The multiple roles of the Mre11 complex for meiotic recombination

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Abstract

During the first meiotic prophase, numerous DNA double-strand breaks (DSB) are formed in the genome in order to initiate recombination between homologous chromosomes. The conserved Mre11 complex, formed of Mre11, Rad50 and Nbs1 (Xrs2 in *Saccharomyces cerevisiae*) proteins, plays a crucial role in mitotic cells for sensing and repairing DSB. In meiosis the Mre11 complex is also required for meiotic recombination. Depending on the organisms, the Mre11 complex is required for the formation of the DSB catalysed by the transesterase Spo11 protein. It then plays a unique function in removing covalently attached Spo11 from the 5′ extremity of the breaks through its nuclease activity, to allow further break resection. Finally, the Mre11 complex also plays a role during meiosis in bridging DNA molecules together and in sensing Spo11 DSB and activating the DNA damage checkpoint. In this article the different biochemical functions of the Mre11 complex required during meiosis are reviewed, as well as the consequences of Mre11 complex inactivation for meiosis in several organisms. Finally, I describe the meiotic phenotypes of several animal models that have been developed to model hypomorphic mutations of the Mre11 complex, involved in humans in some genetic instability disorders.

Introduction

In most sexually reproducing organisms meiotic recombination between homologous chromosomes is needed to ensure a physical connection that allows them to orient properly on the spindle and segregate accurately at the first division. Meiotic recombination is initiated by the formation of programmed DNA double-strand breaks (DSB) catalysed by Spo11, which are then repaired by homologous recombination (reviewed in Keeney 2001). The repair of meiotic DSB utilizes many of the mitotic homologous recombination proteins, but also has some unique specificities, among which a bias towards repair with the homologous chromosome rather than with the sister chromatid, and the need to remove the

Spo11 protein from DSB ends. A central player in the signalling and repair of DSB in mitotic cells is the Mre11 complex. Hypomorphic mutations in two of its components, Nbs1 and Mre11, are responsible for Nijmegen breakage syndrome (NBS) and ataxia-like disorder (ATLD) respectively, two human genetic instability disorders. Not surprisingly, the Mre11 complex is also involved in the meiotic recombination pathway. This review analyses the different studies of the Mre11 complex during meiosis. First, I emphasize the biochemical properties of each member of the Mre11 complex, and detail the studies made in *Saccharomyces cerevisiae* using separation of function mutants, which allowed better understanding of which functions of the Mre11 complex are solicited during meiosis. Then I describe

the evolutionary conservation of the meiotic roles of the Mre11 complex and the meiotic phenotypes of the recent animal models that have been developed for Mre11 complex deficiency.

## Structure and biochemical properties of the Mre11 complex

The conserved Mre11 complex influences each aspect of chromosome break metabolism. This complex contains three proteins, Mre11, Rad50 and Nbs1 (Xrs2 in *S. cerevisiae*). It is involved in telomere maintenance, cell cycle responses to DNA damage and DSB repair by both homologous recombination and non-homologous end joining (reviewed in D'Amours & Jackson 2002). Many recent studies concerning the biochemical properties and structure of the complex have shed light on how it may fulfil its various roles in DSB repair.

The Rad50 protein is a split ABC-type ATPase which contains two heptad repeats in its centre, that fold into a coiled-coil, bringing the two N- and C-terminal ATPase motifs Walker A and B in close proximity (de Jager *et al*. 2001) (Figure 1). These motifs also interact with two Mre11 monomers, forming a globular domain that interacts with DNA (de Jager *et al*. 2001). The apex of the Rad50 coiled-coil contains a zinc hook that may facilitate interactions between Rad50 molecules (Hopfner *et al*. 2002). ATP binding promotes the dimerization between two catalytical Rad50 domains, which is crucial for efficient DNA binding (Hopfner *et al*. 2000) and thus for all functions of the Mre11 complex (Alani *et al*. 1990). The association between two Rad50 protomers can either form a large loop, or can also occur between two complexes bound on two different DNA molecules. Both modes of association have been seen by atomic force and electron microscopy. Upon DNA binding via the globular domain, the intracomplex interaction is prevented and the intermolecular configuration is favoured, which allows DNA tethering (Moreno-Herrero *et al*. 2005). In addition to ATP binding and hydrolysis, the conserved 'signature motif' in the C terminal ATPase domain of Rad50 bears an adenylate kinase activity, which is required for efficient tethering between different DNA molecules, independently of DNA binding (Bhaskara *et al*. 2007). All these observations have led to the proposal that this molecular

bridging by the Mre11 complex via the Rad50 coiled-coils constitutes the molecular basis of the Mre11 complex's influence on recombinational DNA repair.

The Mre11 protein is a DNA-binding protein that contains four conserved N-terminal phosphoesterase motifs (Figure 1). Human and budding yeast Mre11 have single-strand endonuclease activity, 3′-5′ double-strand exonuclease activity, as well as weak DNA unwinding (stem-loop opening) activity (Paull & Gellert 1998, Usui *et al*. 1998, Trujillo *et al*. 2003). The observed 3′-5′ polarity of exonuclease activity was at first surprising because genetic and physical analyses of meiotic recombination and mitotic DSB repair had suggested that the Mre11 complex is involved in 5′-3′ exonucleolytic resection. However, hypomorphic mutations that abolish the nuclease activity of Mre11 do not impair most forms of DSB repair in mitotic cells, indicating that the complex's contribution to that process is largely independent of its nuclease activity (Tsubouchi & Ogawa 1998, Usui *et al*. 1998, Bressan *et al*. 1999, Moreau *et al*. 1999, Lewis *et al*. 2004, Llorente & Symington 2004, Krogh *et al*. 2005). Mre11 also contains two DNA-binding domains, one conserved in the centre of the protein and one less conserved in the C-terminus (Usui *et al*. 1998).

