The MRE11-RAD50-XRS2 Complex, in Addition to Other Non-homologous End-joining Factors, Is Required for V(D)J Joining in Yeast

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Lymphoid cells of the vertebrate immune system rely on factors in the non-homologous end-joining (NHEJ) DNA repair pathway to form signal joints during V(D)J recombination. Unlike other end-joining reactions, signal joint formation is a specialized case of NHEJ that also requires the lymphoid-specific RAG proteins. Whether V(D)J recombination requires the Mre11-Rad50-Nbs1 complex remains an open question, as null mutations in any member of the complex are lethal in mammals. However, Saccharomyces cerevisiae strains carrying null mutations in components of the homologous Mre11p-Rad50p-Xrs2p (MRX) complex are viable. We therefore took advantage of a recently developed V(D)J recombination assay in yeast to assess the role of MRX in V(D)J joining. Here we confirmed that signal joint formation in yeast is dependent on the same NHEJ factors known to be required in mammalian cells. In addition, we showed an absolute requirement for the MRX complex in signal joining, suggesting that the Mre11-Rad50-Nbs1 complex may be required for signal joint formation in mammalian cells as well.

The immune systems of jawed vertebrates assemble a diverse array of immunoglobulin molecules and T-cell receptors by the process known as V(D)J recombination (1). This site-specific recombination reaction is initiated by the lymphoid-specific proteins, RAG1 and RAG2, which together compose the V(D)J recombinase. RAG1/2 bind recombination signal sequences (RSSs)1 flanking the borders of gene segments and create a double-strand break via a two-step transeseification mechanism common to several transposases and retroviral integrases (2, 3). Two distinct types of DNA ends are generated from this mechanism of cleavage: blunt, 5′-phosphorylated, 3′-hydroxyl signal ends and covalently sealed hairpin-coding ends (2, 4, 5). Coding joints are then formed by the imprecise fusion of coding ends, whereas signal joints are formed by joining signal ends without nucleotide loss or addition. Like other double-strand breaks in mammalian cells, repair of RAG1/2-induced breaks requires ubiquitously expressed factors of the non-homologous end joining (NHEJ) repair pathway (6).

However, the repair phase of V(D)J recombination is a specialized case of NHEJ, where the repair of double-strand breaks is dependent not only on components of the NHEJ pathway but also on the RAG proteins themselves. Mutations in both RAG1 and RAG2 have been identified that interfere with V(D)J joining but not V(D)J cleavage in vivo and in vitro (7–10). Furthermore, in vitro reconstitution of the joining phase of V(D)J recombination shows a dependence on RAG1 and RAG2 (11). Although the precise role of the RAG proteins in the post-cleaveage events of V(D)J recombination is not known, RAG1 and RAG2 do remain associated with the DNA ends in a stable, heparin-resistant, synaptic complex post-cleavage in vitro (12–15). Moreover, nuclease-resistant complexes containing the RAG proteins and cleaved signal ends have recently been isolated from mammalian cells (16). These findings have led to the notion that, in addition to DNA cleavage functions, the RAG proteins play at least an architectural role in the joining phase of the reaction either by binding and aligning ends prior to joining, or by protecting ends from random nuclease digestion, or possibly by recruiting repair factors to the post-cleavage complex (10). More recently it has been suggested that in mammalian cells the RAG proteins may actually function post-cleavage as molecular shepherds that guide the repair of broken ends down the NHEJ pathway and away from inappropriate repair by homologous recombination (17).

In addition to RAG1 and RAG2, at least four ubiquitously expressed factors are also known to be required for joining signal ends in mammalian cells: Ku70, Ku80, XRCC4, and DNA ligase IV. These factors and possibly others mediate the fusion of blunt signal ends to one another without loss or addition of nucleotides to create a precise signal joint (6). Exactly how these factors facilitate signal joint formation has not been well defined, but biochemical activities for each of these proteins have been characterized. Ku70 and Ku80, which together compose the Ku heterodimer, bind DNA ends. The DNA end-binding activity of the Ku proteins may function to protect ends from degradation and may also function to recruit other NHEJ factors to the site of the double-strand break (18).

