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Signalling networks in focus

Double functions for the Mre11 complex during DNA double-strand break repair and replication

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ABSTRACT

Defining the factors that lead to genomic instability is one of the most important fields in cancer biology. DNA damage can arise from exogenous sources or as a result of normal cellular metabolism. Regardless of the cause, when damaged DNA is not properly repaired the genome acquires mutation(s). Under normal circumstances, to prevent such chromosome instability the cell activates the checkpoint response, which inhibits cell cycle progression until DNA repair is complete. The Mre11 complex is formed by three components: Mre11, Rad50, and Nbs1/Xrs2 and is involved in the signaling pathways that lead to both checkpoint activation and DNA repair. In response to DNA damage two functions of the complex will be discussed, one involves its role in initiating kinase activation and the second involves its ability to tether and link DNA strands. This review will highlight the functions of the Mre11 complex during the process of DNA double strand break recognition and repair, and during the process of replication. Understanding how the Mre11 complex is working at the molecular level is important for understanding why disruptions in components of the complex lead to genomic instability and cancer predisposition syndromes in humans.

1. Introduction

The Mre11 complex is comprised of three components: Mre11, Rad50, and Nbs1 (Xrs2 in *Saccharomyces cerevisiae*) and is involved in pathways critical for DNA damage repair, DNA replication, meiosis and telomere maintenance. It senses breaks in the DNA and signals for activation of the cell cycle checkpoint and also plays a central role within both the non-homologous end joining (NHEJ) and the homologous recombination (HR) pathways for DNA double strand break (DSB) repair. In addition to repair, the Mre11 complex plays an important role during DNA replication where it has been shown to prevent DSB formation under both normal and stressed conditions.

Defining the precise functions of the Mre11 complex in higher eukaryotes has been particularly challenging because null mutations in any of the three complex members is lethal. Because of this, studies in model organisms have been extremely valuable in the characterization of the Mre11 complex. For example, budding yeast has been particularly useful in determining the temporal recruitment of the Mre11 complex to sites of DNA damage and for understanding the role of Mre11 nuclease activity. As well, experiments in *Xenopus laevis* egg extracts have been instrumental for characterizing the relationship between Mre11 and ATM/ATR (ataxia-telangiectasia mutated/ATM- and Rad3 related) during replication and checkpoint activation (Trenz et al., 2006). In addition to the work done in model organisms, structural studies of the Mre11 complex show that it functions as a scaffold bridge, tethering duplexed DNA such as sister chromatids and the ends of DNA at a DSB (reviewed in Williams et al., 2007). We will review here the known function of the Mre11 complex in checkpoint activation at DSBs and during DNA replication and discuss the involvement of the Mre11 nuclease activity and the scaffolding capabilities of the complex in these processes.

2. Key molecules and functions

2.1. The Mre11 complex

Mre11 is the core complex member and it interacts with itself and both Rad50 and Nbs1/Xrs2. Mre11 has endonuclease and 3′–5′ exonuclease activities that are important in the processing of DNA ends for recognition by DNA repair and cell cycle checkpoint proteins (Jazayeri et al., 2008; Lee and Paull, 2005; Paull and Gellert, 1998). The nuclease activity of Mre11 is specified by four

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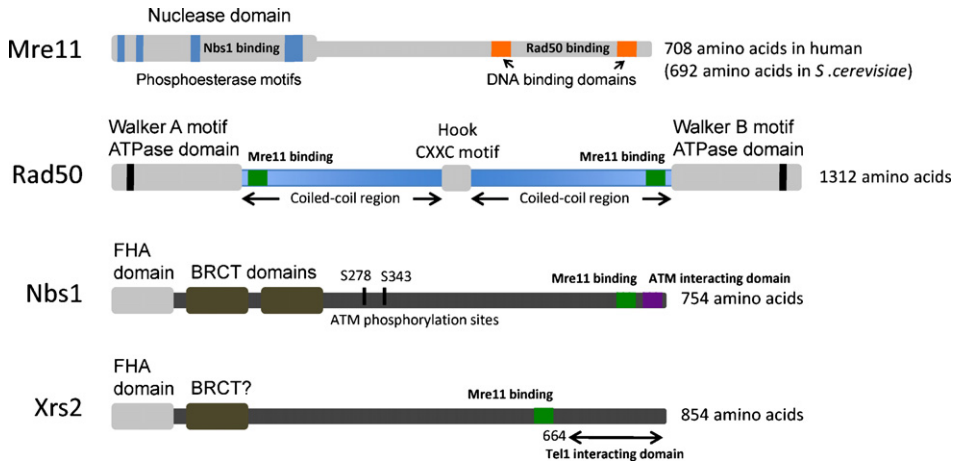

