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The MRE11 complex: starting from the ends

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Abstract

The maintenance of genome stability depends on the DNA damage response (DDR), which is a functional network comprising signal transduction, cell cycle regulation and DNA repair. The metabolism of DNA double-strand breaks governed by the DDR is important for preventing genomic alterations and sporadic cancers, and hereditary defects in this response cause debilitating human pathologies, including developmental defects and cancer. The MRE11 complex, composed of the meiotic recombination 11 (MRE11), RAD50 and Nijmegen breakage syndrome 1 (NBS1; also known as nibrin) proteins is central to the DDR, and recent insights into its structure and function have been gained from *in vitro* structural analysis and studies of animal models in which the DDR response is deficient.

Genomic instability, defined as a reduction in the fidelity with which genetic information is transmitted to daughter cells, is caused by the failure to recognize and repair parental DNA lesions before cell division. DNA double-strand breaks (DSBs) represent a particularly acute danger as they are the underlying cause of chromosomal rearrangements, and can trigger cytotoxic responses or cytostatic responses. DSB formation is intrinsic to normal cell growth, with DSBs arising spontaneously during DNA replication and as intermediates in programmed DNA rearrangements that occur during meiosis and immune system development. DSBs also result from exposure to DNA-damaging agents used in therapeutic settings, including ionizing radiation (IR) and topoisomerase poisons, such as etopo-side and camptothecin, used in cancer treatment. Therefore, the process of DSB metabolism is an integral part of organismal development and the aetiology of myriad disease states; it also determines the response to clastogenic cancer therapies that act by inducing DNA strand breaks.

The DNA damage response (DDR) is initiated upon recognition of the DNA lesion by sensor proteins, followed by rapid and, in many cases, reversible changes in cell behaviour. The DDR can also trigger specialized programmes, such as apoptosis and senescence, to remove or minimize the risk posed by cells with genetic instability. DDR activation is evident in preneoplastic lesions, leading to the hypothesis that it is an inducible barrier to tumorigenesis[1,2]. Consistent with this, hereditary cancer predisposition, as well as other severe pathologies, results from mutations in DDR genes[3,4].

The MRE11 complex consists of meiotic recombination 11 (MRE11), RAD50 and Nijmegen breakage syndrome 1 (NBS1; also known as nibrin), a homologue of Xrs2 in

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Saccharomyces cerevisae, and is a sensor of DSBs that also controls the DDR by governing the activation of the central transducing kinase ataxia-telangiectasia mutated (ATM). In addition, the MRE11 complex regulates DSB repair, through the homology directed repair (HDR), non-homologous end-joining (NHEJ; also known as classical (C)-NHEJ) and alternative non-homologous end-joining (A-NHEJ) pathways (fig. 1; for reviews, see refs 5,6). On balance, its primary role in mitotic cells seems to be the promotion of HDR between sister chromatids to resolve damage that arises during DNA replication[7] (box 1). During meiotic recombination, the HDR functions of the MRE11 complex promote DSB repair events between homologous chromosomes[8,9]. During both meiotic and mitotic repair, the MRE11 complex influences DSB repair structurally, by forming a bridge between the participating DNA molecules, and enzymatically, by promoting the resection of DSB ends[10]. The MRE11 complex is highly conserved, with readily identifiable orthologues of MRE11 and RAD50 evident in eubacterial, archaeal and eukaryal genomes. NBS1 appears to be confined to eukarya and, within that domain, is somewhat less conserved than MRE11 or RAD50.

In this Review, we discuss recent advances that have defined the molecular and structural bases of the MRE11 complex's role in DSB metabolism, telomere homeostasis, meiosis, apoptosis and immune system development. These advances are founded upon approaches ranging from structural analysis to the derivation of new *in vivo* models in yeast and mice. Together, these studies have provided important insights into how this complex influences DDR signalling, DNA repair and tumour suppression.