The online version of this article (available at http://www.jbc.org) contains supplemental Fig. 1.

This paper is available on line at http://www.jbc.org

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1 The abbreviations used are: RSSs, recombination signal sequences; NHEJ, non-homologous end-joining; MRN, Mre11-Rad50-Nbs1 complex; MRX, Mre11p-Rad50p-Xrs2p complex.
joining of DNA ends requires DNA ligase IV, which physically interacts with XRCC4; the interaction with XRCC4 is thought to enhance the stability and activity of DNA ligase IV (19–23). Ku70, Ku80, XRCC4, and DNA ligase IV are also necessary for the joining of coding ends, although the greater complexity of coding joint formation requires at least two additional factors: DNA-PKcs and Artemis (6). Based on genetic and biochemical data, Artemis is thought to be responsible for the nucleolytic opening of hairpin coding ends (24–26). Budding yeast also has an Artemis homologue (PSO2) (27), which proves to be necessary either for the excision of the Activator/Dissociaters (Ac/Ds) transposable element or for processing of hairpin ends generated by Ac/Ds transposon excision (28). Additional factors are also likely to be involved in V(D)J joining but they await identification (29).

Three interesting candidates for involvement in the repair phase of V(D)J recombination are members of the MRN complex: Mre11, Rad50, and Nbs1. The MRN complex is a nuclear protein complex that is thought to be intimately involved in sensing and signaling the presence of double-strand breaks in yeast and mammals (30). The complex has been implicated in an array of cellular responses involving the repair of double-strand breaks: homologous recombination, telomere length maintenance, cell cycle checkpoint signaling, and NHEJ (in *Saccharomyces cerevisiae*) (31). As discussed below, a variety of results from immunofluorescence studies in mammalian cells, genetic analyses in yeast, and biochemical investigations with purified proteins suggest that the MRN complex could potentially be involved in the repair of V(D)J intermediates.

Studies in *S. cerevisiae* have demonstrated an absolute requirement for Mre11p, Rad50p, and the functional orthologue of Nbs1, Xrs2p, for NHEJ. Yeast strains with null mutations in any member of the complex are extremely sensitive to ionizing radiation and severely defective in DNA repair by NHEJ (32, 33). In mammalian cells, Nbs1 is found to localize to an actively recombining locus in developing thymocytes (34), and the complex itself localizes to sites of DNA damage very rapidly after the damage is induced (35). More recent work in mice with conditional null mutations in Nbs1 and in mammalian cells harboring hypomorphic mutations in members of the MRN complex indicate that the complex is involved in another recombination process that requires the repair of double-strand breaks, class switch recombination (36–38). A number of biochemical experiments also suggest that the MRN complex is important for the joining of both compatible and incompatible ends. Purified Mre11 has 3′–5′-exonuclease activity and single-stranded endonuclease activity, which is necessary to facilitate the joining of incompatible ends in vitro (39, 40). In addition, efficient joining of cohesive-ended DNA in vitro by the human Ku proteins, XRCC4, and DNA ligase IV is dependent upon a cellular fraction containing Mre11, Rad50, and Nbs1 (41). Taken together, these findings make the MRN complex an attractive candidate for involvement in V(D)J recombination. However, this has remained a controversial and open question.

Studies in chicken cells and fission yeast have suggested that this complex may not be absolutely required for NHEJ in eukaryotes other than *S. cerevisiae*. Null mutations in *Schizosaccharomyces pombe* Rad50 and the *S. pombe* MRE11 homologue, RAD32, have wild-type levels of NHEJ activity in plasmid repair assays (42). Studies in chicken MRE11−/−/− DT-40 cells engineered to carry a tetracycline-repressible chicken MRE11 transgene indicate that Mre11 repression does not interfere with Ku-dependent end-joining in this system (43). Moreover, hypomorphic mutations in any member of the complex do not appear to decrease V(D)J recombination frequencies in mammalian cells (44–48), and conditional null NBS1 mutant mice have wild-type levels of mature B-cells (36, 38).