Fig. 1. Schemes of the Mre11 complex components. The domains of each component of the complex are shown here. For Mre11, the blue regions represent the relative position of the four phosphoesterase motifs and the DNA binding domains are shown in orange. As well, the regions important for Nbs1 and Rad50 interaction have been indicated. The domain structure of Rad50 shows the Walker A and B motifs at each end of the protein and Mre11 binding sites are shown in green adjacent to the ATPase domains. The coiled-coil regions meet at the hook/hinge region in the center of the protein. This central region in Rad50 contains a CXXC motif that reverses the directionality of the coiled coil, coordinating Zn^{2+} to mediate Rad50 hook-hook assembly. For Nbs1 and Xrs2 the FHA and BRCT domains are shown in the N-terminal half on the protein. Regions in Nbs1/Xrs2 that are important for Mre11 binding are shown in green and the regions that interact with ATM (purple) and Tel1 are also indicated. Also shown in Nbs1 are two ATM phosphorylation sites.

phosphoesterase motifs in the amino terminal end of the protein (Fig. 1). Furthermore, Mre11 binds duplexed DNA (Hopfner et al., 2002) and the ends of linear DNA molecules (de Jager et al., 2001; Usui et al., 1998).

The Rad50 subunit of the complex belongs to a class of proteins known as the 'structural maintenance of chromosome' (SMC) proteins. It has Walker A and B NTP-binding motifs at each end of the protein that are essential for its function. These motifs are separated by two heptad-repeat regions which fold on themselves to form an extended coiled-coil structure bearing a zinc-hook (CXXC motif) at its apex (Fig. 1). Current models indicate the ATPase motif regions of Rad50 together with Mre11 form the head domain of the complex and interact directly with DNA (Hopfner et al., 2002). The CXXC motif is important for interactions with additional Rad50 molecules within a single complex and for binding multiple complexes together. For a detailed review of the structure of the Mre11 complex we refer readers to Williams et al. (2007).

Xrs2/Nbs1 is present in the complex at sub-stoichiometric levels (Paull and Gellert, 1999), and is the most divergent complex member. Nbs1 contains four functional regions: a forkhead-associated (FHA) domain at its amino terminus, a central region with BRCT (BRCA1 carboxy-terminal) domains and ATM phosphorylation sites on serine 278 and 343 (Fig. 1). As well, two C-terminal domains have been defined, one for interaction with Mre11 and one for interaction with ATM (Fig. 1). The typical 70–80 amino acid features that define a BRCT domain are not present in Xrs2.

2.2. PI-3-kinase like kinases and checkpoint activation

Central to checkpoint signalling in *S. cerevisiae* are two phosphoinositide 3-kinase (PI3K) like kinases, Tel1 and Mec1, orthologs of ATM and ATR respectively. These kinases collaborate with other factors to mediate the activation of downstream effector kinases Chk1 (human Chk1) and Rad53 (human Chk2). In response to DNA damage or replication fork defects, Tel1/ATM associates closely with the Mre11 complex and Mec1/ATR interacts with sites of damage through it binding partner Ddc2/ATRIP.

The Mre11 complex localizes to DSBs very rapidly (Lisby et al., 2004; Nelms et al., 1998), recruiting Tel1/ATM kinase (Nakada et al., 2003). The DSB repair functions of the Mre11 complex are both initiated and regulated by Tel1/ATM kinase on a pathway parallel to Mec1–Ddc2/ATR–ATRIP (Fig. 2A; Usui et al., 2001). In humans, the Mre11 complex senses DNA damage and through multistep interactions with ATM activates the DNA damage checkpoint (reviewed in Lee and Paull, 2007).