Structural insights into the MRE11 complex

The MRE11 complex comprises a large central globular domain, in which MRE11 and NBS1 associate with the Walker A and B domains of RAD50, and the extended coiled-coil domain of RAD50, in which the amino-terminal and carboxy-terminal portions of the coils associate in an antiparallel manner (fig. 2a,b). At the apex of the RAD50 coils, where the N-terminal and C-terminal stretches fold back on themselves, is a domain called the RAD50 hook[4,11,12]. Recent insights have been gained into the structural features of the globular domain and the hook domain from crystallographic analyses. The physical properties of the coiled-coil regions in RAD50 have been interrogated with scanning force microscopy[13–16], whereas relatively limited structural information on that domain is available at atomic resolution. As the bulk of structural data have been obtained from *Pyrococcus furiosus*, an archaeon that seems to lack an NBS1 orthologue, some aspects of current models are likely to be revised once structural data from the eukaryotic complexes are obtained. In the following sections, the salient points of these recent studies are summarized.

DNA binding through the globular domain

The MRE11 complex binds DNA via its globular domain, and usually in the context of a higher-order assembly[14,16–20]. This activity primarily requires MRE11 and RAD50, although some data suggest that NBS1 (and Xrs2 in yeast) may bind DNA and also influence the DNA-binding properties of MRE11 and RAD50[19,21,22]. Although structural analyses of each component have provided important insights, an integrated picture of how they assemble into the globular domain and affect DNA binding and enzymatic functions has not yet been established.

The crystal structure of *P. furiosus* Mre11 bound to DNA reveals its binding to DSB ends with two to three base overhangs and branched DNA structures, and provides a basis for understanding the complex's role in NHEJ and DNA end processing[17]. Mre11 dimerization is critical for DNA binding and is mediated by conserved domains in its N terminus. DNA binding by Mre11 is mediated by six DNA recognition loops, in which 17 residues form

sugar–phosphate contacts in the minor groove of DNA. The absence of any base interactions is consistent with the lack of sequence preference for Mre11 in DNA binding. In branched DNA substrates, the single-stranded DNA (ssDNA) is bound similarly by contacts to the phosphodiester backbone. The DNA-binding site also easily accommodates ssDNA, but the double-stranded DNA (dsDNA) and branched substrates appear to be preferred[19]. Rad50 also binds DNA[23], but the relative contributions of Mre11 and Rad50 to DNA binding at the structural level remain to be established.

Mre11 specifies di-manganese-dependent ssDNA endonuclease and 3′–5′ dsDNA exonuclease activities[24,25]. Accordingly, the active site of the *P. furiosus* enzyme (and by extension, orthologous MRE11 species) is structured to accommodate both ssDNA and dsDNA. The exo nuclease function of Mre11 seems to be exerted via melting of the dsDNA terminus, followed by endonucleolytic-type cleavage of the 3′ strand-releasing mononucleotides[17,24–26], suggesting that the extent of melting required for incision is limited.

Homodimerization through the Rad50 hook domain

X-ray crystallographic data from the *P. furiosus* Rad50 homologue suggests that the hook domain functions as a zinc-dependent homodimerization cassette that mediates formation of Mre11 complex assemblies. This domain is conserved in the known Rad50 orthologues, and is characterized by a central sequence motif of CXXC. Alignment of hook domains from 132 species (Pfam database ID: Pf04423) reveals marked preferences for the residues at these sites: most (95%) have either Pro (85%) or Tyr (10%) at the first X position and 80% have Leu or Val at the second X position, indicating that the residues between the invariant Cys are constrained. The two Cys residues from one Rad50 protomer coordinate a zinc atom with the two Cys from a second protomer, resembling the intramolecular coordination of zinc in zinc finger domains[27]. Zinc-dependent interaction within the hook domains of the two proto mers orients their respective coils away from each other at an approximately 140 ° angle, so that the globular domains of each protomer lie at the distal ends of the assembly[28] (fig. 2b).