However, assessing the involvement of the MRN complex in V(D)J recombination based on hypomorphic mutations has been difficult. Hypomorphic mutations in members of the MRN complex can result in reduced expression levels of the proteins and aberrant subcellular distribution, but they do not substantially interfere with MRN complex formation in *vivo* or DNA binding and exonuclease activity in *vitro* (44, 48–50). Thus, it is unclear whether the residual activity of these mutant proteins is facilitating V(D)J recombination in these cells. Moreover, hypomorphic mutations in Mre11 and Nbs1 are associated with an increased frequency of trans-rearrangements between T-cell receptor β and γ loci, indicating that mutations in members of MRN can ultimately result in aberrant V(D)J recombination reactions (48).

Similar to studies utilizing hypomorphic mutations in members of the MRN complex, evaluating the role of the MRN complex in V(D)J recombination in conditional null Nbs1 mouse models is difficult. This is because conditional deletion of NBS1 is incomplete in these models (36, 38, 51), particularly in developing lymphocyte populations (51). Therefore, it is possible that the recombination that is occurring is being mediated by Nbs1 synthesized prior to disruption in these cells or in cells in which NBS1 has not yet been deleted. Although there is no specific block in B-cell development in one conditional null Nbs1 model (38), it is clear that the Nbs1 protein is still present even in the splenic B-cell population, albeit at lower levels. Moreover, in one conditional null Nbs1 mouse model at least 50% of Nbs1 null mutant cells are lost from the bone marrow and even more from the thymus (51), indicating that Nbs1 null lymphocytes are unable to complete essential developmental processes, such as V(D)J recombination and/or DNA replication, and this ultimately results in cell death.

Participation of the MRN complex in the resolution of V(D)J intermediates has been difficult to assess unequivocally because absolute null mutations in any member of the MRN complex in mammalian cells are lethal (hence the reliance on hypomorphic mutations and conditional null mutations in Nbs1). In *S. cerevisiae* however, strains with null mutations in MRE11, RAD50, or XR52 are still viable (32, 33). Yeast can be induced to carry out V(D)J recombination by introducing the V(D)J recombinase (*i.e.* RAG1 and RAG2) and a recombination substrate (52). As in mammalian cells, the signal joints formed in yeast are precise junctions and their formation is dependent on the yeast XRCC4 homologue, LIF1 (53). Moreover, the RAG proteins appear to play a role in the joining process in yeast, as they do in mammalian cells. Therefore, it is possible to test whether the MRX complex is required for this specialized form of double-strand break repair by examining signal joint formation in yeast.

Here we show that signal joining in yeast is dependent on the same factors known to be required in mammalian cells. In addition, we find that the members of the yeast MRX complex, MRE11, RAD50, and XR52, as well as the yeast-specific NHEJ factor, NEJ1 (54–57), are also necessary for precise signal joint formation. None of these factors are required for RAG-mediated transposition, an alternative fate of signal ends in yeast, although the loss of RAD50p affects the types of transposition products that are recovered.

**Experimental Procedures**

**Yeast Strains**—Yeast strains used in these experiments are described in Table I. Strains with disruptions in DNA repair genes were made by standard PCR-based gene replacement techniques. Strains MAV109, MAV134, MAV135-2, MAV144, MAV288, MAV312, MAV320, and MAV321 were derived from parental strain BY4704 by replacing the NEJ1, LIG4, YKU70, PS02, RAD50, RAD52, MRE11, or XR52 open reading frames, respectively, with the kanMX4 cassette. The resulting strains were then each transformed with the RAG expression plasmids.
pCCY3 and pCCY4 and the recombination substrate pACY16 creating the following: ACY176, ACY177, ACY215, ACY217, ACY199, ACY201, ACY221, ACY212, ACY215, ACY222, ACY197, ACY222, ACY199, ACY201, ACY220, ACY221, ACY212, ACY215, ACY222, ACY217, and ACY217, respectively (Table I).