Like components of the Mre11 complex in higher eukaryotes, ATR is an essential protein. The ATR–ATRIP/Mec1–Ddc2 complex is recruited to damage or stalled forks presumably through contact between ATRIP/Ddc2 and Replication Protein A bound single-stranded DNA (RPA-ssDNA). Once recruited, ATR activation requires interaction with topoismerase-binding protein-1 (TopBP1); which is itself localized through binding to the Rad9–Rad1–Hus1 (9-1-1) complex (reviewed in Cimprich and Cortez, 2008). Emerging data suggests that the Mre11 complex is involved in ATR signalling during replication in response to UV damage (Stiff et al., 2005) (Olson et al., 2007). Activation of Chk1 was reduced in cells carrying a nuclease deficient $Mre11^{H129N/\Delta}$ after UV treatment, indicating at least in mammals the nuclease activity of Mre11 is important in the generation of ssDNA for ATR/ATRIP recruitment and activation (Buis et al., 2008). Similarly, data from budding yeast indicates that both Mre11and Exo1 generate ssDNA tracts at forks upon UV treatment to facilitate Mec1 localization (Nakada et al., 2004).

3. Cascades

3.1. Roles of the Mre11 complex in signalling and processing of DSBs – a multistep cross-talk with ATM

DSBs in the genome can arise from endogenous sources such as reactive oxygen species produced as by-products of cellular metabolism and accidental replication fork collapse, as well as from exogenous sources like ionizing radiation or chemicals that directly or indirectly damage DNA. Self-inflicted DSBs are also induced from nucleases during programmed genomic rearrangements including mating-type switching, V(D)J recombination, class switch recombination and meiosis.

The Mre11 complex is one of the first, if not the first, factor to sense DNA DSBs (Fig. 2C [step 1]). This is consistent with its ability to bind and tether together the ends of DNA (de Jager et al., 2001; Hopfner et al., 2002). Once the Mre11 complex is bound at the DSB site, it unwinds the ends of DNA in an ATP-dependent process (Paull and Gellert, 1999). This unwinding is important for localizing ATM