Whereas Mre11 and Rad50 are strongly conserved in bacteria (SbcCD), archaebacteria and eukaryotes, the third member of the complex is less well conserved among species. Nevertheless, NBS1 has a functional homologue, Xrs2, in *S. cerevisiae*. The two proteins share some common motifs: a N-terminal FHA domain, involved in protein–protein interactions and which binds to phosphorylated histone H2AX (Kobayashi *et al*. 2002), phosphorylation sites for the checkpoint protein Atm (Tel1 in yeast) and a conserved C-terminal domain (CCD) involved in interaction with Mre11 (Figure 1; reviewed in Kobayashi *et al*. 2004). Both Nbs1 and Xrs2 proteins enhance Mre11 nuclease activity *in vitro* (Carney *et al*. 1998, Paull & Gellert 1999, Desai-Mehta *et al*. 2001, Tauchi *et al*. 2001). Mutant studies in *S. cerevisiae* have shown that interaction of Xrs2 with Mre11 is crucial to all the functions of the Mre11 complex since a single amino-acid mutation disrupting this interaction results in a Mre11 complex-null phenotype (Tsukamoto *et al*. 2005). Similarly, in NBS patients with the 657del5 allele, the mutated protein is in reduced amounts and hMre11p is mislocalized to
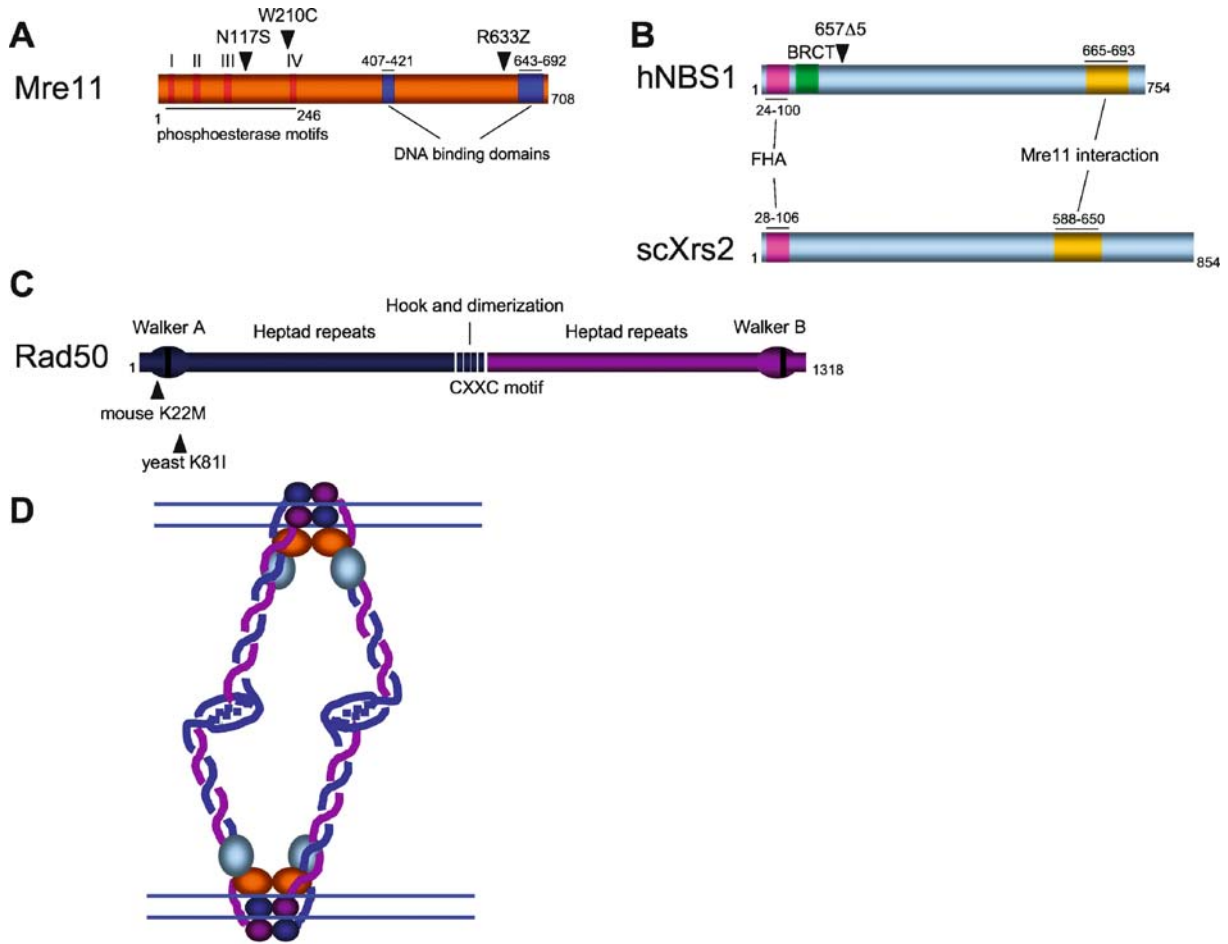
Figure 1. Structure of the Mre11 complex. **A**: Structure of the human Mre11 protein. The two DNA binding domains and the nuclease domain with the four conserved phosphoesterase motifs are indicated. The position of the three mutations involved in human ATLD is also shown. **B**: Structures of the human NBS1 and the budding yeast Xrs2 proteins. The different FHA, BRCT and Mre11 interaction domains are indicated, as well as the mutation found in most NBS patients (657del5), giving rise to a N-terminal truncated protein lacking the FHA and BRCT domains. **C**: Primary structure of the Rad50 protein, comprising two Walker A and B motifs carrying the ATPase activity, two heptad repeats and a central portion forming a zinc hook that facilitates interaction between Rad50 molecules (Wiltzius *et al.* 2005). **D**: Proposed structure of the Mre11 complex. The position of Nbs1/Xrs2 is speculative. Two Rad50/Mre11 dimers (each composed of $Rad50_2Mre11_2$) interact with each other via their hooks, here tethering two DNA molecules, as deduced from electron microscopy studies (Hopfner *et al.* 2002).

