Repair of double-strand breaks by nonhomologous end joining in the absence of Mre11

Michela Di Virgilio and Jean Gautier

Introduction

Mammalian cells rely on the nonhomologous end-joining (NHEJ) pathway to repair most of their DNA double-strand breaks (DSBs), the most harmful type of DNA damage (Khanna and Jackson, 1998). NHEJ is a mechanism in which broken ends at a DSB site are rejoined without requiring extended segments of homology. The main components of the vertebrate NHEJ machinery comprise DNA ligase IV, XRCC4, Artemis, and the DNA-dependent protein kinase (DNA-PK), which is composed of a large catalytic subunit, DNA-PKcs, and the Ku70/80 heterodimer. Defects in NHEJ factors result in radiosensitivity and immunodeficiency syndromes, as vertebrate lymphocytes rely on the NHEJ pathway to complete the rejoining step of V(D)J recombination (Taccioli et al., 1993; Nussenzweig et al., 1996).

The MRN/MRX complex is a multisubunit nuclease that is composed of Mre11, Rad50, and Nbs1 (Xrs2 in Saccharomyces cerevisiae). Mutations in genes encoding these proteins result in genomic instability, increased sensitivity to ionizing radiation, telomere shortening, and meiosis defects (D’Amours and Jackson, 2002). In S. cerevisiae, HO-induced NHEJ-dependent cell survival is reduced in mre11, rad50, or xrs2 mutants (Moore and Haber, 1996). Mutations in any of the MRN/X complex components display a 40–50-fold reduction in the efficiency of plasmid end joining in S. cerevisiae (Boulton and Jackson, 1998) but show no significant defects in Schizosaccharomyces pombe (Manolis et al., 2001).

Mre11, Rad50, and Nbs1 are all essential genes in vertebrates (Xiao and Weaver, 1997; Luo et al., 1999; Zhu et al., 2001). In vitro studies with extracts from mammalian cells indicated a requirement for the MRN complex in the NHEJ pathway (Huang and Dynan, 2002; Zhong et al., 2002). In contrast, no DSB repair deficiency has been observed in human or mouse cells harboring hypomorphic mutations in the Mre11 or Nbs1 gene, which are responsible for Ataxia telangiectasia–like disorder (ATLD) or Nijmegen breakage syndrome, respectively (Varon et al., 1998; Stewart et al., 1999). However, a subset of MRN complex activities is still associated with ATLD and Nijmegen breakage syndrome alleles (Stewart et al., 1999; Theunissen et al., 2003; Costanzo et al., 2004). Therefore, these experiments could not rule out a role for the MRN complex in NHEJ.

Xenopus laevis egg extracts support highly efficient and precise end joining of linear DNA molecules using similar pathways to those found in mammalian cells (Pfeiffer and Vielmetter, 1988; Thode et al., 1990; Sandoval and Labhart, 2002). These extracts have been used extensively to characterize the role of the Ku protein in eukaryotic NHEJ (Labhart, 1999; Sandoval and Labhart, 2002). In this study, we show that in contrast to Ku70-depleted extracts, Mre11-depleted extracts are competent to end join with high efficiency regardless of the
DNA end structure. We find that the kinetics of end joining as well as the sequence of junctions formed is comparable in the absence of Mre11 and in mock-depleted extracts. Together, our data indicate that the MRN complex plays no detectable role in the repair of DSBs by the NHEJ pathway.

Results and discussion

Immunodepletion of Mre11 and Ku70 proteins from X. laevis egg extracts

We investigated the consequences of Mre11 inactivation on NHEJ by depleting Mre11 protein from cell-free extracts with a polyclonal serum specific for X. laevis Mre11 protein (antiXMre11). We previously used these antibodies to demonstrate that genomic DNA accumulates DSBs during DNA replication in Mre11-depleted extracts (Costanzo et al., 2001). Moreover, Mre11 depletion dramatically impairs the ATM (Axatia telangiectasia mutated)-dependent response to DSBs (Costanzo et al., 2004). Importantly, we show that the former defect is rescued by the Mr11 hypomorphic ATL34/3 allele, whereas ATM activation is not (Costanzo et al., 2004). This clearly demonstrates that Mre11-associated activity remains in ATL3 cells. We used two types of cell-free extracts for our study and established that egg cytosol and membrane-free egg cytosol supported NHEJ with similar efficiency (see Figs. 2 and 4).