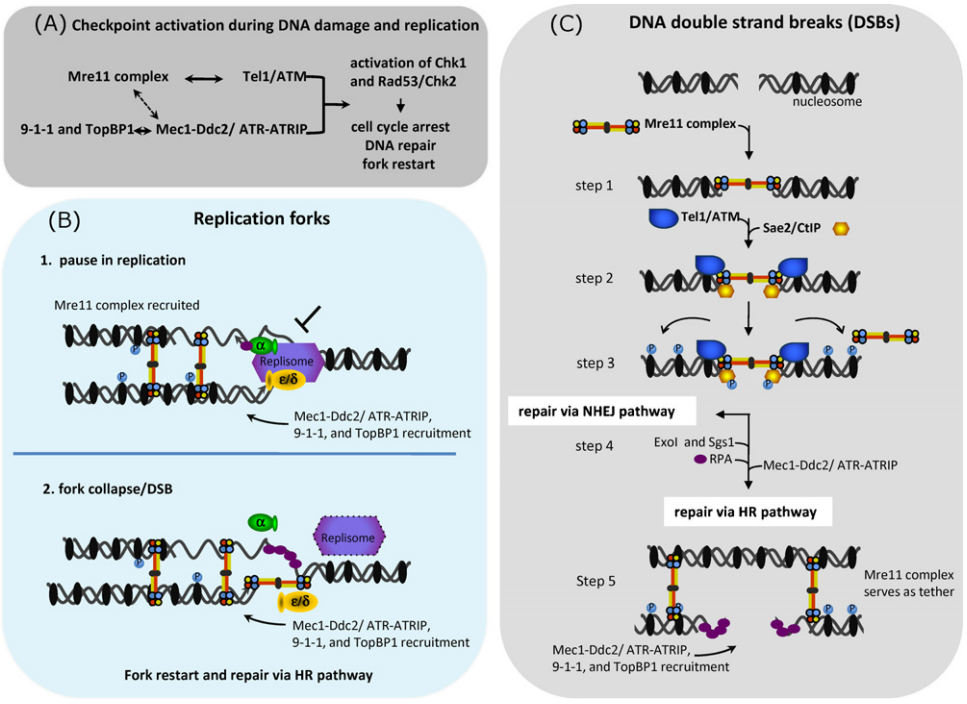

Fig. 2. Mre11 complex functions at DNA DSBs and during mishaps in replication. (A) Functional organization of the checkpoint pathway. Activation of ATM and ATR lead to the phosphorylation of an overlapping set of substrates including Chk1 and Rad53/Chk2 that promote cell-cycle arrest and DNA repair. The Mre11 complex serves to recruit and activate Tel1/ATM. Mec1–Ddc2/ATR–ATRIP and the 9-1-1-TopBP1 complex are recruited independently to sites of damage, but ATR–ATRIP activation is dependent on interaction with TopBP1. The Mre11 complex has been shown to signal ATR–ATRIP activation during UV treatment, indicating interplay between the two canonical pathways. (B) During pauses in replication (1) the Mre11 complex is recruited to the replication fork where it tethers sister chromatids. This promotes sister chromatid cohesion and has a stabilizing affect on the replication fork, allowing the replisome to resume DNA replication once the stress is alleviated. If forks collapse (2), DNA DSBs form and the Mre11 complex not only functions as a tether but functions as it does in Fig. 2A prevent DNA ends from dispersing and signaling checkpoint activation together with Tel1 kinase. (C) Upon DSB formation, the first factor recruited is the Mre11 complex, which binds and unwinds DNA ends (step 1). This allows the recruitment of Sae2/CtIP and the recruitment and activation of the Tel1/ATM PI-3-kinase like kinase (step 2). Activated ATM then phosphorylates its substrates, including histone H2AX, Nbs1/Xrs2, Mre11 and Sae2. This results in a second wave of Mre11 complex recruitment and amplification of the checkpoint response (step 3). Sae2 phosphorylation by ATM results in the activation of Mre11 nuclease activity. Exo1 and the RecQ helicase Sgs1 are necessary for further DSB end resection allowing the binding of RPA to ssDNA (step 4). The presence of RPA-ssDNA serves to recruit Mec1–Ddc2/ATR–ATRIP and its activator, TopBP1 which is bound to the 9-1-1 complex. At this step the Mre11 complex serves as a tether between chromosomes allowing DNA DSB repair by the HR homologous recombination pathway (step 5).

to the site of DNA damage (Fig. 2C [step 2]). Once recruited, ATM physically interacts with the Mre11 complex and in a process that is still not fully understood stimulates the ability of ATM to phosphorylate target substrates (Bakkenist and Kastan, 2003; Lee and Paull, 2004). An important interaction for ATM activation involves the C-terminus of Nbs1 (Fig. 1), and removal of the ATM/Tel1 interacting site in Nbs1/Xrs2 disrupts signalling and the phosphorylation of ATM targets in human cells (Nakada et al., 2003; Uziel et al., 2003; You et al., 2005). In addition to interactions with the Mre11 complex, ATM also requires the presence of linear DNA to initiate checkpoint signalling. Work in *Xenopus laevis* egg extracts had shown that in an Mre11-dependent manner the addition of fragmented DNA strongly activates ATM (Costanzo et al., 2000). As well, *in vitro* studies using purified components showed that both linear DNA and the Mre11 complex were requirements for the activation of recombinant ATM (Lee and Paull, 2005).

Activation of ATM results in the phosphorylation of numerous target proteins including histone H2AX and both Mre11 and Xrs2/Nbs1 on ATM consensus sites (Figs. 1 and 2C [step 3]). Nbs1 binds γH2AX after phosphorylation, resulting in a second wave of Mre11 complex recruitment to sites of damage and thus further amplification of the checkpoint signal (Fig. 2C [step 3]). The protein Sae2/CtIP is also phosphorylated by Tel1/ATM and interacts with the Mre11 complex (Cartagena-Lirola et al., 2006). Sae2 activates the nuclease activity of Mre11 likely through a transient interaction between phosphorylated Sae2 and Rad50. In agreement with this, Sae2 has endonuclease activity *in vitro* that stimulates the ability of Mre11 to cleave hairpin DNA (Lengsfeld et al., 2007). The properties of the *sae2*Δ mutant are identical to those of *mre11* nuclease dead mutants and the *rad50S* ($80^{K\rightarrow I}$) mutant. These phenotypes include a slight sensitivity to DNA damage, no meiotic DSB processing, and a failure to process hairpin DNA or covalent topoisomerase I–DNA complexes after treatment with camptothecin. However, these mutants are not deficient in checkpoint activation, and recently Buis et al. (2008) showed that ATM autophosphorylation and phosphorylation of the ATM substrates Chk2 and SMC1 were not reduced in $Mre11^{H129/\Delta}$ nuclease deficient cells. Taken together, these data indicate that the nuclease activity of the Sae2/Mre11 complex is downstream of DNA break binding and initial ATM/Tel1 activation.

