to prevent mitotic entry by enforcing inhibitory tyrosine phosphorylation on Cdc2/Cdk1 (2–9). In the budding yeast Saccharomyces cerevisiae, an alternative DNA damage checkpoint is enforced by Rad53, a relative of the Chk2 kinase (10, 11). Unlike Chk1, Rad53 elicits a blockade to the metaphase→anaphase transition in mitosis by preventing cohesin cleavage (12). This mode of checkpoint arrest does not occur in most other organisms, including the fission yeast Schizosaccharomyces pombe, the model used here. Rather, the Chk2 kinase in S. pombe, Cds1, is activated at sites of stalled replication (and not DNA damage) and acts to stabilize stalled replication forks (13–15). RPA-coated ssDNA also recruits Cdc24/Dna2, a helicase/nuclease implicated in replication fork processing (16) and subsequent protein A; HR, homologous recombination; SSA, single-stranded annealing; DAPI, 4’,6-diamidino-2-phenylindole; OF, Okazaki fragment; ATR, ATM and Rad3-related.
longer-range DSB resection in an HO–endonuclease-based system (17).

Following complex assembly and checkpoint signaling, HR complexes assemble. Here, a different ssDNA-binding protein, Rad51, replaces RPA in a manner catalyzed by Rad52 and/or Brca2 (18–22) to form a nucleoprotein-filament structure (23, 24). Rad51-bound ssDNA invades the sister chromatid for repair synthesis followed by resolution and re-ligation. An error-prone variation on this repair mechanism is single-stranded annealing (SSA), where the resection events are the same but Rad51 is not recruited. Tandemly repeated sequences within the ssDNA then anneal, and the displaced flaps are removed by flap-endonucleases, thus deleting sequences between the tandem repeats (1, 25).

*S. pombe* has proven to be an excellent model to understand both checkpoint signaling and DSB repair mechanisms. Many genes that control these events were identified from collections of simple recessive loss–of–function mutants that were hypersensitive to DSB-inducing agents. Analysis of these genes, together with work in many experimental models, eventually defined pathways that are highly conserved to humans, highlighting the ancient origin and conserved nature of mechanisms to maintain genome stability. What eluded identification in these screens were the enzymes necessary for 5’-flap endonuclease activity, with the help of DNA helicase(s), 5’-flap endonucleases. There is no implication that there be one end-resecting activity nor that this need be the only function for such enzymes, and indeed such features made their identification challenging.

Recruitment data have argued for an involvement of the 3’→5’ exonuclease activity of MRN, which, importantly, would require an additional ssDNA gap to be formed 3’ to the DSB. However, in *S. pombe*, MRN is not required for Chk1 activation or checkpoint signaling in S- and G2-phases (26–28), and so whatever its activities may be at DSB, this complex is not required for the end resection to produce RPA-bound checkpoint complexes necessary to activate Chk1. Two other proteins, Ctp1/Ctp1 and Dna2, have been implicated to collaborate with MRN in DSB resection, but at least in *S. pombe*, ctp1 and dna2 are also not required for Chk1 activation (16, 29–32). However, the function of these enzymes is complex and may not be easily elucidated by studying single mutants. For example, there is a nuclease-independent role for Mre11, acting in concert with Ctp1, to initiate the processing of an HO endonuclease–induced DSB for eventual resection by Exo1 (and possibly other enzymes), but Mre11 nuclease activity is proposed to displace Ku70/80 and the nonhomologous end joining machinery to promote end resection (33).

In searching for end-resecting enzymes, we performed a checkpoint amplification screen in *S. pombe* to restore checkpoint arrest to a conditional allele of the effector kinase gene *chk1* that is defective in ATR-mediated activation. This identified three 5’→3’ exo- and 5’-flap endonucleases that are related to the canonical 5’-flap endonuclease for nucleotide excision repair, XPG. Thus, we have named these enzymes XPG-related nucleases (XRNs). These nucleases are encoded by *ast1*, *exo1*, and *rad2* in *S. pombe* and by *ASTE1*, *EXO1*, and *FEN1* in humans. Initial analyses showed that no single deletion of these genes ablates end resection, Chk1 activation, or DNA damage checkpoint arrest. Each of these enzymes is recruited to a euchromatic DSB at the *his3* locus, and cells mutant for all three XRNs failed to activate a checkpoint, failed to form damage-induced foci occupied by ssDNA-binding proteins, and failed to resect DSBs generated by irradiation or by the I–PpoI homing endonuclease in the 28S gene of the rDNA (34). Thus, the XRN enzymes fulfill the criteria for the molecules needed for DSB resection.

Notably, Rad2 has been implicated in DSB repair, base excision repair, and Okazaki fragment (OF) processing. Similarly, Exo1 functions in mismatch repair and, with Rad2, is essential for OF processing (35–40). Ast1, which is conserved to humans (ASTE1), was a newly identified gene in that screen. Although *ast1Δ* cells are not themselves DNA damage–sensitive, like all XRN null alleles, they are defective in DSB repair by SSA, which requires resection between the tandem repeats. In addition, *rad2Δ* is synthetically lethal with mutations in the MRN complex, whereas both *exo1Δ* and *ast1Δ* greatly limit the growth and enhance the DNA damage sensitivity of MRN mutants. The latter is suppressed by deleting *pku70* (encoding the Ku70 homolog), implicating the XRN enzymes in unblocking Ku-blocked ends (34).

The potential redundancy between XRN genes, as well as their multiple cellular functions, explains why they were difficult to identify and why teasing apart their relative contributions to DSB resection is complex. This does not imply, however, that they are functionally equivalent and that specificity of function may indeed be present at certain lesions, chromatin environments, and/or genomic loci. The conservation of all three genes to humans suggests there must be unique nonoverlapping functions that select for their evolutionary retention.

Here, we have built on the initial identification of the XRNs to systematically ask if there is a hierarchy by which they are recruited to DSBs versus a simple division of labor between three related enzymes. We show that they are not simply redundant and such a hierarchy of XRN recruitment does indeed exist. Furthermore, there is an ordered compensation of recruitment when one or more XRN enzymes are absent. Finally, there is a specificity for the requirement of XRN function depending on the direction of transcription at the DSB. These studies demonstrate that XRN-dependent end resection is a remarkably complex initiating event in the DNA damage response to DSBs, where the layers of options ensure both the G2 DNA damage checkpoint arrest and repair of these most pathological lesions.

**Results**

**Effects of XRN mutant combination of ssDNA-binding protein foci**

The overall goal of this study was to assess specificity of function for individual XRN enzymes in DSB resection as an explanation for the conservation of three related enzymes. We have previously used the formation of RPA and Rad51 foci at bleomycin-induced DSBs as a marker for (nonsite-specific) ssDNA production at lesions (34). As *exo1Δ rad2Δ* cells are synthetically lethal, we have used a thiamine-repressible “shut-off”...
indicating Ast1 contributes to end resection in the absence of ast1

In cells with bleomycin-induced foci (p < 0.05), but statistically significant reduction was seen (H11011). These data suggest a moderate (H11011) decrease in resection in the absence of Ast1. Thus, without knowing the genomic locations of these foci, we conclude that each XRN enzyme contributes, in concert, to ssDNA production following DSB induction.

