EXPERIMENTAL CELL RESEARCH 329 (2014) 139–147

Available online at www.sciencedirect.com

ScienceDirect

journal homepage: www.elsevier.com/locate/yexcr

Review Article

The Mre11/Rad50/Nbs1 complex: Recent insights into catalytic activities and ATP-driven conformational changes

*Tanya T. Paull**, *Rajashree A. Deshpande*

The Howard Hughes Medical Institute, The Department of Molecular Biosciences, The Institute for Cellular and Molecular Biology, The University of Texas at Austin, Austin, TX 78712, USA

ARTICLE INFORMATION

Article Chronology:
Received 12 June 2014
Accepted 1 July 2014
Available online 9 July 2014

Keywords:
Mre11
Rad50
Nbs1
DNA double-strand break repair

Contents

Introduction

DNA double-strand breaks (DSBs) can arise from internal or external sources of damage, and the rapid detection, processing, and repair of this damage is important for cell viability. Failure to repair DNA damage can result in genomic instability, ultimately increasing the frequency of lymphoid disorders, neurodegeneration, and cancer. The Mre11–Rad50–Nbs1 (Xrs2) complex plays a central and critical

*Corresponding author.
E-mail address: tpaull@utexas.edu (T.T. Paull).

http://dx.doi.org/10.1016/j.yexcr.2014.07.007

role in detection and repair of DSBs and is conserved in all kingdoms of life, as Mre11/Rad50 (MR) in prokaryotes and as MRN/X in eukaryotes [36,74]. The importance of this complex is emphasized by the fact that deletion of any of the three components results in embryonic lethality in mice and loss of proliferative activity in embryonic stem cells ([5,50,88,96]) which is likely related to the role of MRN/X in homologous recombination. Repair of DSBs by homologous recombination involves replication of the broken region using an undamaged template, usually a sister chromatid. Deletions of other genes important for homologous forms of repair also exhibit early embryonic lethality, including Rad51, BRCA1, BRCA2, and CtBP-interacting protein (CtIP) [12,27,46,69]. Hypomorphic mutations in MRN components result into developmental and neurodegenerative disorders in humans, including Ataxia-Telangiectasia-Like Disorder (ATLD), Nijmegen Breakage Syndrome (NBS), and NBS-like syndrome [53,73,80,81], which are related, at least in part, to the role of MRN/X in the activation of cell-cycle checkpoints through the Ataxia-Telangiectasia-Mutated (ATM) protein kinase [43,71]. The roles of MRN/X also extend to the processing of DSBs during meiosis, for which it is essential, and to telomere maintenance [3,36].

Repair of DSBs is achieved through two broadly-defined groups of pathways: non-homologous end joining (NHEJ) and homologous recombination (HR) [35]. The choice between these pathways primarily depends on the cell-cycle phase and the complexity of the damage generated at the break site [9,67]. In the classical NHEJ pathway, ends are bound by the Ku70–Ku80 heterodimer/DNA-dependent protein kinase catalytic subunit (DNA-PKcs) complex which recruits additional factors involved in end modifications and gap filling. DNA ends are ultimately ligated by the NHEJ-specific DNA ligase IV complex [21]. In mammalian cells, the C-NHEJ pathway is not dependent on the MRN complex, although in budding yeast MRX contributes to NHEJ pathway through interactions with Ku70–Ku80 and DNA Lig4 complexes [45]. The MRN complex, in conjunction with CtIP/Sae2, also regulates the alternative NHEJ (A-NHEJ or MMEJ), which utilizes short microhomologies and can result in large deletions [44,92]. In mammalian cells MRN was also shown to interact with DNA ligaseIIIα/Xrcc1, the ligase complex implicated in alternative NHEJ, stimulating intermolecular ligation [20].

In contrast to NHEJ, HR requires the 5′–3′ resection of dsDNA to generate single-stranded DNA tails, a process that is initiated by the MRN complex and CtIP [90]. Extensive resection is perfomed by exonuclease 1 (Exo1), and Dna2 [76], whose activities are also promoted by MRN [7,59,60,89,94,95]. 3′ ssDNA tails thus generated are bound by replication protein A (RPA), which activates ATM- and Rad3-Related (ATR), promoting replication checkpoint arrest and stabilization of replication forks [93]. RPA on these 3′ ssDNA tails is then exchanged for Rad51 to create Rad51 filaments that catalyze homology search and strand invasion, ultimately priming DNA synthesis and resolution of repair intermediates.