Mre11 protein was quantitatively removed by two rounds of immunodepletion and was no longer detectable by Western blotting (Fig. 1 A, lanes 2–5; and B). Mre11 depletion did not affect Ku70 protein level (Fig. 1 A, lanes 2–5). Conversely, Mre11 protein levels were not reduced by Ku70 depletion (Fig. 1 A, lanes 6–9). This demonstrates that there is no significant interaction between the two proteins in solution, and it allows for the independent analysis of Mre11- and Ku70-depleted extracts. Moreover, both Mre11 and Ku70 proteins can be depleted simultaneously from egg extracts (Fig. 1 C).

All assays were performed after two rounds of immunodepletion. The extent of Mre11 depletion was assessed by incubating 20 μl of depleted extracts with linear biotinylated DNA molecules immobilized on streptavidin beads. The total fraction of DNA-bound proteins was then pulled down and analyzed by Western blotting (Fig. 1 A, lanes 2–5; and B). Mre11 depletion did not affect Ku70 protein level (Fig. 1 A, lanes 2–5). Conversely, Mre11 protein levels were not reduced by Ku70 depletion (Fig. 1 A, lanes 6–9). This demonstrates that there is no significant interaction between the two proteins in solution, and it allows for the independent analysis of Mre11- and Ku70-depleted extracts. Moreover, both Mre11 and Ku70 proteins can be depleted simultaneously from egg extracts (Fig. 1 C). Mre11 is not required for efficient NHEJ

Both Mre11- and Ku70-depleted extracts were tested for NHEJ activity. Ku70 depletion provided a control for the removal of a protein required for NHEJ (Fig. 2, A and B). Nine different NHEJ substrates were generated by digesting the pBS KS II plasmid with one or two different restriction enzymes to yield linear DNA molecules (Table SI, available at http://www.jcb.org/cgi/content/full/jcb.200506029/DC1). The NHEJ substrates were individually incubated in extracts at a final concentration of 1 ng/μl, and samples were taken at 0 and 150 min. Upon incubation in control extracts (Fig. 2 A, untreated egg cytosol and mock-depleted extracts), the linear molecules underwent efficient intramolecular NHEJ, generating covalently closed circles (CCs). CCs were the prominent NHEJ product in control extracts (Fig. 2 A and Fig. S1). In addition to recircularization, intermolecular joining of the linear substrates generated minor products: linear dimer (LD) as well as higher multimeric forms (collectively indicated as M). Ku70 depletion dramatically impaired intramolecular end joining, and CC forms were not detected (Fig. 2 A, lanes 10, 11, 21, and 22). As previously described (Labhart, 1999), multimerization of linear substrates was favored in these extracts. This pattern of reduced recircularization and increased multimer formation after Ku70 depletion was observed with all NHEJ substrates (unpublished data). Next, we assessed whether the catalytic activity of DNA-PKcs was required by using NU7026, a specific inhibitor of DNA-PKcs (Hollick et al., 2003). We found that intramolecular end joining was inhibited at doses as low as 0.5 μM, whereas intermolecular end joining was inhibited at 25 μM NU7026 (Fig. S2, available at http://www.jcb.org/cgi/content/full/jcb.200506029/DC1). Our results suggest that DNA-PKcs also required for NHEJ. In
contrast, the profile of NHEJ product formation was not affected by Mre11 depletion and was indistinguishable from that of both untreated and mock-depleted extracts. In the absence of Mre11 protein, the linear substrates were efficiently converted to NHEJ products (Fig. 2 A, lanes 14, 15, 25, and 26). This strongly suggests that Mre11 is dispensable for NHEJ repair of these substrates.

To uncover any possible DNA end structure–dependent role of Mre11 in NHEJ, we determined the repair efficiency for each NHEJ substrate in Ku70- or Mre11-depleted extracts. Repair efficiency was defined as the ratio between recircularized product (CC) and total DNA (NHEJ products and remaining substrate) and was expressed as the percentage of the corresponding efficiency in mock-depleted extracts (Fig. 2 B, percent repair efficiency values). We used circular product formation by intramolecular end joining as a relevant measure for NHEJ activity in the extract because CC formation is dependent on Ku70 and DNA-PKcs (Fig. S2; Labhart 1999). In the absence of Ku70, the efficiency of the repair reaction was reduced to zero for all NHEJ substrates (Fig. 2 B), including cohesive 5′-protruding single strand (PSS) digested with EcoRI or BamHI (unpublished data), which differs from a previous study (Labhart, 1999). In contrast, the mean repair efficiency in Mre11-depleted extracts was 95.1% of the corresponding value in mock-depleted extracts (Fig. 2 B). Similar results were obtained when NHEJ reactions were monitored in extracts supplemented with the recombinant MRN complex at a final concentration up to threefold the level of endogenous MRN complex (Fig. 3 B).