Plasmids, DNA Isolation, Nested PCR Assay, and Ψ1 Nuclease Assay—The RAG expression constructs (pCCY3 and pCCY4) and recombination substrate plasmid (pACY16) are described elsewhere (52). DNA isolation conditions, nested PCR, and Ψ1 nuclease assays were performed as described previously (52). Each PCR was performed on DNA recovered from equal numbers of yeast cells. Roughly one transposition product or signal joint was recovered per 2 million cells. Thus, a rough estimate of the frequency of transposition and signal joint formation in wild-type yeast is \( \frac{1}{2} \times 10^{-6} \) per cell. This number differs from our previous report (49) in which the frequency calculation was based on the number of events per µg of DNA as measured by A_{260}. However, the A_{260} of yeast DNA preparations does not accurately reflect the amount of DNA present in the sample. The precision of signal joint formation was assessed by sequence analysis in all strains.

RESULTS

Previous results suggested that signal joint formation in yeast would be dependent on the NHEJ repair pathway, whereas RAG-mediated transposition would not (52). We extended and confirmed these results through an analysis of the genetic requirements for signal joint formation and RAG-mediated transposition in a series of isogenic yeast strains with disruptions in genes involved in NHEJ (YKU70, LIG4, MRE11, RAD50, XR52, and NEJ1), homologous recombination (RAD52) (58), or interstrand cross-link repair (PSO2). The parental strain BY4704 was used to derive the following null strains: yku70Δ (MAV135-2), lig4Δ (MAV134), and mre11Δ (MAV220, rad50Δ (MAV288), xrs2Δ (MAV321), and nej1Δ (MAV109), rad52Δ (MAV312), or pso2Δ (MAV144) (Table I). Each strain was then transformed with a recombination substrate plasmid (pACY16) and the RAG expression plasmids for core RAG1 (pCCY3) and core RAG2 (pCCY4). RAG cleavage of pACY16 results in the release of a 968-bp blunt, signal-ended fragment from the plasmid backbone. In yeast, this signal-ended fragment can be used as a donor molecule in an intramolecular transposition reaction, creating either an inversion circle or deletion circle. Alternatively, signal ends may be joined to one another to create a signal joint. Signal joints, inversion circles, and deletion circles formed in yeast are detectable by using a nested PCR assay (52). Here this assay was used to assess the ability of the DNA repair-deficient strains to support V(D)J recombination and RAG-mediated transposition following induction of RAG protein expression.

Signal Joining in Yeast Mimics Signal Joining in Mammalian Cells—Fifty nested PCRs were performed on DNA isolated from one induction of each of two independently derived yku70Δ strains induced to express the RAG proteins. In 30 reactions performed from DNA isolated from ACY195 and 20 reactions performed from DNA isolated from ACY209, no signal joints were amplified, although 27 intramolecular transposition products were amplified (Table II). The number of inversion circles (20) and deletion circles (7) recovered in these 50 reactions was similar to the numbers recovered in wild-type strains for NHEJ activity, ACY142 and ACY159 (45 inversion circles and 8 deletion circles from 110 reactions; Table II). Moreover, the ratio of inversion circles to deletion circles in the yku70Δ strains was also similar to wild-type strains, with the formation of inversion circles vastly favored over the formation of deletion circles.