the cytoplasm (Carney *et al.* 1998, Tauchi *et al.* 2001). One of the roles of both Nbs1 and Xrs2 might thus be to maintain an intranuclear localization of Mre11. Another role is to activate Atm autophosphorylation specifically in response to DNA damage induced by ionizing radiation (Difilippantonio *et al.* 2005) and thus the Mre11 complex works not only downstream but also upstream of Atm. This is consistent with findings that purified Mre11 complex can activate Atm *in vitro* (Lee & Paull 2004), as well as studies in yeast and human cells that show

that the Mre11 complex recruits Atm/Tel1 to sites of DNA damage (Nakada *et al.* 2003, Uziel *et al.* 2003). The yeast Xrs2 and mammalian NBS1 proteins interact with Tel1 and Atm, respectively, through their C terminus (Nakada *et al.* 2003, You *et al.* 2005). This interaction is specifically required for telomere elongation (Shima *et al.* 2005, Tsukamoto *et al.* 2005). The role of the FHA domain is less clear. Its interaction in mammals with phosphorylated histone H2AX (Kobayashi *et al.* 2002), which itself is phosphorylated through Atm, suggests that it might

serve to recruit Nbs1 to sites of damage to amplify the checkpoint response to DSB. In yeast the FHA domain is dispensable for all the mitotic functions of the Mre11 complex (Shima *et al.* 2005, Tsukamoto *et al.* 2005).

Roles of the Mre11 complex in budding yeast meiosis

In meiosis the role of the Mre11 complex has been especially well documented in *S. cerevisiae* and the use of separation of function mutants of each member of the complex allowed to identify the different functions of the Mre11 complex required for meiotic recombination. The *mre11* mutation was first described as meiotic recombination deficient (Ajimura *et al.* 1993), and it was shown that deletion of either member of the Mre11 complex leads to an absence of meiotic recombination and extremely low spore viability (Alani *et al.* 1990, Ivanov *et al.* 1992). This absence is due to a lack of meiotic DSB, which are normally catalysed by the Spo11 protein (Alani *et al.* 1990, Johzuka & Ogawa 1995, Shima *et al.* 2005). Some separation of function mutants have shown that the Mre11 complex is also needed once meiotic DSB are formed, for their resection and repair.

The budding yeast complex is required for meiotic DSB formation independently of its catalytic nuclease activity

The three members of the Mre11 complex are among the nine proteins required, in addition to the catalytic component Spo11, for the formation of meiotic DSB (reviewed in Keeney 2001). One specific region of Mre11, in the C-terminus, is strictly required for this: indeed, its deletion leads to an absence of DSB formation and the production of dead spores. In two cases, the *mre11-5* mutant, truncated for the last 137aa, and the *mre11ΔC49* mutant, truncated for the last 49aa, the truncated protein was fully functional for its mitotic functions (Furuse *et al.* 1998, Usui *et al.* 1998). In another case a truncated form of Mre11 lacking the last 128aa (*mre11-T4*) was deficient for DSB formation but also partially deficient for MMS tolerance (Nairz & Klein 1997). However, whenever tested it appears that these truncated Mre11 proteins are able to resect and repair meiotic

DSB made by Spo11, since they show intragenic complementation with *mre11* alleles proficient in DSB formation but deficient in meiotic DSB resection (Nairz & Klein 1997, Usui *et al.* 1998). Chromatin immunoprecipitation studies have shown that, in wild-type cells, Mre11 transiently associates with DSB sites, at the time of meiotic DSB formation (Borde *et al.* 2004). This association does not require DSB formation and still timely occurs in a strain carrying the catalytic mutant *spo11Y135F* allele. Surprisingly, Rad50 is not required for Mre11 association with DSB sites. Thus, the functional complex is not needed. Similarly, loading of Mre11 onto telomeres during late S phase also occurs normally in *rad50* cells (Takata *et al.* 2005). In agreement with the notion that an intact Mre11 complex is not required for meiotic DSB formation is that meiotic DSB are normally formed in a *mre11-58S* mutant, which does not form a complex with Rad50 and is deficient for all the other known functions of the Mre11 complex (Usui *et al.* 1998). Thus, the function of Mre11 for DSB formation is probably exerted through its C-terminal part. When assessed by chromatin immunoprecipitation, this C-terminal part is required for Mre11 association with DSB sites. The fact that all DSB proteins are required for Mre11 association with DSB sites suggests that this interaction does involve direct DNA binding but rather binding to some of these proteins (Borde *et al.* 2004). Consistently, the purified C-terminal part of Mre11 interacts in meiotic extracts with three meiosis-specific proteins of a molecular weight compatible with the size of Rec102, Rec104 and Mer2, three of the proteins required for DSB formation (Usui *et al.* 1998). These results agree well with some two-hybrid results showing that the Mre11 protein interacts mitotically with Mer2 and Rec104, which itself interacts with Rec102 (Arora *et al.* 2004).

Xrs2 interacts by mitotic two hybrid with Mer2 (Arora *et al.* 2004), which indicates that it may also play a non-catalytical, maybe structural, role in the 'pre-DSB' complex. In addition, the meiotic DSB formation phenotype of *xrs2Δ* may partially be explained by the fact that Xrs2 is required for efficient nuclear localization of Mre11 (Shima *et al.* 2005, Tsukamoto *et al.* 2005). Fusing Mre11 to a nuclear localization signal bypasses the requirement for Xrs2 for some functions of the Mre11 complex. This suggests that, in *xrs2Δ* cells, there is not enough Mre11 protein in the meiotic nucleus for DSB

formation. Indeed, Mre11 association with DSB sites requires Xrs2 (Borde *et al.* 2004). However, nuclear localization of Mre11 does not bypass the requirement for Xrs2 in DSB formation, and mutagenesis analyses have shown that a minimal region of 80 amino acids of Xrs2, in addition to binding to Mre11, is required for DSB formation (Tsubouchi & Ogawa 1998). In addition, a limited N-terminal truncation of Xrs2 (the first 84 amino acids) impaired meiotic DSB formation, whereas larger truncations did not (Shima *et al.* 2005, Tsukamoto *et al.* 2005). One explanation is that these N terminal truncations might induce conformational changes that impair the meiotic functions of the Mre11 complex whereas more extensive truncations of Xrs2 remove such impairment (Tsukamoto *et al.* 2005).