Recent work has indicated a role for Mre11 nuclease activity in continued ATM activation. Using *Xenopus laevis* Jazayeri et al. (2008) showed that DSB detection by the Mre11 complex is followed by DNA resection and the production of oligonucleotides. The association of these ssDNA oligos with Mre11 is necessary for continued ATM activation, however their formation does not interfere with the DNA tethering activity of the Mre11 complex. These data suggest that while the nuclease activity of Mre11 may not be required for the initiation of ATM activation, it does contribute to the maintenance of ATM checkpoint signaling.

In yeast it was recently shown that after the initial processing events at the break, Exo1 and/or Sgs1 generate long stretches of ssDNA that are necessary for HR (Mimitou and Symington, 2008). This processing appears to be independent of the Mre11 complex because mutations that abrogate Mre11 nuclease activity have been

shown to have little affect on recombination (Bressan et al., 1999; Moreau et al., 1999). A recent report indicates the situation is different in mammals where the nuclease activity is necessary in the early stages of HR, and where RPA and RAD51 foci formation is greatly reduced in Mre11 nuclease deficient cells (Buis et al., 2008). After resection, Replication protein A (RPA) then binds to the DNA and the resulting RPA-ssDNA filaments recruit the ATR–ATRIP/Mec1–Ddc2 and the 9-1-1-TopBP1 complexes (Fig. 2C [step 4]). The mechanism by which TopBP1 activates ATR is not well understood, but its activation at sites of DNA damage or at replication forks leads to Chk1 phosphorylation and signalling to the rest of the cell that DNA damage has occurred (reviewed in Cimprich and Cortez, 2008).

In addition to its role in DNA processing, the Mre11 complex also serves as a multipurpose DNA tether (reviewed in Williams et al., 2007). Indeed, the structural integrity of the complex is very important for HR, and mutations in the Rad50 CXXC linker region which disrupt the scaffolding abilities of the complex result in an increased sensitivity to genotoxic stress (Hopfner et al., 2002).

In summary, during the process of DNA DSB repair the Mre11 complex is multifunctional. It is recruited rapidly to sites of damage where it unwinds the ends of DNA, allowing ATM recruitment. Physical interactions between ATM and the complex are necessary for the checkpoint response. Finally, downstream of this during HR repair the Mre11 complex serves as a long range tether, linking homologous chromosomes and preventing their separation during repair (Fig. 2C [step 5]).

3.2. Replication and the Mre11 complex

A large body of evidence from many organisms indicates that the Mre11 complex has important functions during the process of DNA replication, however compared to DNA DSBs its role in replication is less well characterized. In mammalian cells, the Mre11 complex colocalizes with proliferating cell nuclear antigen (PCNA) and sites of BrdU incorporation during S phase (Mirzoeva and Petrini, 2003). As well, a physical interaction between the complex and RPA has also been reported to increase during hydroxyurea (HU) treatment, presumably to aid in the recruitment of the Mre11 complex to replication centers during stress (Olson et al., 2007; Robison et al., 2004). The integrity of Mre11 nuclease activity in human cells appears to be extremely important for maintaining genomic stability during replication stress, and the levels of DNA breaks greatly increased in $Mre11^{H129N/\Delta}$ nuclease deficient cells upon treatment with aphidicolin, even though this mutant retains Mre11 complex formation and the amount of Rad50 and NBS1 remain at wild type levels (Buis et al., 2008).