A non-enzymatic, `structural' function of the MRE11 complex in HDR was initially inferred from genetic analyses indicating that the complex promoted recombination-mediated DNA repair between sister chromatids[7,29,30]. The configuration of MRE11 complexes joined at the hook resonates well with those genetic data, as do the DNA bridging functions inferred from scanning force microscopy of the MRE11 complex associated with DNA[15] (fig. 2b). The replacement of the hook domain with an artificial FKBP domain that can induce homodimerization provided direct evidence that the RAD50 hook domain functions as an interaction interface *in vivo*, and further supported the idea that the complex serves to bridge molecules during HDR[31]. These experiments also revealed that the hook domain is required for MRE11 complex-dependent telomere maintenance in vegetatively growing cells and for the induction of DSBs by the DNA topoisomerase 2 (TOP2)-like enzyme, SPO11, during meiotic recombination[31,32]. These functions are not readily attributable to DNA bridging and so this outcome remains somewhat perplexing. Is it simply the case that loss of the hook interaction causes global disruption of the complex? Resolution of this issue will require separation-of-function mutations that distinguish telomeric and meiotic functions from other roles of the MRE11 complex in the DDR.

The coiled-coil domain

The coiled-coil domain of RAD50 remains somewhat enigmatic, as does its functional significance. First, why is it so large? The extended coiled-coil architecture is common to the known RAD50 orthologues, as well as to the analogous structural maintenance of

chromosomes (SMC) protein family that regulates sister chromatid cohesion and chromosome condensation (for recent reviews, see refs 33–36). If fully extended, the coiled-coil domains of eukaryotic RAD50 orthologues could span as much as 500 Å; in the hook-mediated dimeric state, this would be nearly 1,000 Å[16,28] (fig. 2b), a distance that roughly equals three sister chromatids side-by-side. The domains have highly flexible regions embedded within them[13,16] and so the distances spanned may be significantly shorter. Nevertheless, it is surprising that the MRE11 complex would require such long-range actions to affect its diverse functions, and the basis for this apparent requirement is unclear.

Second, it is remarkable that even isosteric mutants of the Cys residues in the CXXC motif have a global effect on MRE11 complex stability, disrupting the association of RAD50 with MRE11 (ref. 11). This observation indicates that the hook domain influences activities at the globular domain, and that the coiled-coil domains, which connect the two, communicate structural perturbations between them. These influences may occur in both directions: upon DNA binding by the human MRE11 complex, the RAD50 coiled-coils seem to become less flexible and long-range interactions with distal RAD50 protomers are favoured[37] (fig. 2b; left versus right panels).

Regulation of the complex by NBS1

NBS1 is important for regulation of the MRE11 complex, influencing DNA binding as well as MRE11 nuclease activity. The N-terminal region of NBS1 contains two phosphopeptide-binding modules commonly found in DDR proteins: a Forkhead-associated domain (FHA domain) and a tandem BRCA1 C-terminal domain (BRCT domain)[4,38,39]. Structural analyses of these N-terminal domains in the *Schizosaccharomyces pombe* Nbs1 protein have revealed a novel modular architecture that allows diverse phosphorylation-dependent protein interactions[40,41]. FHA and tandem BRCT domains generally function as `stand-alone' phosphopeptide-binding domains[42–44]. Thus, Nbs1 is atypical, in that both FHA and tandem BRCT domains are present and could in principle allow three modes of binding to partners: FHA only, BRCT only, or FHA plus BRCT. However, structural analysis has revealed that the FHA domain is fused directly to the tandem BRCT domain[40,41], creating a structural inter-dependence between them that makes it less likely that interactions would occur through the BRCT domain alone. Moreover, engagement of the Nbs1 FHA domain by a phosphorylated partner leads to a dramatic structural transition of the BRCT domains. Whether this potentiates BRCT domain interactions has not been established, but the possibility is appealing. The dynamic behaviour of the *S. pombe* Nbs1 N-terminal domain was revealed by co-crystallization with its binding partner, Ctp1, the orthologue of Sae2 in *S. cerevisae* and CtBP-interacting protein (CtIP) in mammals[40,41,45,46]. Ctp1 contains casein kinase 2 phosphorylation sites (SXT clusters) that mediate binding to the Nbs1 FHA domain. Engagement of the FHA domain is associated with a 20 ° rotation at the BRCT1–BRCT2 interface, causing a 10 Å movement of the C-terminal portion of BRCT2 (ref. 40). Based on the role of the Mre11 complex and Ctp1 in DSB end resection, this structural transition is likely to influence HDR. Genetic evidence suggests that Ctp1 deficiency does not impair activation of the S phase checkpoint in *S. pombe*, supporting the idea that its binding to the Nbs1 FHA domain primarily influences DNA repair[47].