For Rad50 the function required for meiotic DSB formation remains elusive. It is not required for Mre11 to interact with DSB sites (Borde *et al.* 2004); however, in *rad50Δ* cells Mre11 association with DSB sites is unproductive for DSB formation. In the *mre11-58S* mutant cells meiotic DSB are formed (Tsubouchi & Ogawa 1998, Usui *et al.* 1998). *In-vitro* studies have shown that the interaction between this mutated Mre11 and Rad50 is abolished, which led to the suggestion that a complex formation between Mre11 and Rad50 is not required for DSB formation (Usui *et al.* 1998) Similarly, interaction between Mre11 and Rad50 is severely reduced in the nuclease-deficient D16A mutant, which is proficient for meiotic DSB formation (Furuse *et al.* 1998, Krogh *et al.* 2005). However, Rad50 is strictly required for DSB formation. Point mutations in the consensus motif involved in ATP binding, which is crucial for efficient DNA binding (Hopfner *et al.* 2000), completely abolish meiotic DSB formation (Alani *et al.* 1990). By a two-hybrid test, Rad50 does not interact directly with any of the 'DSB proteins' except Mre11 (Arora *et al.* 2004). One clue about the role of Rad50 in DSB formation came from a nice study that examined the role of the dimerization hook domain of Rad50 (Wiltzius *et al.* 2005). Crystallographic studies have shown that this domain allows dimerization between two Mre11 complexes to tether DNA molecules (Hopfner *et al.* 2002). Hook mutants of Rad50 were deficient in meiotic DSB formation, and restoration of dimerization using a conditional dimerization module restored DSB formation (Wiltzius *et al.* 2005). Since meiotic DSB are formed in the chromatin loops rather than on the axial

elements (Zickler & Kleckner 1999, Blat *et al.* 2002), a role for Rad50 might be to bridge sister chromatids in order to establish a proper architecture of the sisters engaged in the pre-DSB complex (Wiltzius *et al.* 2005). Finally, a recent study reported that a mutation affecting the adenylate kinase activity of Rad50, necessary for DNA tethering, also abolishes the formation of viable spores, but this study did not examine if meiotic DSB are formed or not (Bhaskara *et al.* 2007).

Roles of the Mre11 complex for resection and repair of Spo11 DSB

Meiotic DSB differ from most DSB occurring either accidentally during replication or after irradiation or drug treatment, in that they have a covalently-attached Spo11 protein at each of their 5′ ends, that needs to be removed for further processing by 5′ to 3′ resection, and repair by homologous recombination. Thus, in addition to the proteins required for repairing DSB by homologous recombination, they require an additional specific enzymatic activity. The mode of processing of these DSB was recently identified by Scott Keeney's laboratory, where it was shown that removal of covalently bound Spo11 occurs by endonucleolytic cleavage a few bases away from the break site, which frees an oligonucleotide with attached Spo11 (Neale *et al.* 2005). The Mre11 complex is very probably responsible for this activity, both based on its nuclease biochemical activity, and on the long-known phenotype of separation of function mutants as described below. A specific class of separation of function mutants of *RAD50*, named *rad50S*, allow DSB formation but are totally defective for processing of Spo11-induced meiotic DSB, and thus accumulate during meiosis unprocessed DSB with covalently attached Spo11 protein at their 5′ ends (Alani *et al.* 1990, Keeney *et al.* 1997). However, they show almost wild-type resistance to clastogens, such as MMS. The deletion of the *SAE2/COM1* gene, of unknown biochemical function, produces exactly the same phenotypes (McKee & Kleckner 1997, Prinz *et al.* 1997). Accordingly, the Spo11–oligonucleotide complexes are not observed in *rad50S* nor in *sae2Δ* mutants (Neale *et al.* 2005). The mutant Rad50S protein is mutated in the conserved Walker A ATPase motif (Figure 1). How this mutation affects the *in-vitro* functions of the Mre11 complex has not been

determined. On the crystal structure of Rad50, seven of the nine *rad50S* mutations reported (Alani *et al.* 1990) cluster to a narrow surface patch that forms a probable protein interaction site (Hopfner *et al.* 2000). Thus maybe *rad50S* mutations affect the interaction between Rad50 and Sae2 (Rattray *et al.* 2001, Usui *et al.* 2001). Separation of function mutants of Mre11 has also been identified, which allow meiotic DSB formation but do not process DSB (Nairz & Klein 1997, Tsubouchi & Ogawa 1998, Furuse *et al.* 1998, Moreau *et al.* 1999). Mutations lie in the N terminal part of the protein, containing the conserved phosphoesterase motifs. When examined, the mutants showing this phenotype lacked nuclease activity *in vitro* (mutants D16A, D56N, H125N, H213Y (*mre11-58*) and (*mre11-6*)), demonstrating that the nuclease activity of Mre11 is probably the activity required for Spo11 removal and end resection (Furuse *et al.* 1998, Usui *et al.* 1998, Moreau *et al.* 1999). The nuclease-deficient mutants of Mre11 then fall into two categories: those which are affected for the other roles of Mre11 (resistance to damaging agents) and those that are not (no defect in mating type switching or telomere maintenance). Although their meiotic phenotype is identical, nuclease mutants that fail to form a complex with Rad50 and Xrs2 are more affected in mitotic functions (Furuse *et al.* 1998, Usui *et al.* 1998, Lewis *et al.* 2004, Krogh *et al.* 2005). In all cases the activity affected in *rad50S*, *sae2Δ* and the nuclease-deficient *mre11* cells seems to be required for repairing specific kinds of substrates (Rattray *et al.* 2001), processing ('cleaning') certain types of DNA ends, such as hairpins formed in inverted repeats (Lobachev *et al.* 2002), flap structures unprocessed in the absence of Rad27 (Debrauwere *et al.* 2001, Moreau *et al.* 2001), or covalent topoisomerase I-DNA complexes after cell treatment with camptothecin (Deng *et al.* 2005). The meiotic phenotypes of *sae2Δ* and rad50S would suggest that these cells should be hypersensitive to trapped topoisomerase II breaks, which accumulate 5′ covalently linked topoisomerase II at their 5′ ends, similar to Spo11. However, topoisomerase II-linked oligonucleotides, that are observed in vegetative cells and probably reflect spontaneous topoisomerase II-induced DSB, are also observed in *sae2Δ* or *rad50S* mutants (Neale *et al.* 2005). This suggests that the Mre11 complex and Sae2 are not strictly required for the endonucleolytic processing of these topoisomerase II breaks.