However, at the cellular level, a partial response is still sufficient to send a checkpoint-activating signal for Chk1 to arrest the cycle of these cells in G2 phase.

**XRN expression profiles**

To extend the studies of foci formation to direct assays of XRN recruitment and resection, we needed to turn to a site-specific model of DSB induction. The model of choice for this study was the ahTet-driven I–Ppol-induced DSB in the 28S rDNA gene, which is present in ~150 copies in the S. pombe genome (41, 42) and is digested at an efficiency of ≥90% within 90–120 min of ahTet addition (43). We chose this system for three reasons. First, the rDNA is a natural site of DNA damage through replication stress (44). Second, both the cutting efficiency and kinetics of I–Ppol expressed from an ahTet-responsive promoter is far greater than what we and others have achieved with mnt1-driven expression of HO endonuclease (33, 34, 45, 46). Third, we have already shown that simultaneous deletion of all XRNs ablates resection of this lesion (34), indicating some combinatorial role for the XRNs at this locus.

However, we had not studied the recruitment of the individual XRN to this locus, nor the specific requirement of individual XRN in resecting DSBs at this site. Although this model also enabled comparison with our studies of an HO endonuclease–induced DSB at the euchromatic his3 locus, to which all XRN are recruited, however, with a cutting efficiency of only ~20%, coupled with ongoing repair of the break, this was not suitable for statistically significant resection assays. It is important to note that as cutting the 28S genes is a large number of DSBs, resection is never 100%, presumably as the cellular response machinery is saturated (34, 43).

Prior to embarking on these experiments, we first asked whether DSB induction or the complement of XRN genes affected the expression level of each XRN enzyme. To this end, we utilized C-terminally epitope-tagged alleles of ast1, exo1, and rad2 (34) and examined expression in a time course after I–Ppol induction in all viable genetic backgrounds. The epitope tags used (13×Myc on Rad2 and Exo1 and 3×HA on Ast1) where chosen, unlike other epitopes at these positions, had no measurable effect on the function of the tagged proteins using multiple assays involving DNA damage sensitivities and synthetic lethals. These extracts were prepared from flash-frozen cells under highly denaturing conditions to minimize any processing during extraction.

From this analysis, two pertinent observations were made (Fig. 2). First, although Ast1–HA3 was expressed at a single copy of the Mnt1 promoter, to investigate resection in rad2Δ exo1-so and ast1Δ rad2Δ exo1-so triple mutant cells (34); exo1-so is severely attenuated for expression, also synthetically lethal with rad2Δ, but is not a true null allele.

This prior analysis showed little evidence of ssDNA production in response to DSBs in the compound mutants, which was assessed by the formation of RPA and Rad51 foci, as well as physical assays for end resection. We inferred that the single mutants, as well as ast1Δ exo1Δ and ast1Δ rad2Δ double mutants, were proficient in foci formation as they are competent to activate Chk1. However, we did not assay foci formation on these backgrounds. Therefore, we repeated these experiments to assay RPA and Rad51 foci formation on all XRN mutant combinations.

We observed no significant difference in the behavior of WT cells and any of the single mutants: ast1Δ, exo1Δ, exo1-so, or rad2Δ (Fig. 1). Thus, no single XRN is required for ssDNA production in response to global DNA damage. Although checkpoint-proficient, both ast1Δ exo1Δ and ast1Δ rad2Δ showed a moderate (~30%) but statistically significant reduction in cells with bleomycin-induced foci (p = 0.006–0.037), indicating Ast1 contributes to end resection in the absence of Exo1 or Rad2. As observed previously (34), both rad2Δ exo1-so and ast1Δ rad2Δ exo1-so cells showed a more profound foci-forming defect (60–70% lower than WT, p = 0.002–0.0004), although only the triple mutant cells are defective in activating the Chk1-dependent cell cycle arrest, albeit not quite as defective as spores derived from the triple null mutant deleted for all three genes, i.e. Rad2 is critical for end resection in the absence of Exo1. Thus, without knowing the genomic locations of these foci, we conclude that each XRN enzyme contributes, in concert, to ssDNA production following DSB induction. However, at the cellular level, a partial response is still sufficient to send a checkpoint-activating signal for Chk1 to arrest the cycle of these cells in G2 phase.

**Figure 1. Formation of Rad51 and RPA foci following bleomycin treatment.** G2 cells of the indicated strains were left untreated or treated with 0.5 milliunits of bleomycin as described (34). Rad51 foci were imaged by immuno- fluorescence, and RPA foci were imaged by GFP–Rad11. Data are mean ± S.D. from three counts of 100 cells. Numbers in parentheses are p values (Student’s two-sided t test) of strains compared with WT. Absence of parentheses indicates a nonsignificant difference.

allele of exo1 (exo1-so), in which the exo1 promoter is replaced with an attenuated version of the mnt1 promoter, to investigate resection in rad2Δ exo1-so and ast1Δ rad2Δ exo1-so triple mutant cells (34); exo1-so is severely attenuated for expression, also synthetically lethal with rad2Δ, but is not a true null allele.
respectively), suggesting extensive processing and/or modification. A similar complex pattern of Exo1 species was seen with Exo1–HA3 (Fig. 2B), although we were not successful in producing a viable Rad2–HA3 to compare with Rad2–Myc13. Notably, all detected species were specific to the tagging of the XRN gene as indicated by a lack of signal in extracts produced from isogeneic strains lacking the epitope tag (labeled, No Tag).

Second, DSB induction and genetic background had no effect on expression patterns for each of the enzymes. Thus, any observed changes in recruitment or resection kinetics in the following experiments are likely to be either regulated by post-translational mechanisms or regulatory mechanisms functioning in trans without any damage-induced increase in enzyme expression.