Similar results were also obtained in lig4Δ (ACY211 and ACY214) and nej1Δ (ACY176 and ACY177) strains; no precise signal joints were amplified, although transposition products were recovered as expected with the ratio of inversion circles to deletion circles amplified similar to wild type (Table II). One imprecise signal joint that was amplified from DNA isolated

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2 K. Struhl, personal communication.
from the lig4Δ strain ACY211 appeared to have been formed after deletion of 249 bp followed by joining through a 2-bp region of microhomology (Table II). One imprecise signal joint was also amplified from DNA isolated from the nefΔ strain ACY176, although joining in this case did not appear to be microhomology-mediated (Table II). Thus YKU70, LIG4, and NEJ1 are dispensable for RAG-mediated transposition but are required for signal joint formation in yeast.

Although components of the NHEJ pathway were found to be required for signal joint formation in yeast, two other genes required for alternative DNA repair processes were dispensable. The RAD52 gene is necessary for homologous recombination in yeast, a pathway more commonly utilized for DNA repair in this organism (59). However, 70 nested PCRs performed from DNA isolated from rad52Δ strains ACY220 and ACY221 demonstrated that both precise signal joints and transposition products were amplified (Table II), indicating that neither signal joint formation nor RAG-mediated transposition are dependent on the homologous recombination apparatus. Likewise, signal joint formation and RAG-mediated transposition occurred independently of a gene involved in interstrand cross-link repair, PSO2. Both precise signal joints and transposition products were amplified from 49 nested PCRs performed from DNA isolated from psodΔ strains ACY197 and ACY222, similar to wild-type (Table II). Thus, PSO2 is dispensable for signal joint formation and RAG-mediated strand transfer in yeast. However, PSO2 shares a region of homology with Artemis, a factor necessary for the repair of hairpin coding ends in mammalian cells, and has been implicated in the repair of hairpin-ended DNA in yeast (25, 26, 28). It may therefore play a role in the repair of coding end hairpins in yeast.

We have analyzed the ability of the recombination substrate, pACY16, to undergo coding joint formation in wild-type yeast (ACY159) by nested PCR. No definitive coding joints have been detected in yeast by this approach (data not shown), either because coding joint formation is too inefficient to be detected or because coding joint formation involves large scale deletion of DNA sequence from the site of the break prior to joining, thus removing the sequence to which the primers would anneal. Genetic screens that investigate the frequency and genetic requirements for coding joint formation in yeast will form the basis of future work.

The above work demonstrates that signal joint formation in yeast is mediated by the NHEJ repair pathway and not by homologous recombination or a gene involved in interstrand cross-link repair. Previous work demonstrated that precise signal joint formation in yeast is also dependent on the RAG proteins and the XRC4 homologue, LIF1. Thus, both the character of the signal joints and the genetic requirements for their formation in yeast are very similar to that observed in mammalian cells.

### Table II

| Table II: Transposition and signal joining in strains deficient in DNA repair |
|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| Strains         | ACY142 and -159 | ACY195 and -109 | ACY211 and -214 | ACY176 and -177 | ACY197 and -222 | ACY220 and -221 |
| Total reactions | 110             | 50              | 50              | 50              | 49              | 70              |
| Transposition products | 53            | 27              | 34              | 26              | 28              | 27              |
| Inversion circles | 45            | 20              | 34              | 26              | 20              | 24              |
| Deletion circles | 8              | 7               | 10              | 6               | 9               | 3               |
| Signal joints   | 18              | 0               | 1⁸              | 1⁵              | 9⁰              | 8               |
| Precise         | 15              | 0               | 0               | 0               | 8               | 6               |
| Imprecise       | 3⁰              | 0               | 1⁸              | 1⁵              | 1⁰              |