Recent in vitro studies in HeLa and S. cerevisiae reported a direct stimulatory interaction between the MRN complex and NHEJ core components (Chen et al., 2001; Huang and Dynan, 2002). Moreover, studies in fractionated mammalian cell extracts showed that extracts in which Rad50 activity was inhibited were defective in intermolecular end joining (Zhong et al., 2002). Unlike X. laevis extracts, these mammalian cell-free extracts do not support intramolecular end joining, as seen by the exclusive formation of dimers and higher multimers resulting.
from intermolecular ligation. Furthermore, cell-free extracts from X. laevis eggs differ from an in vitro system or a fractionated cell extract in that they contain all cytosolic and nuclear proteins normally present in a cell. These extracts support regulated chromatin assembly, DNA replication, recombination, and repair, and they are not rate limited for the proteins participating in these functions.

Importantly, inhibiting Ku70 activity favors intermolecular joining (Figs. 2 A and 3 A; Labhart 1999). This suggests that multimers are formed by a Ku70-independent pathway reminiscent of what was recently described in mammalian cells (Wang et al., 2003). In X. laevis, the Ku70-independent pathway leading to LD and multimer formation is also independent of Mre11, as intermolecular products are still generated in extracts depleted of both Ku70 and Mre11 (Fig. 3 A).

The kinetics of end-joining reactions is not affected by Mre11 depletion

The aforementioned experiments did not completely rule out that an alternative Mre11-independent NHEJ pathway could substitute for the absence of Mre11. To uncover a hypothetical Mre11-independent pathway, we compared the kinetics of end joining, assuming that different pathways would display different kinetics. We monitored NHEJ activity in Mre11- and mock-depleted extracts at different times during the reaction. NHEJ substrate nonmatching 3'·PSS was incubated in membrane-free egg cytosol, and samples were taken at 0, 0.5, 1, 5, 15, 30, 60, and 120 min. Repaired products were then purified and tested in the colony formation assay. The transforming activity associated with each sample was a read-out of the intramolecular NHEJ activity (circular product formation). At time = 0, no colonies were obtained from either Mre11- or mock-depleted extract samples, thus confirming that all substrate molecules in the input DNA were linear (Fig. 4 A). Repair products were generated as early as 0.5 min in Mre11- and mock-depleted extracts. Furthermore, membrane-free egg cytosol was able to support end joining with similar kinetics in the presence or absence of Mre11. Similar results were obtained when the kinetics of end joining was monitored by Southern blot analysis (Fig. 4 B). We conclude that Mre11 is not required for the rapid NHEJ observed in extracts.

Mre11 is not required for accurate ligation of DNA ends

Next, we examined the fidelity of end-joining reactions in Mre11- and mock-depleted extracts by sequencing the junctions of cloned NHEJ products. We found that all NHEJ substrates were religated without gross rearrangements (Table I). This is in agreement with a previous study (Pfeiffer and Vielmetter, 1988). The specific joining mode was determined by the terminus configuration of the NHEJ substrate. Ends with cohesive 5'· or 3'·PSSs as well as blunt ends were always religated without net nucleotide loss. Nonmatching 3'·PSS substrates were joined after the alignment of ends that allowed the most efficient base pairing. When no base pairing could occur between the partner ends (blunt end + 5'·PSS, blunt end + 3'·PSS, 5'·PSS + 3'·PSS, and completely nonmatching 5'·PSS), the PSSs were usually preserved by fill-in DNA synthesis. Occasional nucleotide additions, mainly adenosine or thymidine residues, and nucleotide losses, which could be caused by exonucleolytic trimming events, were observed. These events contributed to the variety and complexity of junction formation (Table I). With the exception of a few junction types that occurred very rarely, the spectrum of joining products formed in Mre11-depleted extracts was nearly identical to that obtained in mock-depleted extracts. Moreover, the frequency with which these junction types were formed was not altered in extracts lacking Mre11 protein (Table I). In summary, these data suggest that Mre11 is not required for accurate NHEJ in X. laevis egg extracts and does not bias joining toward a specific type of junction.

