Mre11: roles in DNA repair beyond homologous recombination

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The Mre11 protein has well-documented functions in the repair of DNA double-strand breaks via homologous recombination. Now, several new studies reveal that Mre11 also has a role in mammalian DNA double-strand break repair by nonhomologous end joining.

Eukaryotic cells have two well-characterized DNA double-strand break (DSB) repair pathways. Homologous recombination accurately repairs postreplicative DSBs using an intact template from a sister chromatid. In contrast, nonhomologous end joining (NHEJ) ligates DSBs without homology or with short (1–4 bp) junctional homologies referred to as microhomologies (**Fig. 1**). NHEJ is particularly important in the G1 cell-cycle phase, when sister chromatids are not available for homologous recombination. Mre11 is a component of the conserved Mre11–Rad50–Nbs1 (MRN) complex. Together, these proteins sense DSBs, activate the ataxia-telangiectasia mutated (Atm) kinase[1] and trigger DNA damage responses. Although the Atm-dependent DNA damage response has long been known to function in homologous recombination[2], recent work has implicated it as having a significant role in promoting NHEJ[3–6]. In addition, Mre11 has both single-stranded DNA endonuclease activity[7] and 3′→5′ exonuclease activity[8] (**Fig. 2**). Mre11 nuclease activity functions in conjunction with other factors to generate the 3′ single-stranded DNA overhang necessary for strand invasion during homologous recombination[9–12]. However, nuclease-deficient Mre11 still efficiently activates Atm kinase, which has allowed dissection of this function from its DNA resection function during homologous recombination[13]. A diverse set of three new studies in this issue, on V(D)J recombination[14,15], class switch recombination (CSR)[16] and repair of endonuclease-generated DSBs[17,18], have now implicated the MRN complex, and Mre11 in particular, as also having a direct role in NHEJ, a role that extends beyond its function in activation of the DNA damage response. A recent paper in *Nature* reaches similar conclusions by examining the fusion of uncapped telomeres[19].

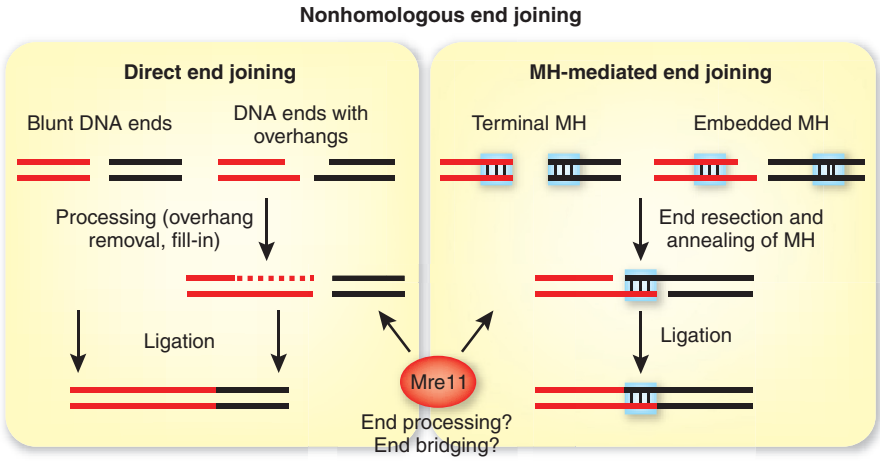

Figure 1 Potential Mre11 functions in C-NHEJ and A-NHEJ pathways. NHEJ can catalyze both direct (left) and microhomology (MH)-mediated end joining (right). Direct joining refers to the joining of blunt DSB ends or DSB ends with overhangs that are processed through fill-in or end resection. MH-mediated joining makes use of base-pairing interactions between short terminal or embedded microhomologies. Mre11 may function in end processing through its nuclease activity.