**Recruitment of XRNs to the 28S rDNA DSB**

Our previous studies showed each XRN was efficiently recruited to an HO endonuclease–induced DSB in the euchromatic his3 locus (34). However, the cutting efficiency at this locus is only ~20% and is too low to rigorously measure resection kinetics. Thus, subsequent studies for resection kinetics were done at the I–Ppol-induced DSB in the 28S rDNA gene (90% efficient). Thus, we first repeated an analysis of XRN recruitment to this sites by chromatin immunoprecipitation (ChiP, Fig. 3), with isogenic untagged strains acting as negative controls. The experiments revealed several pertinent observations. First, both Exo1 and Rad2 (but not Ast1) were significantly enriched in the rDNA prior to the induction of I–Ppol, which was not observed at the euchromatic his3 locus (34). It is notable that the rDNA is a locus of much replication stress (44), and as Okazaki fragment processing and helix–hairpin–helix DNA-binding proteins (47, 48), the presence of Exo1 and Rad2 does not necessarily mean they are actively resecting DNA ends, i.e., a substrate of a free 5’-end or 5’-flap would be required for their activity at this locus. Second, Myc-tagged Exo1 and Rad2 were more significantly enriched over untagged controls than was observed for HA-tagged Ast1; however, the different epitope tags used have been chosen to optimize detection and function of the enzymes by multiple assays. Furthermore, the accessibility of the epitope tags may not be equivalent for each enzyme, and as with all ChiP experiments, we place less weight on the absolute recruitment value compared with (in this case) changes with DSB induction and genetic background (see below). Third, and most importantly, Exo1 was the only XRN that was significantly recruited to both sides of the DSB after I–Ppol induction when both Ast1 and Rad2 are present. This observation suggested Exo1 may be the primary resecting enzyme, at least in the context of the I–Ppol-induced DSB in...
Effects of XRN gene deletion of DSB resection

A defect in end resection should abolish both repair and Chk1-dependent checkpoint responses. This is predicated on the fact that end resection is required for Rad51 nucleoprotein filament formation in order for recombination to proceed (25), and prior to that, checkpoint-signaling filament formation in order for recombination to proceed (25), the fact that end resection is required for Rad51 nucleoprotein complexes to assemble on RPA-coated ssDNA to activate Chk1 (4). However, of the XRN genes, only rad2Δ cells are significantly sensitive to global DNA damage such as UV-C irradiation or treatment with alkylating agents (50). Furthermore, each of the single mutants are proficient in activating a checkpoint response following global DNA damage (34). We assessed I–Ppol-induced checkpoint activation by cell elongation (the cells continue to grow during the G2 checkpoint arrest of nuclear events), as well as an absence of (aberrant) mitotic cells that have escaped a checkpoint arrest. This was seen in 100% of WT, rad2Δ, and ast1Δ cells ((Fig. 4 and not shown) (34). exo1Δ showed a very low number (5.67 ± 1.53%) of cells with mitotic defects or failure to elongate, compared with a chk1Δ control, where 40.67 ± 3.51% (p = 3.65 × 10−5) cells entered lethal mitoses (the “cut” phenotype). Therefore, exo1 is dispensable for checkpoint signaling, at least from this locus. As the signal for Chk1 activation emanates from RPA-coated ssDNA, and in keeping with the Rad51 and RPA foci data (Fig. 1), ssDNA production upon DSB formation at this site cannot be solely dependent on Exo1 activity.

The checkpoint proficiency of exo1Δ cells was somewhat surprising given that Exo1 is the only XRN recruited to this site after DSB formation (Fig. 3). As exo1Δ rad2Δ cells are synthetically lethal, we used exo1-so to investigate resection in exo1-so rad2Δ double mutants and in triple XRN mutants, including ast1Δ (34). This prior analysis showed little resection of this site in these strains (34). Furthermore, the synthetic lethal phenotype of exo1Δ rad2Δ cells is due to induction of a chk1- and ast1-dependent checkpoint arrest, all indicating a concerted action of the XRN enzymes. However, we did not know whether Ast1 or Rad2 were recruited to the DSB in the absence of Exo1, which we next assayed by ChIP (Fig. 5). Unlike in WT cells, Ast1 (but not Rad2) was inducibly recruited to the DSB in exo1Δ cells, which is in keeping with the dependence on Ast1 to activate the lethal checkpoint arrest in exo1Δ rad2Δ cells (34) and the reduction in foci formation in ast1Δ exo1Δ cells (Fig. 1). Thus, Ast1 is a “back-up” activity in the absence of Exo1 at the rDNA. Notably, exo1Δ did not affect the baseline levels of Rad2 in the rDNA, indicating that Exo1 is not required to generate structures that promote the localization of Rad2 to the rDNA.

Effects of exo1Δ and ast1Δ on end resection

Given the above recruitment data, we next explored the effect of deleting exo1 and exo1 plus ast1 on resection of the I–Ppol-induced DSB (Fig. 6). The single deletion of exo1Δ had

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**Figure 4. Low level mitotic abnormality of I–Ppol exo1Δ cells after anhydrotetracycline treatment.** The tet-I–Ppol cells with either WT or exo1Δ background were either treated with 5 μM anhydrotetracycline for 4 h or left untreated (control). Cells were stained with DAPI, and images were captured. Cells lacking exo1 inefficiently elongated (asterisks) and displayed abnormal mitoses (arrow).

**Figure 5. XPG-related nuclease recruitment to an I–Ppol-induced DSB in the rDNA in exo1Δ cells.** Cells with the I–Ppol system and exo1Δ background were grown in exponentially growing cultures in YES medium and either left untreated or treated with 5 μM anhydrotetracycline for 30, 60, or 90 min. ChIP was performed and analyzed for occupancy at the BbsI site 3' to the DSB (A) or occupancy at the MscI site 5' to the DSB (B). Data (mean ± S.E.) are displayed as enrichment over isogenic untagged strains. Ast1–HA occupancy increased by 90 min of DSB induction in cells lacking exo1Δ, *p < 0.05 by Student’s two-sided t test with n = 5.

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Figure 6. DSB resection efficiency at an I–PpoI-induced DSB in the rDNA. Cells with the I–PpoI system in either WT, exo1Δ, or exo1Δ rad2Δ backgrounds were grown to exponential phase in YES medium and were then either left untreated or treated with 5 μM anhydrotetracycline for 2 h. Digests at indicated sites were performed, and qPCR was performed across the respective restriction sites. qPCR data were used to calculate % restriction. Resection efficiency (mean ± S.E., n = 4) was reduced significantly in exo1Δ cells specifically in the direction opposing transcription. *, p < 0.05; **, p < 0.005 WT versus exo1Δ; and #, p < 0.05 for WT versus ast1Δ exo1Δ by Student’s two-sided t test.