Altogether, our data demonstrate that Mre11 is dispensable for all aspects of DSB repair by NHEJ: efficiency, kinetics, and fidelity. This is consistent with results in S. pombe, in which the loss of Rad32, the Mre11 homologue, or Rad50 does not impair either the efficiency or the fidelity of NHEJ (Manolis et al., 2001). In contrast, the MRX complex has been reported to be
important for NHEJ-mediated repair of both chromosomal and plasmid DSBs in *S. cerevisiae* (Moore and Haber, 1996; Boulton and Jackson, 1998). However, the mechanism by which the MRX complex participates in NHEJ has not been described. The differential requirement for the MRN complex is not a result of the DNA template used because our study and the studies in *Saccharomyces cerevisiae* (Moore and Haber, 1996; Boulton et al., 1999) reported a higher radiosensitivity of Mre11-deficient cells compared with wild-type cells from G1 to early S phase, when DSBs are repaired mainly by Ku70-dependent NHEJ in this cell line. However, *MRE11*-null chicken DT40 chicken DT40 cell line (Yamaguchi-Iwai et al., 1999) and the *MRE11*-null *M. cerebralis* all used a plasmid-based assay (Boulton and Jackson, 1998; Manolis et al., 2001).

Our results are consistent with the only study addressing the consequences of a complete loss of Mre11 in vertebrates that was performed with a conditional *Mre11*-null chicken DT40 cell line (Yamaguchi-Iwai et al., 1999). Yamaguchi-Iwai et al. (1999) reported a higher radiosensitivity of *Mre11−/−*/*Ku70−/−* cells compared with *Mre11−/−* cells. They also observed similar levels of radiosensitivity in *Mre11−/−* Mre11−/− and *MRE11−/−* Mre11−/− cells from G1 to early S phase, when DSBs are repaired mainly by Ku70-dependent NHEJ in this cell line. However, *MRE11−/−* cells grew very poorly and displayed a high rate of apoptosis upon Mre11 repression. Therefore, end-joining ability in Mre11-deficient cells could not be assessed directly in this study.

