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SCIENCE DIRECT
DNA Repair 4 (2005) 1281–1294
DNA REPAIR
www.elsevier.com/locate/dnarepair

The Mre11/Rad50/Xrs2 complex and non-homologous end-joining of incompatible ends in *S. cerevisiae*

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Received 29 May 2005; received in revised form 23 June 2005; accepted 23 June 2005
Available online 25 July 2005

Abstract

In *Saccharomyces cerevisiae,* the Mre11/Rad50/Xrs2 (MRX) complex plays important roles in both homologous and non-homologous pathways of DNA repair. In this study, we investigated the role of the MRX complex and its enzymatic functions in non-homologous repair of DNA ends containing incompatible end structures. Using a plasmid transformation assay, we found that *mre11* and *rad50* null strains are extremely deficient in joining of incompatible DNA ends. Expression of the nuclease-deficient Mre11 mutant H125N fully complemented the *mre11* strain for joining of mismatched ends in the absence of homology, while a mutant of Rad50 deficient in ATP-dependent activities exhibited levels of end-joining similar to a *rad50* deletion strain. Although the majority of non-homologous end-joining (NHEJ) products isolated did not contain microhomologies, introduction of an 8 bp microhomology at mismatched ends resulted in microhomology-mediated joining in all of the products recovered, demonstrating that a microhomology exerts a dominant effect on processing events that occur during NHEJ. Nuclease-deficient Mre11p was less efficient in promoting microhomology-mediated end-joining in comparison to its ability to stimulate non-microhomology-mediated events, suggesting that Mre11p influences, but is not essential for, microhomology-mediated repair. When the linearized DNA was transformed in the presence of an intact homologous plasmid to facilitate gap repair, there was no decrease in NHEJ products obtained, suggesting that NHEJ and homologous repair do not compete for DNA ends in vivo. These results suggest that the MRX complex is essential for joining of incompatible ends by NHEJ, and the ATP-dependent activities of Rad50 are critical for this process.

Keywords: Non-homologous end joining; NHEJ; Mre11; Rad50; Xrs2; Double-strand break repair; Microhomology

1. Introduction

Eukaryotic cells employ both homologous and non-homologous mechanisms to repair DNA double-strand breaks. Homology-driven repair of DNA breaks utilizing a sister chromatid or homologous chromosome as a template for repair-initiated replication is the preferred mechanism for repair; however, cells also have the ability to re-attach the severed ends directly without a homologous template. This process of non-homologous end-joining (NHEJ) requires several protein factors to function efficiently in vivo.

The core factors necessary for NHEJ repair in budding yeast include the DNA end-binding heterodimer of Ku70/Ku80 proteins (Hdf1/Hdf2), DNA ligase IV (Dnl4), and a Dnl4-associated factor analogous to Xrcc4 (Lif1) (reviewed by [1]). The Nejl protein (also called Lif2) regulates NHEJ in *Saccharomyces cerevisiae* [2,3], as do the Sir2/3/4 proteins [4], although the effects of the Sir proteins may be primarily mating type-specific [5]. Pol4, a homolog of polβ, has also been shown to play an important role in processing of DNA substrates in NHEJ, specifically at a subset of mismatched ends [6]. The Mre11/Rad50/Xrs2 complex (M/R/X) also stimulates non-homologous joining of cohesive DNA ends in budding yeast and is one of the only complexes that acts in both homologous and non-homologous repair.

Absence of any of the three components of the M/R/X complex in *S. cerevisiae* results in 10- to 100-fold decreased levels of cohesive end-joining using plasmid substrates [7–10]. The M/R/X complex also is critical for the

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doi:10.1016/j.dnarep.2005.06.011

non-homologous repair of double-strand breaks in chromosomal DNA [11], and is required for the recovery of one class of NHEJ junctions formed by unequal alignments of chromosomal cohesive ends [12]. In addition, joining of signal ends produced by the RAG1/RAG2 proteins in yeast was also found to require the MRX complex in vivo [13]. In agreement with the in vivo data from *S. cerevisiae,* a study of DNA end ligation in vitro showed that the M/R/X complex stimulates ligation by recombinant Dnl4/Lif1 in both the absence and presence of Hdf1/Hdf2 [14].