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The MRX Complex Is Necessary for Signal Joint Formation in Yeast—We next asked if members of the MRX complex are required for signal joint formation in yeast. No signal joints, either precise or imprecise, were recovered in mre11Δ, rad50Δ, or xrs2Δ strains, although RAG-mediated transposition products were recovered in all strains (Table III). In mre11Δ strains induced to express the RAG proteins (ACY212 and ACY215), 64 transposition products were amplified from 90 nested PCRs, whereas no signal joints were recovered, well below the expected number of 15 (Table III). Results were similar in xrs2Δ (ACY213 and ACY217) and rad50Δ strains (ACY199 and ACY201). No signal joints were amplified, although 33 transposition products were amplified in 50 nested PCRs performed from DNA isolated from xrs2Δ strains. Similarly, 37 transposition products were amplified from 65 nested PCRs performed from DNA isolated from rad50Δ strains (Table III). The difference in amplification of signal joints between strains disrupted for members of the MRX complex and wild-type strains for NHEJ activity was statistically significant by χ² analysis (mre11Δ strains, p = 0.00002; xrs2Δ strains, p = 0.002; rad50Δ strains, p = 0.0003). Thus, MRE11, RAD50, and XRS2 are required for signal joint formation in S. cerevisiae but dispensable for RAG-mediated transposition.

Although the MRX complex is not required for transposition, RAD50 disruption appears to influence the type of transposition products that are formed. In both mre11Δ and xrs2Δ strains, as in wild-type cells (ACY142 and ACY159), the formation of inversion circle transposition products was vastly favored over the formation of deletion circle products (Table III). By contrast, this ratio of inversion to deletion circle products was significantly altered in rad50Δ strains. In 65 nested PCRs, 18 of 37 transposition products amplified were deletion circles (Table III), well above the expected number of 6 and statistically different from wild-type strains ACY142 and ACY159 by χ² analysis (p = 10⁻⁷). Thus, disruption of RAD50 appears to affect strand choice in RAG-mediated intramolecular transposition reactions, although the mechanism underlying this effect remains to be determined (see “Discussion”).

The number of transposition products amplified from mre11Δ, rad50Δ, and xrs2Δ strains appeared higher than in wild-type strains (Table III and supplemental Fig. 1). This was of interest because it has been suggested that NHEJ factors may play a role in inhibiting RAG-mediated transposition in mammalian cells (60), and it seemed possible that homologues of NHEJ factors may function in this same capacity in yeast. However, although there is a trend toward an increased frequency of amplifying transposition products from mre11Δ, rad50Δ, xrs2Δ, and also lig4Δ strains, these differences did not prove to be statistically significant. Given that the number of transposition products amplified varies from induction to induction (although the ratios of transposition products to signal

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a Data are comprised from Clatworthy et al. (57); 40 additional reactions were also performed for this study.

b Partial RSS still intact in each of these joints and smaller deletions; joining did not appear to be microhomology-based.

c Precise signal joint: 75 bp deleted from 23 RSS; 174 bp deleted from 12 RSS; joining appears to be microhomology-based (2 bp).

d Precise signal joint: 94 bp deleted from 23 RSS; 203 bp deleted from 12 RSS; joining does not appear to be microhomology-based.

e Precise signal joint: 147 bp deleted from 23 RSS; 213 bp deleted from 12 RSS; joining appears to be microhomology based (1 bp).
joints and of inversion circles to deletion circles remains largely invariant), large standard deviations made it difficult to confirm a statistical difference in the incidence of transposition between mutant and wild-type strains (supplemental Fig. 1B). However, yeast NHEJ genes are relatively divergent from their mammalian homologues (53, 61–63). It remains possible that mammalian NHEJ factors may more effectively repress RAG-mediated transposition, a possibility that can now be tested in this yeast assay.