In summary, our study establishes that Mre11 is not required for intramolecular end joining of linear DNA molecules or for the Ku70-independent intermolecular end joining of linear DNA in a vertebrate system.
| NHEJ substrate type | Terminus sequence | Joining intermediate | Joining product | Control | -Mre11 |
|---------------------|-------------------|----------------------|-----------------|---------|--------|
| **Blunt ends (EcoRV)** | 5’-GAT ATC3'-3’ | 5’-GATATC3'-3’ | 5’-GATATC3'-3’ | 1 [8/8] | 1 [8/8] |
| 3’-CTA TAG5'-3’ | 3’-CTATAG5’-3’ | 3’-CTATAG5’-3’ | 3’-CTATAG5’-3’ | 1 [7/7] | 1 [7/7] |
| **Cohesive 5’-PSS (EcoRI)** | 5’-G | AATTC3'-3’ | 5’-GAGATTC3'-3’ | 0.77 [10/13] | 0.92 [12/13] |
| 3’-CTAA G5'-3’ | 3’-CTTAA G5’-3’ | 3’-CTTAA G5’-3’ | 3’-CTTAA G5’-3’ | 0.08 [1/13] | 0.08 [1/13] |
| **Nonmatching 5’-PSS (NotI-EcoRI)** | 5’-C | AATTC3'-3’ | 5’-GCCGCAATC3'-3’ | 0.15 [2/13] | 0.03 [0/13] |
| 3’-GCGG G5'-3’ | 3’-GCCG TGCAC5’-3’ | 3’-GCCG TGCAC5’-3’ | 3’-GCCG TGCAC5’-3’ | 0.15 [2/13] | 0.03 [0/13] |
| **Blunt end + 5’-PSS (BamHI-EcoRV)** | 5’-G | ATC3'-3’ | 5’-GGAATTC3'-3’ | 0.92 [11/12] | 0.92 [12/13] |
| 3’-CTTAA G5'-3’ | 3’-CTTAA G5’-3’ | 3’-CTTAA G5’-3’ | 3’-CTTAA G5’-3’ | 0.08 [1/12] | 0.08 [1/13] |
| **Cohesive 3’-PSS (PstI)** | 5’-G TCGA C5'-3’ | 5’-G GAGATTC3'-3’ | 0.75 [21/28] | 0.63 [17/27] |
| 3’-GACGCTGT5'-3’ | 3’-GAGGTCGTC5’-3’ | 3’-GAGGTCGTC5’-3’ | 3’-GAGGTCGTC5’-3’ | 0.75 [21/28] | 0.63 [17/27] |
| **Nonmatching 3’-PSS (Sacl-KpnI)** | 5’-G TCGA C5'-3’ | 5’-G GAGATTC3'-3’ | 0.64 [9/14] | 0.64 [9/14] |
| 3’-ACC CATGGG5'-3’ | 3’-ACC CATGGG5’-3’ | 3’-ACC CATGGG5’-3’ | 3’-ACC CATGGG5’-3’ | 0.64 [9/14] | 0.64 [9/14] |
| **Nonmatching 3’-PSS (PstI-KpnI)** | 5’-G TCGA C5'-3’ | 5’-G GAGATTC3'-3’ | 0.21 [3/14] | 0.28 [4/14] |
| 3’-ACC CATGGG5'-3’ | 3’-ACC CATGGG5’-3’ | 3’-ACC CATGGG5’-3’ | 3’-ACC CATGGG5’-3’ | 0.14 [2/14] | 0.08 [1/14] |
| **Blunt end + 3’-PSS (Sacl-Smal)** | 5’-GAGCT GGGG3'-3’ | 5’-GAGCTGGCC3'-3’ | 0.77 [10/13] | 0.71 [10/14] |
| 3’-CCGG G5’-3’ | 3’-CCGG G5’-3’ | 3’-CCGG G5’-3’ | 3’-CCGG G5’-3’ | 0.08 [1/13] | 0.07 [1/14] |
| **5’-PSS + 3’-PSS (PstI-XhoI)** | 5’-G TCGA C5'-3’ | 5’-G GAGATTC3'-3’ | 0.64 [9/14] | 0.71 [10/14] |
| 3’-ACC CATGGG5'-3’ | 3’-ACC CATGGG5’-3’ | 3’-ACC CATGGG5’-3’ | 3’-ACC CATGGG5’-3’ | 0.08 [1/13] | 0.07 [1/14] |

○, mismatched base-pair; ●, matched base-pair; ★, fill in DNA synthesis.

*The most likely joining intermediate for each NHEJ substrate, as deduced from the analysis of the junction sequences, is indicated.*
Boulton, S.J., and S.P. Jackson. 1998. Components of the Ku-dependent non-homologous end-joining pathway are involved in telomeric length maintenance and telomeric silencing. *EMBO J.* 17:1819–1828.

Chen, L., K. Trujillo, W. Ramos, P. Sung, and A.E. Tomkinson. 2001. Promotion of Dnl4-catalyzed DNA end-joining by the Rad50/Mre11/Xrs2 and Hdl1/Hdl2 complexes. *Mol. Cell.* 8:1105–1115.

Costanzo, V., K. Robertson, M. Bibikova, E. Kim, D. Grieco, M. Gottesman, D. Carroll, and J. Gautier. 2001. Mre11 protein complex prevents double-strand break accumulation during chromosomal DNA replication. *Mol. Cell.* 8:137–147.

Costanzo, V., T. Paull, M. Gottesman, and J. Gautier. 2004. Mre11 assembles linear DNA fragments into DNA damage signaling complexes. *PLoS Biol.* doi: 10.1371/journal.pbio.0020110.

D’Amours, D., and S.P. Jackson. 2002. The Mre11 complex: at the crossroads ofDNA repair and checkpoint signalling. *Nat. Rev. Mol. Cell Biol.* 3:317–327.

Hollick, J.J., B.T. Golding, I.R. Hardcastle, N. Martin, C. Richardson, L.J. Rigoreau, G.C. Smith, and R.J. Griffin. 2003. 2,6-disubstituted pyran-4-one and thiopyran-4-one inhibitors of DNA-dependent protein kinase (DNA-PK). *Bioorg. Med. Chem. Lett.* 13:3083–3086.