In mammals, mediator of DNA damage checkpoint 1 (MDC1) also binds NBS1 via its FHA domain[48]. As with Ctp1, MDC1 contains SXT phosphorylation site clusters that are required for this interaction and influence MRE11 complex retention at sites of DNA damage[48–51]. Biochemical evidence suggests that MDC1 SXT clusters engage the FHA and BRCT domains simultaneously[41,48–50]; thus, this interaction appears to represent an FHA plus BRCT binding mode. It is an appealing possibility that the phospho-binding-induced dynamic structural transitions in NBS1 form the basis of its regulatory influence on the

MRE11 complex. Also, this structural information lays a solid foundation for testing this idea at the molecular level.

The MRE11 complex in DNA metabolism

The MRE11 complex has multiple roles in the metabolism of DSBs that involve both its enzymatic and structural functions. The 3′–5′ exonuclease and ssDNA endonuclease activities of MRE11 do not depend on RAD50 and NBS1 but are enhanced when MRE11 is in the holocomplex[52]. Although a comprehensive view of the physiological significance of the MRE11 nuclease activity remains to be established, the MRE11 complex and its orthologues are clearly important for both the clearance of covalently attached proteins from DNA termini and promotion of DSB end resection en route to the production of 3′ ssDNA tails required in HDR and checkpoint activation.

Biochemical analysis of the MRE11 and RAD50 orthologues SbcC and SbcD of *Escherichia coli*, as well as the influence of the MRE11 complex on adenovirus replication intermediates, has implicated the MRE11 complex in the removal of covalently attached proteins to promote repair[53,54]. Some of the most-detailed evidence for the MRE11 complex's role in this process has come from studies of meiosis in yeast. During meiotic recombination, the requisite first step of DSB induction is catalysed by Spo11 (refs 32,55,56), which cleaves dsDNA and remains covalently bound to the 5′ strands of the ensuing breaks. The Mre11 complex subsequently mediates endonucleolytic removal of two differently sized Spo11-DNA oligo species, suggesting that Mre11 cleaves Spo11-bound termini asymmetrically[57]. Spo11 removal is impaired in Mre11 nuclease mutants and in cells lacking Sae2, which exhibit nuclease activity *in vitro*[58]; however, nuclease activity may not be sufficient for Spo11 cleavage. Certain alleles of Mre11 and Rad50 — termed `S alleles' for separation of meiotic and mitotic function — block Spo11 cleavage but are unlikely to be defective for Mre11 nuclease activity[57–59]. Studies in *S. pombe* are also consistent with this; however, in *S. pombe*, only a single species of DNA oligo was recovered, suggesting that cleavage is symmetric rather than asymmetric[60–62].

Based on its role in SPO11 removal and the sensitivity of nuclease-deficient MRE11 alleles to topoisomerase poisons, the nuclease activity of MRE11 has been proposed to affect removal of covalent TOP1–DNA and TOP2–DNA intermediates[63]. Null alleles, as well as *Mre11* nuclease and *Rad50*^{S} mutants, are sensitive to both Top1 and Top2 inhibitors, but *Mre11* nuclease and *Rad50*^{S} mutants show only mild sensitivity to methyl methanesulphonate (MMS) and IR when compared with null mutants[63]. Similarly, mouse cells expressing a *Rad50*^{S} allele exhibited sensitivity to both TOP1 and TOP2 poisons but not other DSB-inducing agents such as IR[64].