**Genetic Requirements for Repair of Deletion Circles—**RAG-mediated transposition results in the formation of a product where the DNA directly adjacent to each RSS is single-stranded. Previously, it was determined that the single-stranded regions adjacent to each RSS in inversion circles are not repaired in yeast, whereas the single-stranded regions in deletion circles are filled in and repaired by a \( \text{LIF1} \)-independent mechanism (52). We sought to further characterize the genetic requirements for the repair of deletion circles in yeast by analyzing deletion circle sensitivity to \( S1 \) nuclease in strains disrupted for NHEJ (\( \text{ku70}^{\Delta} \) strains \( \text{ACY195} \) and \( \text{ACY209} \) and \( \text{rad50}^{\Delta} \) strains \( \text{ACY199} \) and \( \text{ACY201} \)). Briefly, DNA isolated from each of the above strains induced to express the \( \text{RAD52} \) protein was divided in half; one-half was treated with \( S1 \) nuclease and the other half was left untreated. The number of deletion circles amplified by nested PCR was then compared between DNA that was treated with \( S1 \) nuclease and DNA that was not treated. Deletion circles were recovered from \( S1 \) nuclease-treated DNA from \( \text{ku70}^{\Delta} \) (\( S1 \)-treated DNA, 5 deletion circles/30 reactions; untreated DNA, 6 deletion circles/30 reactions) and \( \text{rad50}^{\Delta} \) strains (\( S1 \)-treated DNA, 12 deletion circles/30 reactions; untreated DNA, 6 deletion circles/30 reactions). Thus, \( \text{YKU70} \) and \( \text{RAD50} \) are dispensable for the repair of deletion circles, indicating that deletion circle repair is not mediated by NHEJ.

**DISCUSSION**

Previous work demonstrated that signal ends in yeast can be resolved by two alternative pathways: signal joint formation or RAG-mediated transposition (52). That work determined that the yeast \( \text{XRCC4} \) homologue, \( \text{LIF1} \), is necessary for precise signal joint formation in yeast but is dispensable for RAG-mediated strand transfer. Here we find that \( \text{YKU70} \) and \( \text{LIG4} \) are also required for precise signal joint formation but are also dispensable for RAG-mediated transposition. On the other hand, genes involved in homologous recombination (\( \text{RAD52} \)) or the repair of interstrand cross-links (\( \text{PSO2} \)) are not required for either process. Thus, signal joining in yeast resembles signal joint formation in mammalian cells by all known criteria; signal joints in yeast are precise fusions of signal ends, and their formation is dependent on homologues of the NHEJ genes known to be required for joining in mammalian cells. Moreover, whereas signal joining is precise in this strain, joining of restriction enzyme-generated blunt ends is not (49), indicating that the RAG proteins are indeed influencing the joining step and affecting the character of blunt-end repair. Taken together, these results strongly suggest that the signal joints observed in yeast are bona fide V(D)J products, and it is striking then that signal joint formation in yeast also requires the members of the MRX complex.

Although NHEJ in \( \text{S. cerevisiae} \) is known to require MRX, it was by no means clear that V(D)J recombination in yeast, a specialized case of NHEJ, would as well. The specific steps of the joining process for V(D)J joining and typical NHEJ must be distinct, as the RAG proteins are involved in the joining phase of V(D)J recombination but are obviously not part of the normal NHEJ pathway. The finding that the MRX complex, in addition to the RAG proteins, is required for signal joint formation provides strong support for the idea that the MRX complex will play some role in V(D)J recombination in mammalian cells as well.