Huang, J., and W.S. Dynan. 2002. Reconstitution of the mammalian DNA double-strand break end-joining reaction reveals a requirement for an Mre11/Rad50/NBS1-containing fraction. *Nucleic Acids Res.* 30:667–674.

Khanna, K.K., and S.P. Jackson. 2001. DNA double-strand breaks: signaling, repair and the cancer connection. *Nat. Genet.* 27:247–254.

Labhart, P. 1999. Ku-dependent nonhomologous DNA end joining in *Xenopus* egg extracts. *Mol. Cell. Biol.* 19:2585–2593.

Luo, G., M.S. Yao, C.F. Bender, M. Mills, A.R. Blall, A. Bradley, and J.H. Petrini. 1999. Disruption of mRad50 causes embryonic stem cell lethality, abnormal embryonic development, and sensitivity to ionizing radiation. *Proc. Natl. Acad. Sci. USA.* 96:7376–7381.

Manolits, K.G., E.R. Nimmo, E. Hartsuiker, A.M. Carr, P.A. Jeggo, and R.C. Allshire. 2001. Novel functional requirements for non-homologous DNA end joining in *Schizosaccharomyces pombe*. *EMBO J.* 20:210–221.

Moore, J.K., and J.E. Haber. 1996. Cell cycle and genetic requirements of two pathways of nonhomologous end-joining repair of double-strand breaks in *Saccharomyces cerevisiae*. *Mol. Cell. Biol.* 16:2164–2173.

Nussenzweig, A., C. Chen, V. da Costa Soares, M. Sanchez, K. Sokol, M.C. Nussenzweig, and G.C. Li. 1996. Requirement for Ku80 in growth and immunoglobulin V(D)J recombination. *Nature.* 382:551–555.

Pfeiffer, P., and W. Vielmetter. 1988. Joining of nonhomologous DNA double strand breaks in vitro. *Nucleic Acids Res.* 16:907–924.

Sandoval, A., and P. Labhart. 2002. Joining of DNA ends bearing non-matching 3’-overhangs. DNA Repair (Amst.). 1:397–410.

Stewart, G.S., R.S. Maser, T. Stankovic, D.A. Bressan, M.I. Kaplan, N.G. Jaspers, A. Raams, P.J. Byrd, J.H. Petrini, and A.M. Taylor. 1999. The DNA double-strand break repair gene hMRE11 is mutated in individuals with an ataxia-telangiectasia-like disorder. *Cell.* 99:577–587.

Taccioli, G.E., G. Rathbun, E. Oltz, T. Stamato, P.A. Jeggo, and F.W. Alt. 1993. Impairment of V(D)J recombination in double-strand break repair mutants. *Science.* 260:207–210.

Theunissen, J.W., M.I. Kaplan, P.A. Hunt, B.R. Williams, D.O. Ferguson, F.W. Alt, and J.H. Petrini. 2003. Checkpoint failure and chromosomal instability without lymphomagenesis in Mre11(1ATLD1/ATLD1) mice. *Mol. Cell.* 12:1511–1523.

Thode, S., A. Schafer, P. Pfeiffer, and W. Vielmetter. 1990. A novel pathway of DNA end-to-end joining. *Cell.* 60:921–928.

Varon, R., C. Vissinga, M. Platzer, K.M. Cerosaletti, K.H. Chrzanowska, K. Saar, G. Beckmann, E. Seemanova, P.R. Cooper, N.J. Nowak, et al. 1998. Nibrin, a novel DNA double-strand break repair protein, is mutated in Nijmegen breakage syndrome. *Cell.* 93:467–476.

Wang, H., A.R. Perrault, Y. Takeda, W. Qin, and G. Iliakis. 2003. Biochemical evidence for Ku-independent backup pathways of NHEJ. *Nucleic Acids Res.* 31:5377–5388.

Xiao, Y., and D.T. Weaver. 1997. Conditional gene targeted deletion by Cre recombinase demonstrates the requirement for the double-strand break repair Mre11 protein in murine embryonic stem cells. *Nucleic Acids Res.* 25:2985–2991.

Yamaguchi-Iwai, Y., E. Sonoda, M.S. Sasaki, C. Morrison, T. Haraguchi, Y. Hirao, Y.M. Yamashita, T. Yagi, M. Takata, C. Price, et al. 1999. Mre11 is essential for the maintenance of chromosomal DNA in vertebrate cells. *EMBO J.* 18:6619–6629.