Figure 7. DSB resection efficiency at a PpoI-induced DSB in the euchromatic lys1 locus. WT cell expression in Exo1–Myc (plus untagged controls) with an I–PpoI site at lys1 (but lacking from the 28S rDNA gene) was grown to exponential phase and then either left untreated or treated with 5 μM anhydrotetracycline. A, DSB cutting efficiency at an I–PpoI-induced DSB in the lys1 ORF. Input ChIP DNA was analyzed by qPCR performed with primers across the PpoI cut site (PpoLysCut, see Table 2). qPCR of the ORF of the ade6 gene was used as a control for DNA content. Data are mean ± S.E., n = 3. Cutting efficiency rises to ~80% by 90 min of anhydrotetracycline treatment. B, ChIP of Exo1–Myc to the lys1 I–PpoI site. Data (mean ± S.E.) are displayed as enrichment over an isogenic untagged strain. Exo1–Myc occupancy increased by 90 min of DSB induction in cells lacking exo1.* , p < 0.05 by Student’s two-sided t test with n = 5. C, WT and exo1Δ cells with an I–PpoI site at lys1 (but lacking from the 28S rDNA gene) were treated with 5 μM anhydrotetracycline for 2 h, and resection was assayed at the indicated restriction sites. Data are mean ± S.E., n = 3. Resection efficiency was reduced significantly in exo1Δ cells in the direction opposing transcription of the lys1 5′ region. *, p < 0.05 WT versus exo1Δ by Student’s two-sided t test.

a small but statistically insignificant reduction in resection 3′ to the DSB (i.e. in the direction of transcription). This ssDNA production is thus likely to be sufficient for the checkpoint arrest in ~95% of these cells (Fig. 4) and probably for the observed RPA- and Rad51-foci formation (Fig. 1). However, exo1Δ cells were defective in resecting 5′ to the DSB (against the direction of transcription), even though Ast1 is recruited to DNA on this side of the break (Fig. 5). In exo1Δ ast1Δ cells, resection of both sides of the DSB was severely attenuated, similarly to what we previously observed for rad2Δ exo1-so and ast1Δ rad2Δ exo1-so cells (34).

Combining these data with our previous study (34), Exo1 is thus essential for resection of this DSB against the direction of rDNA transcription, and both Ast1 and Rad2 also function in end resection at these breaks. Such an asymmetry in DSB repair has not been previously observed. However, Exo1 is dispensable for resection in the same direction as transcription; on this side of the DSB, resection is largely Ast1-dependent, and as exo1-so rad2Δ cells fail to resect (34), is also dependent on Rad2 already present in the rDNA prior to DSB formation. These data confirm that all XRN enzymes contribute to DSB resection and add another layer of complexity to the response to DSBs, i.e. coordinating their processing for repair, and checkpoint signaling has a transcriptional directionality, in this case for RNA polymerase I.

These data further highlight that any conclusion of redundancy from more macro-cellular phenotypes, including survival data, can be and, in this case, are misleading.

Exo1-dependent resection at a euchromatic locus

We next asked whether the recruitment of Exo1 to the DSB in the 28S gene, together with the dependence on Exo1 to resect against the direction of transcription, could be observed at a euchromatic locus under the control of RNA polymerase II transcription. To this end, we selected 1-bp insertions in the 28S gene, rendering it I–PpoI-resistant, and then integrated an I–PpoI site at the lys1 locus (43). Upon induction of I–PpoI expression, DSB formation at this locus was ~80% efficient within 90 min (Fig. 7A), which was associated with significant accumulation of Exo1 at this DSB (Fig. 7B). Resection assays showed exo1Δ cells to be as efficient as WT cells 3′ to the DSB (in the direction of transcription), but as seen in the 28S gene, they have significantly less resection 5′ to the DSB (against the direction of transcription). Thus, the dependence on Exo1 to resect against the direction extends to this RNA polymerase II
gene and may therefore be a general feature of DSB resection within the transcribed DNA.

**Recruitment of Rad2 to rDNA and the DSB**

The final combination to investigate for recruitment was Rad2 as the sole XRN in ast1Δ, exo1Δ cells (Fig. 8). We performed this experiment by ChIP and made two important observations. First, the baseline levels of Rad2 in the rDNA (without the DSB) were reduced 3-fold in ast1Δ, exo1Δ cells, suggesting the combined activity of these XRN enzymes generates structures that recruit or retain the high baseline levels of Rad2. Second, Rad2 is now inducibly recruited to both sides of the I–PpoI-induced DSB. However, the recruitment is not quite statistically significant (p = 0.1) for the Mscl site 5’ to the DSB (Fig. 8) and is consistent with the minimal amount of resection in ast1Δ, exo1Δ cells (Fig. 6).

**Competition recruitment to the DSB**

Our observations suggested that Exo1 may compete with Rad2 and/or Ast1 for recruitment to the DSB. We therefore tested whether Ast1 and/or Rad2 could displace endogenous Exo1 when overexpressed. We therefore sought to complement our loss–of–function studies with competitive gain–of–function. To this end, Rad2 and Ast1 were overexpressed from the nmt1 promoter, which increased mRNA levels ~100–200-fold over endogenous mRNA levels (Fig. 9, A and B). Under these conditions, we then assayed recruitment of Exo1 following I–PpoI induction, testing whether the overexpressed Rad2 and/or Ast1 could compete for binding with the endogenous Exo1 at the 28S gene I–PpoI site. We observed almost complete induction in cells lacking exo1 and ast1, but not Ast1 overexpression, abrogated the recruitment of Exo1–Myc to the DSB. *, p < 0.05 by Student’s two-sided t test with n = 5.

Discussion

The identification of the XRN enzymes as DSB end-resecting molecules presented the conundrum as to why there are three distinct but functionally overlapping molecules. As all three are distinctly conserved from S. pombe to humans, this is not a case of redundancy arising from duplicated genes. This is in stark contrast to other DSB-processing enzymes and binding proteins, which exist either as single genes, or highly related and functionally equivalent homologs, albeit that some homologs are only expressed in the meiotic cell cycle.

Our previous work (34) showed that deletion of rad2 and exo1 led to a synthetic lethal cell cycle arrest. This arrest is promoted by Chk1 activation, which occurs on RPA-coated ssDNA generated at lesions, and is in turn dependent on ast1,
i.e. there is no Chk1 activation or checkpoint arrest in cells lacking all XRNs. Coupled with this, there were three observations that suggested the potential for redundancy among the XRNs. First, all three enzymes were recruited to an HO-induced DSB in the his3 gene. Second, cells lacking all XRNs failed to produce ssDNA following DNA damage, as assessed by native Southern blotting and the formation of RPA and Rad51 foci, the two ssDNA-binding proteins at DSBs. Finally, cells simultaneously lacking all XRNs failed to resect a DSB in the 28S rDNA gene. Thus, we initially concluded that the existence of three XRNs was selected for by other functions (e.g. Okazaki fragment processing and excision repair pathways) that contribute to the fitness advantage that ensured conservation from S. pombe to humans. Our findings here, however, show that this conclusion is not valid, and although one enzyme can substitute for the absence of others, the recruitment to and resection of this DSB in the rDNA occur in a hierarchical and nonsymmetrical manner.

As rad2 is the only XRN gene required for resistance to DNA-damaging agents (34), it was natural to expect Rad2 to be the primary DSB end resector. On the contrary, at least for this DSB, Rad2 is only recruited in the absence of both Exo1 and Ast1 (Fig. 8). However, when overexpressed, Rad2 can compete with Exo1 for DSB binding (Fig. 9). Thus, although Rad2 can bind to and resect this DSB in the rDNA, albeit with lower affinity than Exo1, the resection of DSBs at other regions of the genome, and/or the processing of other structures such as 5'-flaps, is likely to be more important in surviving genotoxins.