**What Role Might the MRN(X) Complex Play in V(D)J Recombination?**—The MRN(X) complex has been assigned numerous activities that are likely to be important for DNA repair: nucleolytic end-processing, DNA end-bridging, and cell-cycle checkpoint signaling (31). Whereas any of these activities (or ones yet to be described) could be involved in V(D)J joining, certain activities seem less likely to be critical for signal joining. Since the signal joints recovered in yeast are primarily precise fusions of the signal ends, there is no overt evidence to suggest that signal ends are nucleolytically modified before joining (although the infrequent and imprecise signal joints detected in wild-type strains for NHEJ activity indicate that nucleolytic modification can occur). Therefore, nuclelease activity of \( \text{Mre11p} \) may be dispensable for precise signal joint formation in yeast. Indeed, it is not absolutely required for NHEJ in yeast in general (64–66). The cell cycle checkpoint activities of the MRX complex would also not seem to come into play. Both \( \text{Mre11} \) and \( \text{Nbs1} \) (\( \text{Xrs2p} \)) are phosphorylated by ATM (Tel1p) in response to DNA damage, and phosphorylation of Nbs1 by ATM is required for the S-phase checkpoint in mammalian cells (67–72). However, signal ends do not typically trip cell cycle checkpoint controls, and ATM is dispensable for signal joint formation in mammalian cells (73). Therefore, it seems unlikely that the cell cycle checkpoint activities of the MRX complex would be important for the repair of signal ends. Instead, the DNA end-alignment activity for the MRX complex is likely to be important for joining signal ends in yeast.

Atomic and scanning force microscopy studies of yeast and human \( \text{Mre11}/\text{Rad50} \) show that they have DNA end-bridging/alignment activity (74, 75). DNA ends can be directly bound by the \( \text{Mre11p}/\text{Rad50p} \) complex and thus physically tethered to one another by the flexible arms of Rad50p, which interact with one another through a zinc-hook in Rad50p (74, 76). Thus, one could imagine a scenario where the MRX complex must bind and align signal ends in order to facilitate joining by \( \text{Ku70p}/\text{Ku80p}, \text{Lig4p}, \) and \( \text{Lif1p} \). However, one might have expected that any end-alignment activity necessary for

| Strains | Transposition and signal joining in \( \text{mre11}^{\Delta}, \text{rad50}^{\Delta}, \) and \( \text{xrs2}^{\Delta} \) strains |
|---------|------------------------------------------------------------|
|          | \( \text{ACY212 and -215} \) (\( \text{mre11}^{\Delta} \))   | \( \text{ACY199 and -201} \) (\( \text{rad50}^{\Delta} \))   | \( \text{ACY213 and -217} \) (\( \text{xrs2}^{\Delta} \))   | \( \text{ACY142 and -159} \) (wild type)* |
| Total reactions | 90 | 65 | 50 | 110 |
| Transposition products | 64 | 37 | 33 | 53 |
| Inversion circles | 49 | 19 | 26 | 45 |
| Deletion circles | 15 | 18 | 7 | 8 |
| Signal joints | 0 | 0 | 0 | 18 |
| Precise | 0 | 0 | 0 | 15 |
| Imprecise | 0 | 0 | 0 | 3b |

* Data are repeated from Table II.
* Partial RSS still intact in each of these joints and smaller deletions; joining did not appear to be microhomology-based.
the joining of signal ends could be supplied by the RAG proteins themselves, making the end-association activity of MRX dispensable for the V(D)J-joining pathway. Our results indicate that any end-association activity supplied by the MRX complex and the RAG proteins is not functionally redundant in the context of signal joint formation in yeast.

Most surprisingly, although the MRX complex is not required for the strand-transfer step of RAG-mediated transposition or for the repair of deletion circles, the disruption of Rad50 does have a significant impact on transposition. In wild-type strains, as well as mre11Δ and xrs2Δ strains, inversion circle transposition products are vastly favored over deletion circles. This bias for inversion circle over deletion circle formation is also observed in intramolecular transposition reactions in vitro using purified RAG1/2 proteins (77), and thus one might have assumed that strand choice is enforced by the architecture of the transposase-DNA complex itself. However, inversion circles and deletion circles are observed at roughly equal frequencies in rad50Δ strains, demonstrating that factors involved in DNA repair can affect strand choice. Such an influence could either be direct (e.g., binding of components of the MRX complex to the signal ends prior to transposition sterically affecting strand choice) or indirect (with components of the complex affecting the stability of transposition products and/or influencing the frequency of their recovery). In either case, it suggests that the MRX complex may be intimately involved in the processing of RAG1/2-generated DNA ends.

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