Finally, the Mre11 complex may have a role during resection after the initial endonucleolytic removal of Spo11-linked oligonucleotide. However, studies of *mre11* mutant alleles on HO-endonuclease induced DSB in vegetative cells indicate that this function is redundant with at least one other protein (Llorente & Symington 2004). Exo1 is the strongest candidate for being involved in the lengthening of the resection tract from a few bases to hundreds of bases, both in vegetative and in meiotic cells (Moreau *et al.* 2001, Tsubouchi & Ogawa 2000).

In addition to meiotic DSB formation and the nuclease activity, another aspect of the Mre11 complex function that is important in meiosis is sensing of DSB and checkpoint activation. This is difficult to separate from the biochemical activity of Mre11, although some mutants have been identified that are deficient in nuclease activity but proficient in checkpoint activation.

Rad50S, *sae2Δ* and certain nuclease mutants of *MRE11* show delayed meiosis I division, suggesting that a checkpoint is activated (Alani *et al.* 1990, McKee & Kleckner 1997, Nairz & Klein 1997, Prinz *et al.* 1997). Indeed, the unprocessed DSB that accumulate in *rad50S* mutants cause a cell cycle delay that is controlled by the Rad53 paralogue, Mre4/Mek1 (Xu *et al.* 1997). In mammalian cells the Mre11 complex is a sensor of DSB, that activates the Atm protein and is then phosphorylated in response to DNA damage (Stracker *et al.* 2004). In yeast the Mre11 complex is also involved in checkpoint activation, via the Atm homologue, Tel1 (D'Amours & Jackson 2001, Usui *et al.* 2001). In *sae2Δ* (or *rad50S*) *mec1* double mutants, mitotic cells become resistant to MMS. This is because, in these cells, a checkpoint is permanently activated, probably because some ends are unprocessed. This checkpoint requires both Mre11 and Tel1, and its activation is accompanied by Mre11 and Xrs2 phosphorylation (Usui *et al.* 2001). In meiotic cells the same checkpoint is activated upon unprocessed DSB accumulation in *sae2Δ* or *rad50S* cells, requiring a 'structurally functional' Mre11 complex and Tel1 (Usui *et al.* 2001). In meiotic cells accumulating unprocessed DSB, Mre11 accumulates at DSB, as seen by chromatin immunoprecipitation or cytologically as foci (Usui *et al.* 1998, Borde *et al.* 2004), which also requires a complex formation but is independent of checkpoint activation (Usui *et al.* 2001). The Mre11 complex is required for Tel1

activation and autophosphorylation (Nakada *et al.* 2003). When the Tel1 checkpoint is activated, the Sae2 protein becomes phosphorylated, and then dephosphorylated as recombination proceeds (Cartagena-Lirola *et al.* 2006) (Figure 2). Both Tel1 and Mec1 play redundant roles for Sae2 phosphorylation. Mutation of the preferred canonical motifs for phosphorylation by ATM/ATR kinases completely abolished Sae2 phosphorylation during meiosis, and resulted in the accumulation of unprocessed DSB (Cartagena-Lirola *et al.* 2006). Moreover, deletion of both *TEL1* and *MEC1* also induced the accumulation of unprocessed DSB. Thus, Tel1/Mec1-mediated Sae2 phosphorylation is probably necessary to activate the nuclease activity of Mre11, probably through a transient interaction between

phosphorylated Sae2 and Rad50 (Figure 2). However, this has not been formally proven, since it was not investigated whether the unresected ends formed in tel1 mec1 double mutants still have Spo11 attached. Sae2 phosphorylation may also be necessary to switch the checkpoint off by limiting Mre11-dependent recombination checkpoint signalling.