In WT cells, Exo1 was the only XRN inductively recruited to this DSB (Fig. 3) and is especially important to resect the broken end against the direction of transcription (5' to the DSB, Fig. 6). These observations were corroborated with DSB resection at the lys1 locus (Fig. 7), showing directionality was not limited to sites of RNA polymerase I transcription. The proficiency of exo1Δ cells in resecting the DSB in the same direction as transcription, which in turn depends on Ast1 and Rad2 (Fig. 6) (34), suggests that this region may be the major site for assembly of ssDNA-binding proteins and checkpoint signaling complexes following cleavage of the 28S gene in exo1Δ and exo1Δ ast1Δ cells. The asymmetry of resection may explain why each XRN single mutant cell is deficient in repair of lesions between tandemly repeated ade6 heteroalleles (34). In these assays, the repair is via the SSA mode of homologous recombination and requires resection in both directions of the region between the heteroalleles to expose homologous sequences to anneal independently of Rad51's function. Although this pathway also requires 5'-flap removal, the predominant enzyme for this step is the XPF (Rad1/10) nuclease (encoded by rad16 and swi10 in S. pombe) (34, 51–54). At this stage, we do not know whether a small amount of resection 5' to the DSB (Fig. 6) is sufficient for conventional Rad51-dependent HR-mediated repair, but this seems likely as exo1Δ cells are by themselves not hypersensitive to genotoxins (55).

Exo1 homologs have been implicated in end-resection in S. cerevisiae and in humans (47, 56–60). However, our studies and others show Exo1 is not, by itself, essential for DSB end resection at least in S. pombe. Exo1 knockout mice are viable, but have reduced longevity and are cancer-prone, which has been attributed to mismatch repair deficiency (61). This phenotype is in contrast to Chk1 knockout mice, which die prior to implantation (62), suggesting there must also be other mechanisms to resect DNA ends for Chk1 activation in mammals. Furthermore, the combined loss of Exo1 with Rad2 homologs (Rad27 in S. cerevisiae and Fen1 in humans) and Ast1 (ASTE1 in humans, although not present in S. cerevisiae) has not been studied in any other system in the context of end resection. However, exo1Δ rad27Δ double mutants are also synthetically lethal in S. cerevisiae, which has been attributed to defective Okazaki fragment processing (47, 63, 64). However, our DSB resection assays have been performed in (predominantly) G2 cells after Okazaki fragments are processed (34). Thus, any involvement of Fen1 homologs in DSB resection would also need to be performed in G2 cells, and to study the Fen1 plus Exo1 loss would require conditional inactivation of one of the enzymes after Okazaki fragment processing. The same argument applies to many other enzymes that could participate in end resection but also function at sites of DNA replication, such as DNA helicases.

It is also important to note, however, that the findings presented here for this I–Ppol-induced DSB may not be mirrored at other loci or at other types of DNA ends (I–Ppol leaves a 4-bp 3’ overhang (43)). Such a notion is consistent with the observed formation of Rad51 and RPA foci following bleomycin treatment (Fig. 1). Different requirements and hierarchies of XRN recruitment for resection at other loci may also contribute to the fitness advantage that has conserved all three genes, together with the roles for Rad2/Fen1 and Exo1 homologs in Okazaki fragment processing and excision repair pathways. Most probably because there is no Ast1 homolog in S. cerevisiae, a system that has been at the cornerstone of DNA repair genetics, relatively little is known about this enzyme. Ast1 is required for DNA repair by single-stranded annealing, for DNA damage resistance and unblocking of ends in MRN mutants, and for checkpoint activation in rad2Δ exo1Δ cells (34). Ast1 also contributes to Rad51- and RPA-foci formation in the absence of Exo1 or Rad2 (Fig. 1) and contributes to DSB resection in the 28S gene in exo1Δ cells (Fig. 5). Despite this, however, Ast1 is not required for cellular resistance to a large range of genotoxins that induce genome-wide DNA damage (although neither is Exo1) nor for meiotic recombination, and so its conservation to humans suggests the yet to be uncovered functions that maintain its selection, and it will be critical to define these; of course this may include DSB resection in certain contexts that can be compensated for by Exo1 and/or Rad2. Moreover, the fact that overexpressed Ast1 (unlike Rad2) cannot compete with Exo1 for DSB recruitment (Fig. 9) suggests that there is more to the regulation of Ast1 activity than we currently know, and such regulation is crucial for Ast1 to substitute for Exo1.

Our observations show that DSB end resection is a multilayered process, and may be combinatorially more complicated than indicated by this initial analysis. In any case, this begs the question of how the hierarchy is established. It is notable that many species of Rad2 and Exo1 are expressed (Fig. 2), and post-translational modifications that regulate XRN recruitment to, or activity at, DSBs may be another layer of complexity. More-
**Table 1**

| Primer set | Reverse | Forward |
|------------|---------|---------|
| Act1       | CCATTGACCACGGTATCTTC | ACGGCTTCACTGCAAAGGC |
| Ast1       | ATTCAAGGCTGACGACGCAA | CTTGAAGCAGAAATGGAAGCA |
| Rad2       | CCTGTAAGCCGACAGCTTA | AGACAAAGTGTCCAGTTC |

**XRN recruitment to DSBs**

over, *trans*-acting factors such as histone modifications and associated chromatin accessibility and/or transcriptional status may also be a determining factor in XRN specificity.

There is clearly a lot to learn about the early aspects of DSB repair and checkpoint signaling and how these events are regulated in time and space. These events are at the foundation of the processes that maintain the stability of the genome, and thus gaining a detailed view of DSB end resection has profound implications that are central to normal cellular homeostasis and diseases of genome instability.

**Experimental procedures**

**Standard S. pombe protocols**

All strains used were derived from 972h- and 97sh+. Standard classical and molecular genetic protocols and growth conditions were followed as described previously (65). Genetic crosses were performed by tetrad analysis, and multiple isolates of each strain were analyzed. Cell pellets were frozen in liquid nitrogen, and total protein was extracted in 8 M urea, 100 mM NaPO4, 10 mM Tris, pH 8.0. Expressed proteins were detected by Western blotting, as described previously (66), with antibodies against the HA epitope (12CA5, Roche Applied Science), the c-Myc epitope (9E10, Roche Applied Science), or α-tubulin (B-5-1-2, Sigma) and with horseradish peroxidase–coupled anti-mouse IgG antibodies (Life Technologies, Inc.) and developed with Clarity Reagent (Bio-Rad). The functionality of all epitope-tagged alleles was confirmed by multiple genetic assays, and the integrity of the tagged loci was confirmed by PCR/sequencing and Southern blotting (34). For nuclear morphometry microscopy, cells were fixed in 3.7% formaldehyde and stained with 1 µg/ml 4',6-diamino-2-phenylindole (DAPI).