NHEJ repair pathways are divided into the well-characterized, major classical NHEJ pathway (C-NHEJ) and a much less characterized alternative NHEJ (A-NHEJ) pathway (or pathways), revealed by the observation of NHEJ in cells deficient for C-NHEJ[20]. So far, seven C-NHEJ factors have been identified[21]. There are four evolutionarily conserved, 'core' C-NHEJ factors: Ku70 and Ku80, which form a DSB recognition complex, and Xrcc4 and DNA ligase 4, which form a ligation complex. Two additional C-NHEJ factors, DNA-dependent protein kinase catalytic subunit (DNA-PKcs) and Artemis, are found only in vertebrates and are required for joining a subset of DNA ends that need end processing before joining, such as the hairpin-coding ends generated during V(D)J recombination. XLF (also known as Cernunnos) is a more recently described C-NHEJ factor of unknown function[22–24]. The

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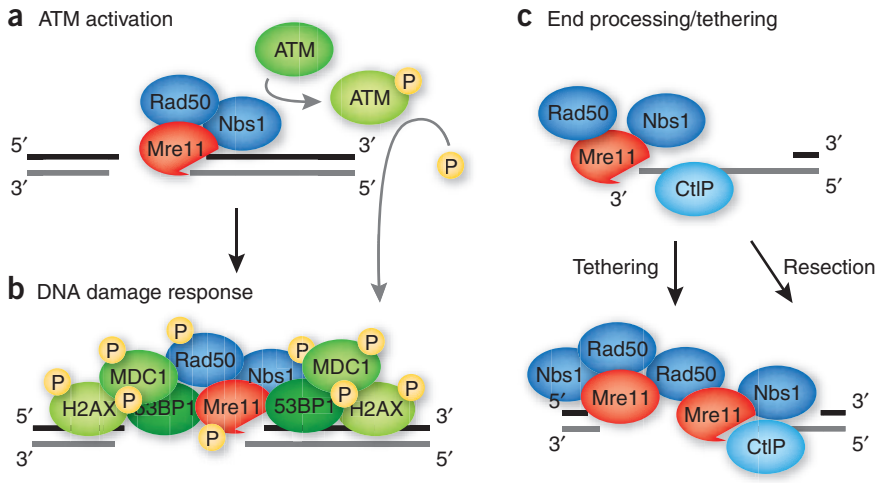

Figure 2 The multiple functions of Mre11. Mre11 interacts with Nbs1 and Rad50 to form the MRN complex, which activates Atm kinase (**a**), participates in the DNA damage response with other Atm substrates (**b**) and also can function to tether broken ends, and may function with CtIP and potentially other factors to resect the broken ends (**c**). Both Mre11-related DNA damage response function and the direct tethering role of the MRN complex may help to hold broken chromosomal ends in proximity to promote NHEJ.

importance of C-NHEJ in mammalian cells is underscored by the severely impaired DSB repair and greatly increased genomic instability of cells deficient for C-NHEJ factors[25].

Mammalian cells deficient for core C-NHEJ factors (for example, Ku80 or Xrcc4) are still capable of joining DNA ends, albeit often at reduced efficiency, leading to the description of alternative NHEJ[20]. A-NHEJ has gained much attention, in part due to the fact that it has been implicated in catalyzing oncogenic translocations in C-NHEJ deficient cells[26–28]. Recently, A-NHEJ was found to robustly repair chromosomal DSBs induced by I-SceI endonuclease in Ku- or Xrcc4-deficient hamster cells[29,30] and to repair physiological chromosomal DSBs introduced into the IgH locus in B cells during immunoglobulin CSR[31,32]. In normal B cells, CSR junctions that are direct (that is, lack any homology at the join; **Fig. 1**) or have very short microhomologies are found in roughly equal numbers, consistent with the known properties of C-NHEJ[31]. However, in Xrcc4-deficient B cells, CSR occurs at 20–50% of wild-type levels and the vast majority of junctions have microhomologies, a proportion of which are longer than those expected for C-NHEJ[31]. In fact, increased use of microhomology has often been noted in the context of junctions from C-NHEJ–deficient cells, leading to the notion that microhomology-mediated end joining (MMEJ) represents a major form of A-NHEJ[20]. However, in some studies, when analyzing the joining of I-SceI–cleaved ends in Ku80-deficient cells, a large proportion of A-NHEJ junctions were found to be direct[29,30]. There could be several explanations for differential recovery of microhomology-containing junctions from different C-NHEJ–deficient cells in different experimental systems, including influences of the types of ends being joined (for example, some substrates provide more microhomology) or multiple pathways of A-NHEJ of which some are more dependent on microhomology. In this context, Mre11 has been speculated to have a role in MMEJ in mammalian cells[20], owing to its preferential processing of mismatched ends *in vitro*[33] and in *Saccharomyces cerevisiae*[34].