Strikingly, although it is absolutely required for meiotic DSB processing in *S. cerevisiae*, no homologue of Sae2 has been found in other species, based on sequence similarity. However, there is a functionally equivalent protein in *Arabidopsis thaliana*, which shows no sequence similarity but whose mutation shows identical meiotic phenotypes as that in *S. cerevisiae*, including pronounced Spo11 foci, no Rad51 foci and DNA fragmentation from anaphase I
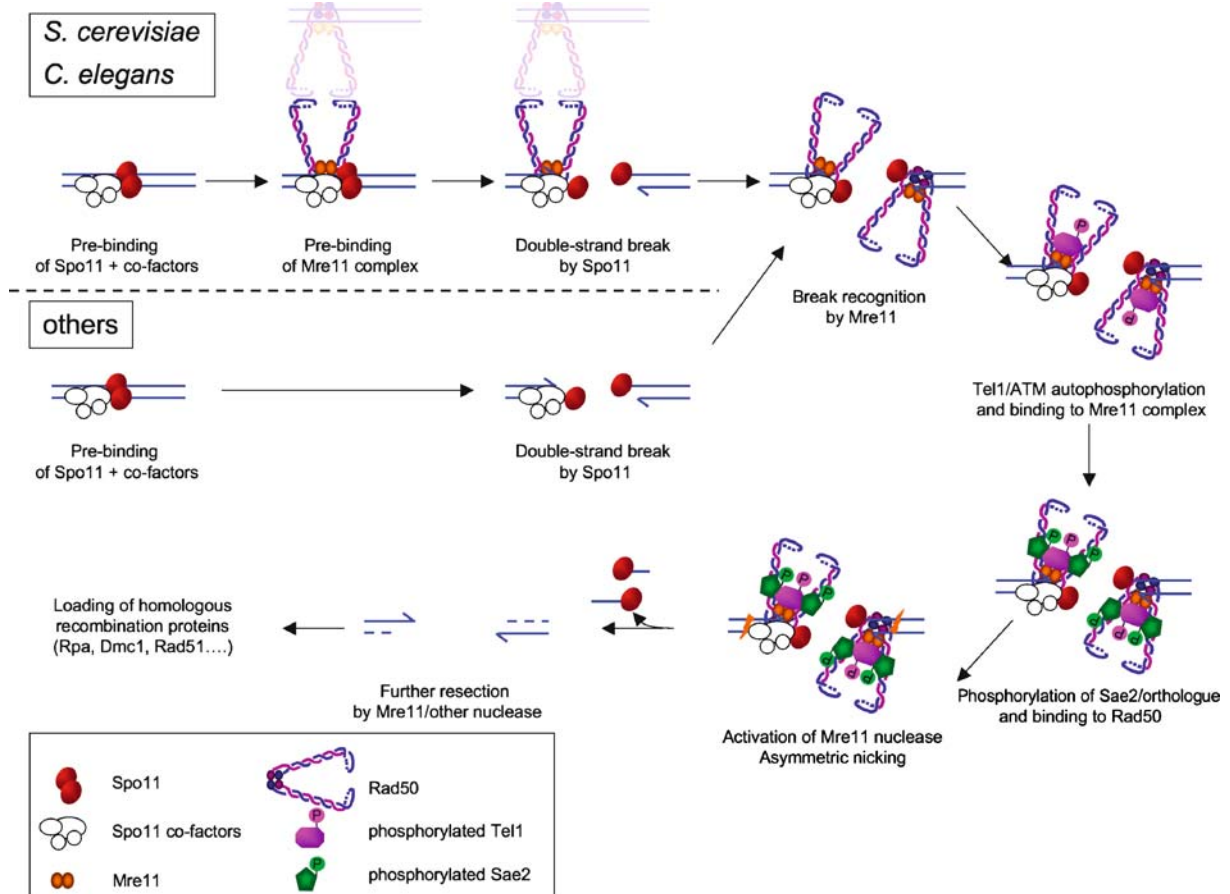

Figure 2. The different steps at which the Mre11 complex is involved during meiotic recombination. For simplicity the Nbs/Xrs2 protein is not drawn. The nature of all the Spo11 'co-factors' is not known for many organisms, but some have been described in *S. cerevisiae*, *S. pombe*, *A. thaliana* and in the mouse. Except in *S. cerevisiae* and *C. elegans*, Mre11 seems to be involved only after DSB formation by Spo11 (see text for details).

onwards (Peter Schloegelhofer, personal communication). This indicates that the function of Sae2 for activating Mre11 nuclease activity during meiotic recombination may be conserved.

Conservation of the roles of the Mre11 complex for meiotic recombination in other organisms

The phenotype of mutations of the Mre11 complex in vertebrates has long been precluded, since each member of the complex is essential, their deletion leading to embryonic lethality (Stracker *et al.* 2004). However, the recent discovery of viable hypomorphic mutants, involved in some rare autosomal diseases, has allowed study of the roles of Mre11 complex in different aspects of chromosomes metabolism using mouse models (Stracker *et al.* 2004). In other eukaryotes the phenotypes of mutations of the Mre11 complex have been described, since the Mre11 complex is not essential. They point to a partial conservation of the meiotic roles defined in *S. cerevisiae*. In particular, all organisms tested show defects in meiotic recombination in Mre11 complex mutants, e.g. in *C. elegans* (Chin & Villeneuve 2001), in *Coprinus cinereus* (Ramesh & Zolan 1995, Gerecke & Zolan 2000, Merino *et al.* 2000), in *Arabidopsis thaliana* (Gallego *et al.* 2001, Bundock & Hooykaas 2002, Puizina *et al.* 2004), in *Aspergillus nidulans* (Semighini *et al.* 2003, Malavazi *et al.* 2005) or in *Schizzosaccharomyces pombe* (Young *et al.* 2004).

In all organisms studied so far, apart from *C. elegans* and *Drosophila*, formation and correct processing of homologous recombination intermediates are necessary to promote proper homologous chromosomes synapsis. Thus, cytological observations of meiotic cells allow inferring defects in homologous recombination.

In *C. elegans*, two different mre11 alleles produced identical phenotypes. One contains a point mutation in a perfectly conserved amino acid (E139K) in the second phosphoesterase motif and the other one a truncation after amino acid 270 (171 in *S. cerevisiae*), which truncates before the fourth phosphoesterase motif and is predicted to be a null, based on *S. cerevisiae* studies (Usui *et al.* 1998). During meiosis both mutants show intact chromosomes, but with no crossing over or chiasmata, suggesting that meiotic recombination did not occur (Chin & Villeneuve 2001). Irradiation of *mre11* germ cells during meiotic prophase induced chromosome fragmentation, indicating a defect in repair of chromosome damage. The fact that, despite their lack of chiasmata, chromosomes in *mre11* are intact suggests that, in *C. elegans*, Mre11 may, like in *S. cerevisiae*, have a dual role in both generating meiotic DSB and repairing them.