**Methods for assaying the formation of bleomycin-induced RPA**

**Table 2**

| Primer set | Reverse | Forward |
|------------|---------|---------|
| nmt1       | AAAGTTGATTTATTTGCTTG | CACACTGACGCTACATGAC |
| Ast1       | GCCGGGAGTTGAGATTGTTT | CCCTAGTAAAGTCCTTTTT |

**DSB resection analysis**

Cells were treated with 5 µM anhydrotricine for 2 h or left untreated. Cells were collected by centrifugation and resuspended in 5–25 ml of cold stop buffer (150 mM NaCl, 50 mM NaF, 10 mM EDTA, 1 mM sodium azide) and left on ice for at least 5 min.

Cells were digested in CSE buffer (20 mM citrate-phosphate, pH 5.6, 40 mM EDTA, 1.2 M sorbitol) with 5 mg/ml Zymolyase 20T at 37 °C for 2–4 h. Cells were lysed in 5× TE with 1% SDS (50 mM Tris, pH 8.0, 5 mM EDTA, 1% SDS) at 65 °C for 1–1.5 h. SDS was precipitated by adding 1/3 volume of 5 M potassium acetate and incubation on ice. Tubes were spun for 15 min at 16,100 × g; supernatants were collected, and nucleic acid was precipitated by adding 1 volume of cold isopropyl alcohol and incubated at 4 °C overnight. RNA was digested by incubation of nucleic acid precipitates in 1× TE (10 mM Tris, pH 8.0, 1 mM EDTA) with 1 mg/ml RNase A at 37 °C for 1–1.5 h. Phenol/chloroform extraction was then performed. DNA was pre-
cipated by adding a half-volume of 7.5 mM ammonium acetate and then 1 volume of cold ethanol. DNA precipitates were resuspended in water.

100 ng of DNA was digested with restriction enzymes using 20 units of restriction enzyme in a total volume of 40–100 μl at 37 °C for 4 h. Restriction enzymes were inactivated by raising samples to 95 °C for 5 min. As an undigested control, DNA was incubated in the respective restriction enzyme buffer by the same protocol. Both controls and reaction mixtures were then analyzed by qPCR with primers that amplify across the respective cut site using the conditions described above.

The amount of DNA was determined by use of a standard curve relating DNA amount and Ct value: ΔDNA = 96.4e^(−0.678(Ct_{digested} − Ct_{undigested})). To determine percent resection, Equation 1 was used.

% resection = 200(1 − (ΔDNA_{digested}/ΔDNA_{control}))/((2 − (ΔDNA_{digested}/ΔDNA_{control}))) (Eq. 1)

Data points represent mean values from at least three experiments. Error bars represent standard error of the mean. qPCR primers used for end resection and ChIP experiments are listed in Table 2 (46).

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Table 2
qPCR primers used for end resection and ChIP experiments

| Primer set | Forward | Reverse |
|------------|---------|---------|
| 28S rDNA   | TCGAAGCAGCTCTTACTGTGGAG | GCCGATTCTCGTCTATAG |
| BglI       | GAAGTCTGCTGACACATGAC   | GCCGATTCTCGTCTATAG |
| AatII      | TGGCGTATGACTGCGATG     | GCCGATTCTCGTCTATAG |
| BamHI      | TAAGGAAACCTGAGCTATG   | GCCGATTCTCGTCTATAG |
| MscI       | CACCACTTATGCAGCTGAG   | GCCGATTCTCGTCTATAG |
| BsuI       | TCTCCTCTACTGAGCCACTG   | GCCGATTCTCGTCTATAG |
| NruI       | TCACACGCTGAGCTGAG      | GCCGATTCTCGTCTATAG |
| EcoRI      | TCGGTAATTTTATATGCTA   | GCCGATTCTCGTCTATAG |
| SacII      | ACCGAGGAGACGCTTATTA   | GCCGATTCTCGTCTATAG |
| 28S Cut    | CGCAATTTTATATGCTATAG  | GCCGATTCTCGTCTATAG |

lys1

PpoLysR | CCGCTGCTCTTACTGTGGAG | GCCGATTCTCGTCTATAG |
| PpoLysCut| GAAGTCTGCTGACACATGAC | GCCGATTCTCGTCTATAG |
| HindIIIFar| TAAGGAAACCTGAGCTATG | GCCGATTCTCGTCTATAG |
| Agel     | AACCTTGCTGCTATGGAA   | GCCGATTCTCGTCTATAG |
| HindII   | CAACCATTGCTGCTGCTGACT | GCCGATTCTCGTCTATAG |
| AatII    | TGGCGTATGACTGCGATG    | GCCGATTCTCGTCTATAG |
| PstI     | GCTCCTCTTACTGTGGAG    | GCCGATTCTCGTCTATAG |
| SacII    | GCTGTTCTCTTACTGTGGAG | GCCGATTCTCGTCTATAG |
| EcoRIFar | GCGCTGCTCTTACTGTGGAG | GCCGATTCTCGTCTATAG |

References

1. Symington, L. S., and Gautier, J. (2011) Double-strand break end resection and repair pathway choice. Annu. Rev. Genet. 45, 247–271 CrossRef Medline
2. O’Connell, M. J., Walworth, N. C., and Carr, A. M. (2000) The G2-phase DNA-damage checkpoint. Trends Cell Biol. 10, 296–303 CrossRef Medline
3. MacDougall, C. A., Byun, T. S., Van, C., Yee, M. C., and Cimprich, K. A. (2007) The structural determinants of checkpoint activation. Genes Dev. 21, 898–903 CrossRef Medline
4. O’Connell, M. J., and Cimprich, K. A. (2005) G2 damage checkpoints: what is the turn-on? J. Cell Sci. 118, 1–6 CrossRef Medline
5. Walworth, N. C., and Bernards, R. (1996) rad53 kinase-dependent response of the chk1-encoded protein kinase at the DNA damage checkpoint. Science 271, 353–356 CrossRef Medline
6. Capasso, H., Palermo, C., Wan, S., Rao, H., John, U. P., O’Connell, M. J., and Walworth, N. C. (2002) Phosphorylation activates Chk1 and is required for checkpoint-mediated cell cycle arrest. J. Cell Sci. 115, 4555–4564 CrossRef Medline
7. Rhind, N., Furnari, B., and Russell, P. (1997) Cdc2 tyrosine phosphorylation is required for the DNA damage checkpoint in fission yeast. Genes Dev. 11, 504–511 CrossRef Medline
8. O’Connell, M. J., Raleigh, J. M., Verkade, H. M., and Nurse, P. (1997) Chk1 is a weel kinase in the G2 DNA damage checkpoint inhibiting cdc2 by Y15 phosphorylation. EMBO J. 16, 545–554 CrossRef Medline
9. Latif, C., den Elzen, N. R., and O’Connell, M. J. (2004) DNA damage checkpoint maintenance through sustained Chk1 activity. J. Cell Sci. 117, 3489–3498 CrossRef Medline
with Mre11 complex to control double-strand break repair by homologous recombination. *Mol. Cell* **28**, 134–146 CrossRef Medline