The 5′–3′ resection of DSB ends underlies the initiation of checkpoint responses and is required for the initiation of HDR[10] (fig. 1). The rate of DSB resection was found to be reduced in Mre11- and Rad50-deficient strains, suggesting the possibility that the Mre11 complex is somehow involved in end resection[29,65,66]. Paradoxically, Mre11 specifies 3′–5′ exonuclease activity — the opposite polarity to that required for resection — and nuclease-deficient alleles of *mre11* exhibited milder resection defects than *mre11*$^{\Delta}$ (refs 63,67). However, analysis of DSB resection *in vivo* in *S. cerevisiae* reveals that the Mre11 complex and Sae2 catalyse the initiation of resection through the removal of a short tract of ssDNA. This initial resection is followed by the bulk resection of DNA by either the 5′–3′ exonuclease 1 (Exo1) or DNA2 in conjunction with the helicase Sgs1 (refs 68,69). Bulk resection was required for efficient induction of G2 arrest, as well as repair by single-strand annealing (SSA) or HDR repair of lesions and cell survival[10].

The available evidence in mammalian cells suggests that the MRE11 complex, in conjunction with CtIP, the mammalian orthologue of Sae2 and Ctp1, mediates analogous functions in DSB resection[70]. An intriguing possibility is that the initial incision step by MRE11 or CtIP, which produces a 3′ ssDNA overhang of 50–100 bases, discourages engagement of the DSB end by the Ku heterodimer, and thereby inhibits repair of the DSB by NHEJ. An analogous function in blocking NHEJ has been ascribed to components of the Fanconi anaemia (FA) pathway[71,72], perhaps consistent with the observation that FA proteins have been shown to physically and functionally interact with the MRE11 complex[73–76]. Separation of function alleles of *S. cerevisiae* Sae2 reveal that Spo11 removal and camptothecin resistance can be separated genetically from the opening of hairpins at DSB ends[77]. These data suggest that Sae2 and Mre11 are not completely redundant in DSB end processing. The ability of the MRE11 complex to promote early steps of nucleolytic resection is clearly important for the efficient induction of meiotic and mitotic recombination but whether resection is catalysed by the enzymatic activities of MRE11, Sae2 or CtIP, or an as yet unidentified factor, remains an important question to resolve.

The molecular requirements for reconstitution of the *S. cerevisiae* DSB resection process *in vitro* are generally consistent with the *in vivo* studies. At the core of the resection machinery is the 3′–5′ helicase Sgs1 that unwinds the DSB end, and the ssDNA thus formed is digested by the nuclease activity of Dna2. The activity of Dna2 is directed to the 5′ strand, and away from the 3′ strand by the ssDNA-binding protein replication protein A (RPA); the net result is resection of the 5′ strand of the DSB end to produce the 3′ ssDNA tail. Sgs1 activity is enhanced by the Mre11 complex and the Rmi1–Top3 complex, although the enzymatic functions of these complexes are not required for resection[78,79]. These findings in yeast echo those obtained with *P. furiosus* proteins[80]. Resection *in vitro* requires *P. furiosus* Mre11 and Rad50, as well as HerA, a bidirectional helicase, and NurA, a 5′–3′ exonuclease, all four of which are encoded by the same operon in the *P. furiosus* genome. The role of RPA in the resection process seen in *S. cerevisiae* has not been examined in *P. furiosus*.

Neither the yeast nor the *P. furiosus in vitro* systems exhibit the two-step mechanism observed *in vivo*. This would suggest that the first step is dispensable for resection. Presumably, factors present *in vivo* underlie the requirement for the initial Mre11 complex-dependent step. As noted above, the possibility that this first incision step may regulate the binding of Ku and other factors to the DSB end must be considered.