All of the studies of M/R/X and end-joining thus far have utilized cohesive or blunt ends generated by a single restriction enzyme. Broken DNA ends created in vivo by ionizing radiation or chemical agents are likely to create ends lacking homologous overhangs, however, and in these cases, processing of the ends must occur prior to the ligation step. The enzymatic properties of Mre11 and Rad50 suggest a possible role for the complex in these processing events. Mre11 contains a highly conserved phosphoesterase domain that possesses both exo- and endo-nucleolytic activities [15–20]. The Rad50 component of the complex contains ATPase subunits that facilitate nucleotide-dependent DNA binding [21,22] and limited DNA unwinding activity [16]. In addition, Mre11/Rad50 complexes from yeast and humans have been shown to tether linear DNA molecules [14,23,24], potentially providing end-bridging functions for NHEJ.

Support for a possible role of the Mre11 nuclease in end-joining comes from studies in vitro, showing that ligation of mismatched DNA ends can occur in the presence of Mre11 and a DNA ligase [15]. The junctions isolated from these reactions contained small regions (2–5 bp) of homology between the otherwise non-homologous ends. In addition, the distributive exo-nuclease activity of Mre11 was also shown to be affected by the presence of heterologous DNA ends, with the enzyme in some cases apparently pausing its degradation at positions of homology [25].

Previous studies have examined the effects of nuclease-deficient alleles of Mre11 on NHEJ and found no significant differences from wild-type [9,10]; however, all of these assays were performed with cohesive or blunt DNA ends that would not necessarily require M/R/X processing activities. In the experiments presented here we specifically investigated the role of the M/R/X complex in joining of incompatible DNA ends in vivo, using a plasmid transformation assay in *S. cerevisiae.* Mutants of Mre11 lacking nuclease activity were tested in this assay, specifically, *mre11–H125N* [10], as well as a mutant of Rad50 deficient in ATP-dependent activities, *rad50–S1205R* [26].

Our analysis of end-joining using this assay shows that the presence of the M/R/X complex is required for recovery of NHEJ products originating from mismatched ends, regardless of the structure of the mismatch. Surprisingly, Mre11 nuclease activity is not required for mismatched end-joining, and in fact appears to antagonize this process in vivo. In contrast, the *rad50–S1205R* mutant behaves similarly to a *rad50* deletion strain, yielding very few NHEJ products with mismatched end junctions. These results indicate that the M/R/X complex clearly plays an essential role in facilitating ligation of mismatched ends, perhaps, through DNA unwinding and end-bridging, but that Mre11 nuclease activity is not essential for processing of mismatched nucleotides on the ends of DNA prior to ligation.

2. Materials and methods

2.1. Strains

The strains used in this work are shown in Table 1 and are all derivatives of W303α [27]. KRY88 and KRY78 strains [28] were gifts from Tom Petes. W1588–4A is a RAD5 derivative of W303α from Klein [29]. LSY0780 and LSY1398 are derivatives of W1588–4A made by Sylvie Moreau, Alicia Lam, and Lorraine Symington. TP1991 is a derivative of W303α with the *rad50–S1205R* allele replacing the genomic copy of RAD50. TP1991 was made by integrating pTP659 into the chromosome at the *RAD50* locus. pTP659 was linearized within the *rad50–S1205R* coding region with *Nhe*I, transformed into W303α and selected for URA+ transformants.

Correct integration of the plasmid at the *RAD50* locus was confirmed by PCR and Southern blotting. The

Table 1
Yeast strains used in this study

Strain	Relevant genotype	Strain construction or reference[a]
W303α (TP1218)	Wild-type *rad5–535*	Thomas and Rothstein [27]
KRY88 (TP1220)	W303α *mre11::kanMX*	Richie and Petes [28]
KRY78 (TP 1219)	W303α *rad50::hisG*	Richie and Petes [28]
TP1991	W303α *rad50–S1205R*	This study
W1588–4A (TP1745)	wild-type *RAD5*	Klein [29]
LSY0780 (TP1751)	W1588–4A *mre11::LEU2*	L. Symington
LSY1398 (TP1755)	W1588–4A *mre11–H125N*	L. Symington
TP1264	W303α*/(CENARS URA3)*	W303α + pRS316
TP1265	KRY88*/(CENARS URA3)*	KRY88 + pRS316
TP1266	KRY88*/MRE11(CEN ARS URA3)*	KRY88 + pTP165
TP1267	KRY88*/mre11–H125N (CEN ARS URA3)*	KRY88 + pTP178

[a] All strains in the study are isogenic with W303α.