Studies in *Xenopus laevis* egg extracts have been instrumental in characterizing the Mre11 complex during DNA replication. Using the method of fluorescence resonance energy transfer (FRET) it was shown that during replication stress, the Mre11 complex localizes to stalled forks in an ATM- and ATR-dependent manner early during the period of fork recovery (Trenz et al., 2006). In addition, through depletion experiments the Mre11 complex was shown to be essential for preventing the formation of DSBs in newly replicated DNA in the absence of damage (Trenz et al., 2006). It has been proposed that one essential role of the Mre11 complex in higher eukaryotes is to rapidly repair fork-associated lesions (Fig. 2B [2]), preventing the accumulation of damage (Trenz et al., 2006).

Finally, in budding yeast, disruption of any member of the Mre11 complex results in sensitivity to replication stress (D'Amours and Jackson, 2001; Shor et al., 2002). However, in contrast to what has been observed in human cells and by FRET in *X. laevis*, microscopy on living yeast cells have shown no Mre11 foci formation after HU treatment (Lisby et al., 2004). This could represent a difference between higher eukaryotes and yeast, however an alternative explanation is that the levels of Mre11 present at HU-paused forks in yeast are below the levels of detection by microscopy. Indeed, by chromatin immunoprecipitation (ChIP) we have found that the complex is recruited to HU paused forks in wild type cells, prior to replisome breakdown or fork collapse and that replication patterns are altered in cell lacking Mre11 during HU treatment (Tittel-Elmer et al., submitted for publication). We have proposed that the Mre11 complex promotes sister chromatid cohesion (SCC) through tethering newly synthesized daughter during replication stress, promoting recovery and deterring fork collapse (Fig. 2B [1]).

During replication stress, the Mre11 complex is also involved in checkpoint activation. The efficiency of Rad53 checkpoint activation after HU treatment is dependent on the nuclease activity of Mre11 (D'Amours and Jackson, 2001). As well, both Xrs2 and Mre11 are phosphorylated in a Tel1-dependent manner and genetic analyses indicate that the Mre11 complex and Tel1 are in the same epistasis group, working parallel to Mec1 for S phase checkpoint activation (D'Amours and Jackson, 2001).

To summarize, the Mre11 complex has a role during replication. First, it prevents fork associated damage during both 'normal' replication and under conditions of stress. Secondly, the nuclease activity of Mre11 is important for checkpoint activation on a pathway with Tel1/ATM in response to replication stress. Lastly, the Mre11 complex likely has a scaffold function to maintain the fork in a competent conformation to resume progression during pauses in replication.

4. Associated pathologies and therapeutic implications

Two human syndromes exhibit cellular defects similar to ataxia-telangiectasia (A-T), a disorder resulting from mutations in ATM. They are Nijmegen breakage syndrome (NBS) and A-T like disorder (ATLD), which occur from mutations in NBS1 and MRE11 respectively. All three disorders share clinical and cellular phenotypes that include checkpoint deficiencies, chromosome instability, radio-resistant DNA synthesis and a hypersensitivity to ionizing radiation (IR). These syndromes clearly establish the importance of the Mre11 complex in preventing cancer development and underscore the functional significance of the Mre11–ATM interaction.

Characterization of the Mre11 complex during DNA repair and replication has and will continue to provide valuable insight into both Mre11-interacting proteins, and the molecular functions essential for Mre11-dependent DNA metabolism and checkpoint activation. Understanding where and how Mre11 functions in cells will be crucial for identifying the cause of tumourigenesis, not only in the above mentioned syndromes, but also for understanding cancer predisposition in general. Recently, knowledge of the interaction between the Mre11 complex and ATM proved to be important in understanding results from a chemical screen for inhibitors of the pathway. In a small molecule screen, Dupre et al. (2008) identified mirin as an inhibitor of DSB-induced ATM activation. It was shown that mirin prevents MRN-dependent activation without affecting ATM protein kinase activity. This recent example shows how knowledge about the Mre11 complex–ATM interaction(s) facilitated the characterization of this inhibitor. Indeed, in-depth understanding of the checkpoint response in combination with drug screening will almost certainly be valuable for identifying new chemotherapeutic drugs.

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