The MRE11 complex in telomere homeostasis

The ends of linear chromosomes consist of telomeres and are bound by an array of proteins that prevent them from being recognized as DSBs. This protein assembly, called shelterin in mammals, together with the unique DNA sequences at chromosome ends, defines the telomere[81,82]. Genetic analyses in *S. cerevisiae* have clearly established that the Mre11 complex regulates telomere length, most likely via its effect on telomerase recruitment[81]. The MRE11 complex also localizes to mammalian telomeres independently of shelterin or ATM[83,84], and recent data offer some clues as to its functions there. first, in contrast to the Mre11 complex in budding yeast, the mammalian MRE11 complex does not appear to exert a strong effect on telomere length homeostasis[85]. Second, as is the case with interstitial DSB damage, the MRE11 complex is required for activating the ATM-dependent response at dysfunctional telomeres (fig. 3). This induces the rapid assembly of DDR components into telomere-dysfunction-induced foci (TIFs) which can be visualized by immunofluorescence[86]. Small hairpin RNA-mediated depletion or conditional deletion of key components of the shelterin complex results in its removal from telomeres and TIF formation[86]. This induction of TIF formation is impaired in most *Nbs1*- or *Mre11*-defective cells[85,87,88]. ATM activation by the MRE11 complex and TIF formation are independent of

MRE11 nuclease activity[89]. However, because the fusion of dysfunctional telomeres completely depends on ATM[90], it is unclear whether this phenotype indicates a direct role of the MRE11 complex in the end-joining process, or simply reflects its effects on ATM activity.

Finally, the MRE11 complex seems to promote resection of telomeric DNA to create the single-stranded 3′ overhang that is typically found at the telomere (fig. 3). Although telomere fusions are markedly reduced upon acute telomere dysfunction in MRE11 complex mutants, they are not completely abolished. Also, virtually all of the rare telomere fusions observed in MRE11 complex mutants occur between telomeric ends replicated by the leading strand polymerase[88]. In contrast to the lagging strand telomere, the leading strand is a blunt end that forms immediately following replication. The lagging strand telomere is not blunt because lagging strand DNA synthesis cannot fully replicate DNA ends; removal of the RNA primer used to initiate Okazaki fragment synthesis leaves behind a stretch of ssDNA. The 3′ overhang inhibits NHEJ[91], and if MRE11 complex hypomorphism were to impair resection of the blunt end it would make it a better end-joining substrate, thus accounting for the observed bias toward leading-end fusions; however, this interpretation awaits experimental validation.

The MRE11 complex in human disease

The identification of mutations affecting the MRE11 complex in human genomic instability syndromes provided the first hints of an intimate relationship between the MRE11 complex and ATM-mediated checkpoint signalling. Inherited mutations in *MRE11*, *NBS1* and *RAD50* cause ataxia-telangiectasia-like disease (ATLD), NBS and NBS-like disorder (NBSLD), respectively (fig. 4a). The clinical and cellular features of these syndromes underscore the importance of the MRE11 complex in the DDR, and the corresponding animal models have provided tractable systems for genetic analysis[4]. NBS and ATLD cells exhibit phenotypic similarity to those from patients with ataxia-telangiectasia (A-T), including hypersensitivity to DSB-inducing clastogens, defects in DNA damage-dependent cell-cycle checkpoint arrest, and chromosomal fragility. However, the clinical presentations of patients with NBS and patients with ATLD are distinct. Patients with NBS present with microcephaly and `bird-like' facial features, which are more similar to Seckel syndrome, and are highly predisposed to cancer. Recently, it was reported that a patient with NBSLD resulting from heteroallelic mutations in RAD50 also exhibited microcephaly[92]. Morphological abnormalities are not characteristic of patients with A-T or ATLD who exhibit neurodegeneration and ataxia. Although cancer occurs with high frequency in patients with A-T, it has been reported in only two patients with ATLD so far[93]. However, given the limited number of patients with ATLD that have been identified, it is difficult to exclude the possibility that cancer predisposition is a primary feature of this disease. The implication of all three members of the MRE11 complex in distinct but clinically overlapping syndromes solidifies the concept that MRE11, RAD50 and NBS1 function as a unit, and argues against the possibility that any of the members mediate autonomous functions outside the complex.