Earlier this year, two groups reported a role for Mre11 and Nbs1 in V(D)J recombination, a process that normally absolutely requires C-NHEJ[35] and does not use A-NHEJ at all, because the Rag endonuclease shepherds the joining phase of the reaction into C-NHEJ and excludes other repair pathways[36]. The Sleckman laboratory previously showed that Atm, potentially via its downstream substrates, stabilizes DSB complexes to facilitate proper NHEJ during chromosomal V(D)J recombination[6]. Recently they further showed that hypomorphic mutations of Mre11 or Nbs1 also compromise chromosomal V(D)J recombination similar to, but to a lesser degree than, Atm deficiency[15]. Their finding that Atm activation was not overtly affected by the Mre11 or Nbs1 hypomorphic mutations used further suggested that Mre11 and Nbs1 have functions in V(D)J recombination downstream of Atm phosphorylation; however, upstream roles were not formally excluded[15]. In another recent study, the Roth group used a mutated Rag2 protein that permits A-NHEJ–mediated repair of V(D)J breaks[36] to reveal a role for Nbs1 in V(D)J coding-joint formation in the absence of Artemis or DNA-PKcs[14]. One obvious function for Nbs1 in this context might be to function via the MRN complex to support Mre11 nuclease processing of coding end hairpins. However, Mre11 nuclease domain mutants could support A-NHEJ of V(D)J coding joints[14], excluding such possibility. Together, these two studies indicated that Mre11 and Nbs1 might have novel roles in C-NHEJ and A-NHEJ. The most recent studies support and extend this notion to more general DSB repair, beyond the rather specialized V(D)J recombination process.

The articles in this issue of *Nature Structural & Molecular Biology* firmly establish a role for Mre11 in C-NHEJ and A-NHEJ. Two of these, from the Lopez and the Scully groups, assess the role of Mre11 in NHEJ by studying the repair of I-SceI endonuclease–induced DSBs. Depletion or inhibition of Mre11 reduces end-joining efficiency up to 40% in both wild-type and *Xrcc4*$^{-/-}$ cells, indicating that Mre11 promotes both C-NHEJ and A-NHEJ[17,18]. In this context, the MRN complex and the MRN-interacting protein CtIP, an endonuclease implicated in repair pathway choice during the cell cycle[37], probably work together, because depletion of Nbs1 (refs. 18,38), Rad50 (ref. 17) or CtIP[17,18] in wild-type or Xrcc4-deficient cells also leads to a similar decrease in overall end joining.

In the third article, the Ferguson group tested the role of Mre11 in CSR[16]. Similar to what was previously reported for Nbs1 (refs. 38,39), they found that conditional deletion of *Mre11* led to major decrease in CSR. Moreover, the decreased CSR in Mre11-deficient B cells was accompanied by the presence of chromosome breaks that originated within the IgH locus[16]. The latter finding clearly demonstrates a role for Mre11 in end joining during CSR, reminiscent of that previously shown via this assay for Atm and other Atm DSB response substrates[4]. The severe CSR defect in Mre11-deficient B cells is consistent with potential roles for Mre11 in both C-NHEJ and A-NHEJ[16], as it is known that A-NHEJ carries out CSR at up to 50% of wild-type levels in the absence of C-NHEJ[31,40]. However, given the relatively low frequency of IgH chromosomal breaks in activated Mre11-deficient B cells, the severe CSR impairment in Mre-11 deficient B cells may also reflect the putative role for the MRN complex in generating DNA breaks at abasic sites created by AID and uracil DNA glycosylase[41]. Likewise, it is possible that other defects associated with Mre11 deficiency, not directly linked to CSR (for example, impaired proliferation (see below)), might contribute to the severity of the CSR defect.

An important question is whether the role of Mre11 in NHEJ of I-SceI breaks or CSR is upstream, downstream or independent of Atm activation. Downstream functions might reflect, among other things, the known involvement of the MRN complex along with other Atm DSB response substrates in suppressing separation of broken chromosomal ends to promote normal NHEJ, as best illustrated in the context of CSR[3–5] or functions related to the Mre11 nuclease activity. In this regard, an Mre11 function upstream of Atm in CSR seems likely: the Ferguson group shows that Mre11 nuclease–deficient B cells, which activate Atm normally, have more modest CSR defects that were not associated with IgH breaks and thus may be related, at least in part, to more general effects of the mutations such as the substantial proliferation defects of the cells[16]. Also, given the known sensitivity of CSR to cellular proliferation defects, the finding that both *Mre11*-null and nuclease-deficient B cells showed significant proliferation defects[16], along with the proliferation and CSR defects for B cells deficient for the Nbs1 component of the MRN complex[38,39], raises the possibility that a portion of the CSR defects in both Mre11 mutants might reflect a cellular proliferation impairment. If so, it is conceivable that the death of activated B cells lacking Mre11 nuclease activity might mask the accumulation of a low level of IgH chromosome breaks, reflective of a more modest role of this activity in NHEJ during CSR.