The MRN complex plays important and diverse roles in DNA double-strand break repair and signaling. Here we review recent evidence elucidating the structures and regulation of the Mre11/Rad50 complex, focusing primarily on the enzymatic activities of MRN and the role of ATP-driven conformational changes in Rad50.

Mre11 nuclease activity

The Mre11 protein is related to a family of phosphoesterases that includes lambda phosphatase, protein phosphatase-2B, PP2A, PP1, calcineurin, and purple acid phosphatases ([34]) (Fig. 1). This family of enzymes binds two metal ions in the active site and cleaves either phosphomonoester or phosphodiester bonds. Mre11 is conserved in all species and exhibits manganese-dependent 3′ to 5′ exonuclease and endonuclease activities on double-stranded DNA in vitro [13,33,62,78,79]. The roles of these activities in cells have been widely debated and it is still not entirely clear what the biologically relevant activity is, but it is likely that this depends on the structure and context of the DNA ends. Experiments in budding yeast have shown that the nuclease activity of Mre11 is dispensable for the resection of enzymatically-generated DSBs but is absolutely required for meiosis when DSBs are covalently bound by the Spo11 protein, as well as for the processing of cruciform structures in vegetatively growing cells [48,57,64]. Mre11 nuclease activity also contributes to (but is not essential for) the survival of radiation damage and topoisomerase conjugates in budding yeast [57], although at least some of its activities are redundant with Dna2 [4]. In vitro experiments show that a nuclease-deficient MRN complex can promote Exo1-mediated resection in the presence of Ku and DNA-PKcs equivalently to that of a wild-type complex [89,95], further suggesting that Mre11 nuclease activity is not essential for resection of enzymatically generated DSBs. Similarly, the nuclease activity of MRN not necessary for compatible end ligation by DNA ligase IIIα/Xrcc1, but is critical for the ligation of incompatible DNA ends that require processing [20].

The role of Mre11 3′ to 5′ exonuclease activity has been unclear, in part because it requires millimolar levels of manganese in vitro (higher than would ever be encountered in vivo) and because the polarity of the exonuclease is opposite to the 5′ to 3′ resection required for creation of long 3′ ssDNA tails that are ultimately bound by Rad51. Recent support for a physiological role of the exonuclease activity came from a study of meiotic DSB repair in which breaks are created by and covalently linked to Spo11 on the 5′ strands [26]. Although Mre11 nuclease activity was known to be required for removal of Spo11, Neale et al. showed in this work that the 3′ to 5′ exonucleolytic activity of Mre11 is specifically required to resect the Spo11-linked strand after an endonucleolytic break is first made, approximately 300 nt from the Spo11 cut; 5′ to 3′ exonucleolytic degradation from the nick was found to be dependent on Exo1 (Fig. 2).

A similar model was proposed for HR in mammalian cells, where the roles of Mre11 exo- and endonuclease activites were assessed through the use of small molecule inhibitors that specifically affect each of these activities [70]. It was suggested that Mre11 endonucleolytic activity initiates resection, followed by Mre11-dependent 3′ to 5′ exonuclease and Exo1/BLM-dependent bidirectional resection from the site of the nick. The catalytic functions of Mre11 appear to be much more important in mammals than they are in yeast, as a transgenic mouse model expressing nuclease-deficient Mre11 exhibits early embryonic lethality [5]. In further support of a physiological role of Mre11 exonucleolytic activity in mammalian cells, Mre11 was found to be responsible for the degradation of nascent strands at stalled replication forks in the absence of BRCA2 [68].

Mre11 endonucleolytic activity in manganese has been observed in vitro with all Mre11 orthologs studied [15,29,63,78]. A weak but detectable endonuclease activity was also observed on 5′ strands of DSBs in the presence of magnesium using *Pyrococcus furiosus* MR (PfMR)[33]. The oligonucleotide products