31. Limbo, O., Porter-Goff, M. E., Rhind, N., and Russell, P. (2011) Mre11 nuclease activity and Ctp1 regulate Chkl activation by Rad3ATR and Tel1ATM checkpoint kinases at double-strand breaks. *Mol. Cell. Biol.* **31**, 573–583 CrossRef Medline

32. Farah, J. A., Cromie, G. A., and Smith, G. R. (2009) Ctp1 and exonuclease 1, alternative nucleases regulated by the MRN complex, are required for efficient meiotic recombination. *Proc. Natl. Acad. Sci. U.S.A.* **106**, 9356–9361 CrossRef Medline

33. Langerak, P., Mejia-Ramirez, E., Limbo, O., and Russell, P. (2011) Release of Ku and MRN from DNA ends by Mre11 nuclease activity and Ctp1 is required for homologous recombination repair of double-strand breaks. *PLoS Genet.* **7**, e1002771 CrossRef Medline

34. Kurtz, K., and O’Connell, M. J. (2013) Initiation of DNA damage responses through XPG-related nucleases. *EMBO J.* **32**, 290–302 CrossRef Medline

35. Lehmann, A. R., Wallick, M., Grittiths, D. J., Murray, J. M., Watts, F. Z., McCready, S., and Carr, A. M. (1995) The rad18 gene of *Schizosaccharomyces pombe* defines a new subgroup of the SMC superfamily involved in DNA repair. *Mol. Cell. Biol.* **15**, 7067–7080 CrossRef Medline

36. Memisoglu, A., and Samson, L. (2000) Contribution of base excision repair, nucleotide excision repair, and DNA recombination to alkylation resistance of the fission yeast *Schizosaccharomyces pombe*. *J. Bacteriol.* **182**, 2104–2112 CrossRef Medline

37. Balakrishnan, L., and Bambara, R. A. (2013) Flap endonuclease 1. *Annu. Rev. Biochem.* **82**, 119–138 CrossRef Medline

38. Marti, T. M., Kunz, C., and Fleck, O. (2002) DNA mismatch repair and mutation avoidance pathways. *J. Cell Physiol.* **191**, 28–41 CrossRef Medline

39. Sun, X., Thrower, D., Qiu, J., Wu, P., Zheng, L., Zhou, M., Bachant, J., Wilson, D. M., 3rd, and Shen, B. (2003) Complementary functions of the *Saccharomyces cerevisiae* Rad2 family nucleases in Okazaki fragment maturation, mutation avoidance, and chromosome stability. *DNA Repair* **2**, 925–940 CrossRef Medline

40. Tsutakawa, S. E., Lafrange-Vanasse, J., and Tainer, J. A. (2014) The cutting edges in DNA repair, licensing, and fidelity: DNA and RNA repair nucleases sculpt DNA to measure twice, cut once. *DNA Repair* **19**, 95–107 CrossRef Medline

41. Wood, V., Gwilliam, R., Rajandream, M. A., Lyne, M., Lyne, R., Stewart, A., Sgouros, J., Peat, N., Hayles, B., Baker, S., Basham, D., Bowman, S., Brooks, K., Brown, D., Brown, S., et al. (2002) The genome sequence of *Schizosaccharomyces pombe*. *Nature* **415**, 871–880 CrossRef Medline

42. Wood, V., Harris, M. A., McDowall, M. D., Rutherford, K., Vaughan, B. W., Staines, D. M., Aslett, M., Lock, A., Bähler, J., Kersey, P. J., and Oliver, S. G. (2012) PomBase: a comprehensive online resource for fission yeast. *Nucleic Acids Res.* **40**, D695–D699 CrossRef Medline

43. Sunder, S., Greeson-Lott, N. T., Runge, K. W., and Sanders, S. L. (2012) A new method to efficiently induce a site-specific double-strand break in the fission yeast *Schizosaccharomyces pombe*. *Yeast* **29**, 275–291 CrossRef Medline

44. Murray, J. M., and Carr, A. M. (2008) Smc5/6: a link between DNA repair and unidirectional replication? *Nat. Rev. Mol. Cell Biol.* **9**, 177–182 CrossRef Medline

45. Du, L. L., Nakamura, T. M., Moser, B. A., and Russell, P. (2003) Retention of Ctp1 nuclease activity and Ctp1 gene involved in DNA repair and telomere maintenance. *Mol. Cell. Biol.* **23**, 6553–6563 CrossRef Medline

46. Hartsuiker, E., Vaessen, E., Carr, A. M., and Kohli, J. (2001) Fission yeast Rad50 stimulates sister chromatid recombination and links cohesion with repair. *EMBO J.* **20**, 6660–6671 CrossRef Medline

47. Akcasabas, A. A., Osborn, A. J., Bachant, J., Hu, F., Werler, P. J., Boussert, K., Furuya, K., Diffee, J. F., Carr, A. M., and Elledge, S. J. (2001) Mrc1 transduces signals of DNA replication stress to activate Rad53. *Nat. Cell Biol.* **3**, 958–965 CrossRef Medline

48. Allen, J. B., Zhou, Z., Siede, W., Friedberg, E. C., and Elledge, S. J. (1994) The *sox3* gene of *Escherichia coli* encodes a phosphatase that blocks DNA damage-induced transcription in yeast. *Genes Dev.* **8**, 2401–2415 CrossRef Medline

49. Cohen-Fix, O., and Koshland, D. (1997) The anaphase inhibitor of *Schizosaccharomyces pombe*. *Curr. Biol.* **7**, 314–317 CrossRef Medline

50. Yang, H., et al. (2002) Smc5/6: a link between DNA repair and telomere maintenance. *Mol. Cell. Biol.* **22**, 10729–10736 CrossRef Medline

51. Thorslund, T., and West, S. C. (2007) BRCA2: a universal recombinase protein involved in DNA double-strand break repair in association with the Mre11 complex to control double-strand break repair by homologous recombination. *Mol. Cell* **28**, 134–146 CrossRef Medline

52. Mortensen, U. H., Lisby, M., and Rothstein, R. (2009) Rad52. *Curr. Biol.* **19**, R676–R677 CrossRef Medline

53. Short, J. M., Liu, Y., Chen, S., Soni, N., Madhusudhan, M. S., Shiviji, M. K., and Venkitaraman, A. R. (2016) High-resolution structure of the presumptive RAD51 filament on single-stranded DNA by electron cryo-microscopy. *Nucleic Acids Res.* **44**, 9017–9030 CrossRef Medline

54. Zhao, L., Xu, J., Zhao, W., Sung, P., and Wang, H. W. (2018) Determining the RAD51–DNA nucleoprotein filament structure and function by cryo-electron microscopy. *Methods Enzymol.* **600**, 179–199 CrossRef Medline