In *Coprinus cinereus*, Mre11 expression is induced during prophase I of meiosis. An mre11 mutant (mre11-1, truncated after amino acid 315) shows many meiotic defects including incomplete pachytene chromatin condensation, a block in meiotic progression, and defects in chromosome synapsis: some synapsis was observed, but it was not complete and sometimes aberrant synapsis was observed (Gerecke & Zolan 2000). This is reminiscent of the phenotype observed in *mre11S*, *rad50S* or *sae2Δ* mutants in yeast, which can form, but cannot process meiotic DSB and show a less severe defect in synapsis than mutants that form no DSB, e.g. *spo11Δ*, *rad50Δ* or *mre11Δ* (Alani *et al.* 1990, Nairz & Klein 1997, Prinz *et al.* 1997). Similarly, the *Coprinus* rad50-4 allele, although strongly defective in synapsis, shows more SC formation than the spo11 mutant, predicted to be completely deficient in DSB formation (Merino *et al.* 2000). Thus, in *Coprinus*, the Mre11 complex seems not to be required for meiotic DSB formation, but is probably required for the initiation of their processing.

In the plant *Arabidopsis thaliana* a truncated mutant of Rad50, lacking the last 266 amino acids, is hypersensitive to MMS and sterile (Gallego *et al.* 2001). Similarly, a Mre11 mutant (the mre11-1 allele, truncated after amino acid 261, in the phosphoesterase motif IV) is viable, but sterile (Bundock & Hooykaas 2002). These mutants show no normal pachytene stage and a defect in homologous chromosome pairing and synapsis, and extensive meiotic chromosome fragmentation (Bleuyard *et al.* 2004, Puizina *et al.* 2004), which is not seen in a spo11 mutant (Grelon *et al.* 2001). In addition, the fact that this fragmentation in mre11 mutant is Spo11-dependent clearly suggests that DSB are formed by Spo11 in the *Arabidopsis* Mre11 complex mutants, but that they are not repaired (Puizina *et al.* 2004). Finally, the mre11-2 mutant, in which the last 191 amino acids of the Mre11 protein are deleted, is fertile (Bundock & Hooykaas 2002). Thus, unlike in *S. cerevisiae*, no

meiotic function is associated with the C-terminal part of Mre11 in plants.

Finally, in the fission yeast *S. pombe*, Rad50 and Rad32 (the homologue of Mre11) appear not to be required for meiotic DSB formation, although break frequency is reduced. However, both are strictly required for DSB processing, as monitored physically and genetically, the very low spore viability being suppressed by mutations blocking DSB formation (Young *et al.* 2004).

In conclusion, in all organisms where the effects of mutations of the Mre11 complex could be examined, the complex is required for meiotic recombination, most probably for processing the Spo11-generated DSB. In addition, in *S. cerevisiae* and maybe *C. elegans*, the Mre11 complex is also required for DSB formation, through a structural rather than catalytical mechanism.

Animal models for mutations in the Mre11 complex

The embryonic lethality of the disruption of any member of the Mre11 complex in mammals has precluded the study of null mutations (Stracker *et al.* 2004). However, the discovery that some hypomorphic mutations of Mre11 and Nbs1 are responsible for human genetic instability disorders has allowed the development of mouse models of Mre11 complex deficiency. Three genetic disorders, A-T (mutated in the checkpoint ATM gene), ATLD (mutated in MRE11 gene) and NBS (mutated in NBS1) show patients sharing common cellular features, such as hypersensitivity to ionizing radiation, radioresistant DNA synthesis (RDS) and abrogation of ATM-dependent events (Stracker *et al.* 2004). Clinical phenotypes of A-T and NBS are quite distinct but the cellular phenotypes are similar; A-T and ATLD share both clinical and cellular phenotypes. A-T patients are sterile. In Atm−/− mice mutant spermatocytes have chromosome synapsis defects and undergo apoptosis during meiotic prophase I, and ovaries in Atm−/− are devoid of oocytes (Xu *et al.* 1996), which are eliminated in response to the presence of persistent DSB (Di Giacomo *et al.* 2005). ATLD is due to either one of three known mutations in the MRE11 gene: one truncation at R633Z, removing the last 75 amino acids, one mutation at N117S, an invariant

amino acid located between phosphoesterase motifs III and IV (Stewart *et al.* 1999) or the W210C mutation, mutating an invariant amino acid just before phosphoesterase motif IV (Fernet *et al.* 2005) (Figure 1). In the first two cases, patients show strongly reduced levels of the three members of the Mre11 complex, whereas in the third only Nbs1 is strongly reduced. Fertility is not reported for these patients. Both R633Z and N117S mutations have been modelled in *S. cerevisiae* (Q623Z and N113S mutations, respectively). The C-terminal truncation mutant showed wild-type resistance to irradiation and produced only inviable spores, as expected for a C-terminal truncation that makes no meiotic DSB; the N113S mutation conferred moderate IR sensitivity, and produced viable spores, but to a reduced frequency, which may reflect a correct DSB formation but a partial defect in Mre11 nuclease activity (Stewart *et al.* 1999). A mouse model reproduced the C-terminal truncated allele (R633Z). Mice recapitulated all cellular features of ATLD patients, including radiation sensitivity, intra-S checkpoint failure and chromosome instability (Theunissen *et al.* 2003). However, no strong meiotic defect was observed in these mice, apart from a slower progression in meiotic prophase and a higher proportion of incompletely synapsed bivalents (Cherry *et al.* 2007). Female mice were subfertile, showing high levels of embryonic lethality, but meiotic recombination was not defective, showing the presence of chiasmata. Thus, the C-terminal region of mammalian Mre11, required in *S. cerevisiae* for meiotic DSB formation, is clearly not required for either formation or repair of meiotic DSB in mice. The fact that ATLD mice were fertile suggests two alternative hypotheses: either the reduced amounts of the mutated Mre11 protein in the cells impair some of its mitotic functions, but its nuclease activity is intact and still sufficient to remove Spo11 from meiotic DSBs; or the catalytic nuclease activity of Mre11 is not required for meiotic recombination in mammals. The first hypothesis is probably true, because Spo11–oligonucleotide complexes were seen in mice (Neale *et al.* 2005), and because the modelled N113S mutation in yeast showed at least a partial DSB repair activity, which requires the nuclease activity (Stewart *et al.* 1999).