Telomeres, which lie at the end of mammalian chromosomes, are normally protected by the shelterin protein complex, which also participates in telomere maintenance through interaction with telomerase[42]. Loss of the telomere double-stranded DNA–binding protein Trf2, a component of the shelterin complex, leads to the 'uncapping' of the telomeric end into a DSB-like structure that elicits an Atm-dependent DNA damage response and promotes C-NHEJ–mediated end-to-end fusion of uncapped telomeres[42]. Mre11 had been previously shown to interact with Trf2 (ref. 43), but the molecular function of this interaction was unknown. In a recent paper in *Nature*, the Chang group shows that, in Mre11-deficient cells, TRF2 depletion fails to trigger an Atm-dependent DNA damage response and efficient telomere fusion[19]. Thus, the DNA damage sensor function of Mre11 is clearly important for C-NHEJ in the context of telomere fusions. This study also shows that the nuclease activity of Mre11 is required for telomere fusion in the absence of Trf2 and functions in that context by resecting the 3′ overhanging ends before ligation[19]. However, it should be noted that the Ercc1–XPF complex may also serve this function in other contexts, as indicated by earlier studies[44]. Overall, this study of Mre11 in the context of telomeres has provided direct proof that Mre11 functions to mediate NHEJ through both its nuclease activity and Atm-dependent DNA damage response complex (**Fig. 2**).

One might consider the Mre11 end resection role in telomere fusion to be a highly specialized case because of the long 3′ single-stranded DNA overhang of telomere ends potentially representing optimal resection substrates. In fact, the Roth group showed that Mre11 nuclease–deficient mouse fibroblasts supported extrachromosomal V(D)J recombination at wild-type levels[14]. However, the possibility remains that the impact of the Mre11 nuclease deficiency on chromosomal V(D)J recombination might be different. In this context, the Scully group shows that knockdown of *Mre11* in Xrcc4-deficient cells reduced the extent of end resection, providing evidence that the nuclease function of Mre11 may indeed be involved in end resection in the context of the joining I-SceI–generated chromosomal breaks. Notably, Mre11-mediated resection did not seem to promote MHEJ, as evidenced by little[18] or no changes[15–17] in the proportion of direct versus microhomology-mediated junctions after Mre11 depletion in wild-type cells. However, to more firmly elucidate how Mre11 may contribute to A-NHEJ, it would be most useful to determine the junctional patterns of I-SceI or CSR breaks in C-NHEJ–deficient cells.

In summary, this recent set of studies has clearly documented a role for Mre11 in the NHEJ branch of mammalian DNA DSB repair, both in C-NHEJ and also in the A-NHEJ that occurs in the absence of C-NHEJ. In addition, several of these studies have also suggested a role for the Mre11 nuclease activity in NHEJ. However, the precise roles of Mre11 in NHEJ remain to be sorted out. In particular, does Mre11 function in NHEJ upstream of Atm activation, as an Atm downstream phosphorylation substrate, or potentially in Atm-independent roles that involve DNA end binding or nuclease activities (**Fig. 2**)? Similarly, although the current work represents a tremendous leap forward, there is much to be done to elucidate the nature and degree of the various potential Mre11 functions, including in joining of different types of DSB ends, specific functions in the C-NHEJ pathway versus A-NHEJ pathways and specific functions in the physiological V(D)J recombination and CSR processes that repair DSBs via NHEJ.