55. Krogh, B. O., and Symington, L. S. (2004) Recombination proteins in yeast. *Annu. Rev. Genet.* **38**, 233–271 CrossRef Medline

56. Wilson, S., Warn, N., Taylor, D. L., and Watts, F. Z. (1999) The role of telomere-related proteins in *Schizosaccharomyces pombe*. *Cell* **99**, 134–146 CrossRef Medline

57. Ueno, M., Nakazaki, T., Akamatsu, Y., Watanabe, K., Tomita, K., Lindsay, H. D., Shinagawa, H., and Iwasaki, H. (2003) Molecular characterization of the *Schizosaccharomyces pombe* nip1+ gene involved in DNA repair and telomere maintenance. *Mol. Cell. Biol.* **23**, 653–656 CrossRef Medline

58. Hartsuiker, E., Vaessen, E., Carr, A. M., and Kohli, J. (2001) Fission yeast Rad50 stimulates sister chromatid recombination and links cohesion with repair. *EMBO J.* **20**, 6660–6671 CrossRef Medline

59. Akamatsu, Y., Murayama, Y., Yamada, T., Nakazaki, T., Tsutsui, Y., Ohba, K., and Iwasaki, H. (2008) Molecular characterization of the role of the *Schizosaccharomyces pombe* nip1+ gene in DNA double-strand break repair in association with the Mre11–Rad50–Nbs1 complex. *Mol. Cell. Biol.* **28**, 3639–3651 CrossRef Medline

60. Limbo, O., Chahwan, C., Yamada, Y., de Bruin, R. A., Wittenberg, C., and Russell, P. (2007) Ctp1 is a cell cycle-regulated protein that functions...
quirement for multiple nucleases and a novel Rad18 function. Genetics 175, 1585–1595 CrossRef Medline

50. Murray, J. M., Tavassoli, M., al-Harithy, R., Sheldrick, K. S., Lehmann, A. R., Carr, A. M., and Watts, F. Z. (1994) Structural and functional conservation of the human homolog of the Schizosaccharomyces pombe rad2 gene, which is required for chromosome segregation and recovery from DNA damage. Mol. Cell. Biol. 14, 4878–4888 CrossRef Medline

51. Osman, F., Fortunato, E. A., and Subramani, S. (1996) Double-strand break-induced mitotic intrachromosomal recombination in the fission yeast Schizosaccharomyces pombe. Genetics 142, 341–357 Medline

52. Fishman-Lobell, J., and Haber, J. E. (1992) Removal of nonhomologous DNA ends in double-strandbreak recombination: the role of the yeast ultraviolet repair gene RAD1. Science 258, 480–484 CrossRef Medline

53. Ivanov, E. L., and Haber, J. E. (1995) RAD1 and RAD10, but not other excision repair genes, are required for double-strand break-induced recombination in Saccharomyces cerevisiae. Mol. Cell. Biol. 15, 2245–2251 CrossRef Medline

54. Toh, G. W., Sugawara, N., Dong, J., Toth, R., Lee, S. E., Haber, J. E., and Rouse, J. (2010) Mec1/Tel1-dependent phosphorylation of Slx4 stimulates Rad1-Rad10-dependent cleavage of non-homologous DNA tails. DNA Repair 9, 718–726 CrossRef Medline

55. Szankasi, P., and Smith, G. R. (1995) A role for exonuclease I from S. pombe in mutation avoidance and mismatch correction. Science 267, 1166–1169 CrossRef Medline

56. Llorente, B., and Symington, L. S. (2004) The Mre11 nuclease is not required for 5′ to 3′ resection at multiple HO-induced double-strand breaks. Mol. Cell. Biol. 24, 9682–9694 CrossRef Medline

57. Mimitou, E. P., and Symington, L. S. (2008) Sae2, Exo1 and Sgs1 collaborate in DNA double-strand break processing. Nature 455, 770–774 CrossRef Medline

58. Mimitou, E. P., and Symington, L. S. (2009) DNA end resection: many nucleases make light work. DNA Repair 8, 983–995 CrossRef Medline

59. Mimitou, E. P., and Symington, L. S. (2009) Nucleases and helicases take center stage in homologous recombination. Trends Biochem. Sci. 34, 264–272 CrossRef Medline

60. Mimitou, E. P., and Symington, L. S. (2010) Ku prevents Exo1 and Sgs1-dependent resection of DNA ends in the absence of a functional MRX complex or Sae2. EMBO J. 29, 3358–3369 CrossRef Medline

61. Wei, K., Clark, A. B., Wong, E., Kane, M. F., Mazur, D. J., Parris, T., Kolas, N. K., Russell, R., Hou, H., Jr., Kneitz, B., Yang, G., Kunkel, T. A., Kolodner, R. D., Cohen, P. E., and Edelmann, W. (2003) Inactivation of exonuclease 1 in mice results in DNA mismatch repair defects, increased cancer susceptibility, and male and female sterility. Genes Dev. 17, 603–614 CrossRef Medline

62. Liu, Q., Guntuku, S., Cui, X. S., Matsuoka, S., Cortez, D., Tamai, K., Luo, G., Carattini-Rivera, S., DeMayo, F., Bradley, A., Donehower, L. A., and Elledge, S. J. (2000) Chk1 is an essential kinase that is regulated by Atr and required for the G(2)/M DNA damage checkpoint. Genes Dev. 14, 1448–1459 Medline

63. Tishkoff, D. X., Boerger, A. L., Bertrand, P., Filosi, N., Gaida, G. M., Kane, M. F., and Kolodner, R. D. (1997) Identification and characterization of Saccharomyces cerevisiae EXO1, a gene encoding an exonuclease that interacts with MSH2. Proc. Natl. Acad. Sci. U.S.A. 94, 7487–7492 CrossRef Medline

64. Tran, P. T., Erdeniz, N., Dudley, S., and Liskay, R. M. (2002) Characterization of nuclease-dependent functions of Exo1p in Saccharomyces cerevisiae. DNA Repair 1, 895–912 CrossRef Medline

65. Moreno, S., Klar, A., and Nurse, P. (1991) Molecular genetic analysis of fission yeast Schizosaccharomyces pombe. Methods Enzymol. 194, 795–823 CrossRef Medline

66. Calonge, T. M., and O'Connell, M. J. (2006) Antagonism of Chk1 signaling in the G2 DNA damage checkpoint by dominant alleles of Cdr1. Genetics 174, 113–123 CrossRef Medline

67. Maudrell, K. (1993) Thiamine-repressible expression vectors pREP and pRIP for fission yeast. Gene 123, 127–130 CrossRef Medline

68. Tapia-Alveal, C., Lin, S. J., Yeoh, A., Jabado, O. J., and O'Connell, M. J. (2014) H2A.Z-dependent regulation of cohesin dynamics on chromosome arms. Mol. Cell. Biol. 34, 2092–2104 CrossRef Medline