NBS patients show impaired development of gonads and ovarian failure for female patients; 95% of cases are due to the 657Δ5 allele, producing a N-terminally truncated protein, the Nbs1 p70 protein,

expressed through an alternative translation site (Figure 1). Two mice models of NBS have been developed, producing reduced amounts of a N-terminal truncated Nbs1 protein equivalent to the human p70 (Kang *et al.* 2002, Williams *et al.* 2002). These models recapitulated most of the cellular phenotypes of NBS patients. In both studies spermatogenesis was normal and no severe meiotic defects were detected, and consistently NBS male mice were fertile. In the first study the females were sterile and showed oogenesis failure, 8-week-old mice ovaries completely lacking any oocytes and follicles (Kang *et al.* 2002). In the second NBS model both males and females were fertile (Williams *et al.* 2002). Maybe the discrepancies between these two outcomes come form the difference in the alleles produced. Finally, the meiotic phenotypes associated with Nbs1 dysfunction were studied in more detail in null Nbs1 knockout mice complemented with various alleles of the human NBS1 gene (Difilippantonio *et al.* 2005). The main allele of NBS (hNbs1$^{657\Delta5}$) rescued the lethality of Nbs1 $-/-$ mice. Males were fertile although showing a delayed appearance of germ cells. Females were sterile, the adult ovaries being devoid of oocytes. However, meiosis was initiated, and probably meiotic DSB were formed and repaired, since chromosomes were fully synapsed, but the oocytes were blocked in pachytene (Difilippantonio *et al.* 2005). Thus, the NBS1 allele is blocked in meiotic progression, but probably not in meiotic recombination. It is possible that the N-terminal truncated NBS1 could provide enough of the NBS1 activity required to allow normal spermatogenesis, since from *S. cerevisiae* studies of Xrs2, the region deleted in NBS patients is not required for meiotic recombination (Shima *et al.* 2005, Tsukamoto *et al.* 2005). It is also possible that the essential role of ATM in meiosis is not dependent on NBS1 since ATM activates a number of other DNA repair pathways upon DNA strand-break damage. In this context, Brca1, which is also activated by ATM through phosphorylation after DNA strand-break damage, is required for homologous recombination and thus might mediate ATM-dependent functions during meiosis (Cortez *et al.* 1999, Moynahan *et al.* 1999, Li *et al.* 2000).

No mutations in the human RAD50 gene, which is also essential for viability, have been described. A hypomorphic 'Rad50S' mutation has been modelled in the mouse. In the original report concerning budding yeast *rad50S* alleles, several point mutants of *RAD50*, all occurring in the N terminal portion of Rad50 containing the ATP binding domain, were called 'S' for separation of function, because they showed almost no mitotic phenotype but a complete absence of meiotic DSB repair (Alani *et al.* 1990). Two of the three Rad50S alleles tested (K6E and R83I, equivalent to yeast K6E and K81I, respectively) were embryonic lethal. Thus, the Rad50K22M allele (equivalent to yeast R20M) was studied (Figure 1). The 'Rad50S' mice showed not much meiotic phenotype, and were fertile: although there was a cellular attrition in testes, there was no meiotic recombination defect, normal meiotic progression and chromosome morphology (Bender *et al.* 2002). Amazingly, these mice showed almost no cellular phenotype, unlike the other Mre11 complex hypomorphic mutations: cells were not sensitive to DNA damage and showed no radioresistant DNA synthesis (RDS). There were wild-type levels of protein, but a profound impact on the organismal level (Bender *et al.* 2002). In these mutants there is a constitutive damage (increase of chromosome breakage and constitutive histone H2AX phosphorylation) (Bender *et al.* 2002) and a constitutive activation of PIPK3 kinases, probably due to unprocessed 'physiological' damage (Bender *et al.* 2002, Morales *et al.* 2005). This aspect is reminiscent of the phenotype in yeast, where in *mec1* cells, *rad50S* or *sae2Δ* mutation produces constitutive Tel1 phosphorylation (Usui *et al.* 2001). Since Rad50 is so well conserved, the enzymatic functions impaired by the murine S mutations are probably the same as the corresponding *S. cerevisiae* mutations. The fact that the Rad50S mice are fertile is puzzling. However, a subtle defect in meiotic recombination might not have been detected in the study (Bender *et al.* 2002). Maybe the K22M activity is sufficient to allow some Mre11 nuclease activity for removing Spo11 from meiotic breaks, whereas the other two S alleles tested were too strongly affected in resection of a diverse spectrum of substrates to be viable. In support of a conservation of the effect of the 'canonical' Rad50S mutation is the fact that, when modelled in the fission yeast *Schizosaccharomyces pombe*, the K81I *rad50S* mutation was viable and behaved as in *S. cerevisiae*, showing formation but no resection of meiotic DSB,

and thus no viable spores (Young *et al.* 2002). Since Spo11–oligonucleotide complexes, most probably resulting from endonucleolytic release by the Mre11 complex, have been detected during mouse meiosis (Neale *et al.* 2005), it would be interesting to see if this intermediate exists in the same amounts in the meiotic cells of the different Mre11 complex mutations mouse models, or if there might be an alternative way of processing meiotic DSB in mice.

As a conclusion, the Mre11 complex plays essential roles during meiosis, in signalling meiotic DSB and activating the Tel1/Atm checkpoint, and also a unique catalytic role in removing the Spo11 protein from break ends to allow their resection and repair by homologous recombination. The different viable mouse models used so far to model Mre11 complex deficiencies have not shown a clear meiotic recombination defect. However, it is only possible to use hypomorphic mutants, and it is very likely that the catalytic nuclease function of the Mre11 complex, used for Spo11 removal, is essential for embryonic development and cell viability in mammals. This could be related to the involvement of the nuclease activity of Mre11 in preventing the generation of chromosome inverted duplications due to failures in processing hairpins and in preventing amplification of palindromes (Rattray *et al.* 2001, Lobachev *et al.* 2002). Meiosis-specific knockout of the Mre11 complex should allow one to study specifically the meiotic roles of this complex in mammals.

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