1. Lee, J.H. & Paull, T.T. *Science* **304**, 93–96 (2004).
2. Lavin, M.F. *Nat. Rev. Mol. Cell Biol.* **9**, 759–769 (2008).
3. Bassing, C.H. & Alt, F.W. *DNA Repair (Amst.)* **3**, 781–796 (2004).
4. Franco, S. *et al. Mol. Cell* **21**, 201–214 (2006).
5. Ramiro, A.R. *et al. Nature* **440**, 105–109 (2006).
6. Bredemeyer, A.L. *et al. Nature* **442**, 466–470 (2006).
7. Paull, T.T. & Gellert, M. *Genes Dev.* **13**, 1276–1288 (1999).
8. Paull, T.T. & Gellert, M. *Mol. Cell* **1**, 969–979 (1998).
9. Hopkins, B.B. & Paull, T.T. *Cell* **135**, 250–260 (2008).
10. Sartori, A.A. *et al. Nature* **450**, 509–514 (2007).
11. Williams, R.S. *et al. Cell* **135**, 97–109 (2008).
12. Costanzo, V. *et al. Mol. Cell* **8**, 137–147 (2001).
13. Buis, J. *et al. Cell* **135**, 85–96 (2008).
14. Deriano, L., Stracker, T.H., Baker, A., Petrini, J.H. & Roth, D.B. *Mol. Cell* **34**, 13–25 (2009).
15. Helmink, B.A. *et al. J. Exp. Med.* **206**, 669–679 (2009).
16. Dinkelmann, M. *et al. Nat. Struct. Mol. Biol.* **16**, 808–813 (2009).
17. Rass, E. *et al. Nat. Struct. Mol. Biol.* **16**, 819–824 (2009).
18. Xie, A., Kwok, A. & Scully, R. *Nat. Struct. Mol. Biol.* **16**, 814–818 (2009).
19. Deng Y., Guo X., Ferguson D.O., & Chang S. *Nature* advance online publication, doi:10.1038/nature08196 (26 July 2009).
20. McVey, M. & Lee, S.E. *Trends Genet.* **24**, 529–538 (2008).
21. Sekiguchi, J.M. & Ferguson, D.O. *Cell* **124**, 260–262 (2006).
22. Ahnesorg, P., Smith, P. & Jackson, S.P. *Cell* **124**, 301–313 (2006).
23. Buck, D. *et al. Cell* **124**, 287–299 (2006).
24. Li, G. *et al. Mol. Cell* **31**, 631–640 (2008).
25. Rooney, S., Chaudhuri, J. & Alt, F.W. *Immunol. Rev.* **200**, 115–131 (2004).
26. Wang, J.H. *et al. J. Exp. Med.* **205**, 3079–3090 (2008).
27. Zhu, C. *et al. Cell* **109**, 811–821 (2002).
28. Wang, J.H. *et al. Nature* **460**, 231–236 (2009).
29. Guirouilh-Barbat, J. *et al. Mol. Cell* **14**, 611–623 (2004).
30. Guirouilh-Barbat, J., Rass, E., Plo, I., Bertrand, P. & Lopez, B.S. *Proc. Natl. Acad. Sci. USA* **104**, 20902–20907 (2007).
31. Yan, C. *et al. Nature* **449**, 478–482 (2007).
32. Soulas-Sprauel, P. *et al. J. Exp. Med.* **204**, 1717–1727 (2007).
33. Paull, T.T. & Gellert, M. *Proc. Natl. Acad. Sci. USA* **97**, 6409–6414 (2000).
34. Ma, J.L., Kim, E.M., Haber, J.E. & Lee, S.E. *Mol. Cell. Biol.* **23**, 8820–8828 (2003).
35. Taccioli, G.E. *et al. Science* **260**, 207–210 (1993).
36. Corneo, B. *et al. Nature* **449**, 483–486 (2007).
37. Yun, M.H. & Hiom, K. *Nature* **459**, 460–463 (2009).
38. Kracker, S. *et al. Proc. Natl. Acad. Sci. USA* **102**, 1584–1589 (2005).
39. Reina-San-Martin, B., Nussenzweig, M.C., Nussenzweig, A. & Difilippantonio, S. *Proc. Natl. Acad. Sci. USA* **102**, 1590–1595 (2005).
40. Pan-Hammarström, Q. *et al. J. Exp. Med.* **201**, 189–194 (2005).
41. Larson E. *et al. Mol. Cell.* **20**, 367–375 (2005).
42. Palm,W. & de Lange,T. *Annu. Rev. Genet.* **42**, 301–334 (2008).
43. Zhu, X.D., Kuster, B., Mann, M., Petrini, J.H. & de Lange, T. *Nat. Genet.* **25**, 347–352 (2000).
44. Zhu, X.D. *et al. Mol. Cell* **12**, 1489–1498 (2003).