Insights from MRE11 mouse models

Null mouse mutants of *Mre11*, *Rad50* and *Nbs1* are not viable[89,94,95]. This essential nature of MRE11 complex components has necessitated the derivation of conditional hypomorphic alleles and hypermorphic alleles, the design of which has been guided by the corresponding human mutations or *in vitro* studies (fig. 4a). The phenotypic analyses of mouse models for the human syndromes as well as a hypermorphic allele of *Rad50* have been reviewed elsewhere[4,96] (table 1). We focus here on recent mouse models that are based on molecular information obtained through structural and biochemical analyses, and on clinical features of

the human syndromes that suggest physiological systems in which MRE11 complex functions are particularly significant.

NBS1 mouse models

Mouse mutants harbouring *Nbs1* alleles that affect protein interaction domains of NBS1 have been established. The phenotypes observed are generally consistent with predictions made by the molecular data, but in some of the cases, the data indicate that existing models of NBS1 function might need to be revised.

`Humanized' transgenic mice have been used to carry out structure-function analysis *in vivo*: in this approach, human bacterial artificial chromosomes (BACs) containing mutant alleles of the *Nbs1* locus are used as transgenes to complement $Nbs1^{\Delta/\Delta}$ mice. For example, mice created with the human $Nbs1^{H45A}$ mutation, which alters a conserved residue in the NBS1 FHA domain, partially phenocopied $Nbs1^{\Delta B}$, $Nbs1^{657\Delta 5}$ and $Nbs1^{m}$ mice, which model the common allele found in NBS patients, and encode an N-terminally truncated NBS1 protein lacking the FHA domain altogether[97–99]. It seems likely that a reduction in the recruitment of CtIP, which interacts with the FHA domain[40,41], accounts for many of the cell-cycle checkpoint and repair defects observed in these mutants, as mice lacking MDC1, which also interacts with this domain, showed only subtle defects in checkpoint responses[100]. However, the fact that Ctp1 deficiency in *S. pombe* does not affect S phase checkpoint activation may suggest that $Nbs1^{H45A}$ impairs additional protein interactions relevant to checkpoint function[47].

The C terminus of NBS1 contains a 24 amino acid conserved motif that interacts with ATM[101,102] (fig. 4a). Complementation of cells from patients with NBS with a cDNA lacking this domain failed to rescue phosphorylation of some ATM substrates and intra-S and G2/M checkpoint defects, although ATM activation was normal[101]. Whereas NBS1 was dispensable for ATM activation *in vitro* using purified human MRE11 and RAD50 (ref. 103), the nbs1 C-terminal domain was required for DNA-dependent ATM activation in *Xenopus laevis* extracts[102]. Moreover, in mice that lack the NBS1 C-terminal domain, produced using either the BAC transgenic approach (to create the $Nbs1^{tr735}$ allele) or conventional targeted mutation (the $Nbs1^{\Delta C}$ allele)[104,105], ATM activation was unaffected, MRE11 complex protein levels and subcellular localization were unchanged, and no impairment of MRE11 complex association with DNA damage was evident. Accordingly, checkpoint functions were also largely unaffected save for a mild defect in the intra-S phase checkpoint[105].

Strikingly, thymocytes from $Nbs1^{\Delta C}$ and $Nbs1^{tr735}$ mice were defective in ATM-dependent IR-induced apoptosis. This defect correlated with defects in the ability of ATM to phosphorylate SMC1 and BH3-interacting domain death agonist (BID), which are effectors of the intra-S phase checkpoint and ATM-dependent apoptosis, respectively[106–108]. The discrepancies between findings in the mouse versus *in vitro* systems and overexpression in human cells may in part reflect species-specific differences. It is also likely that the presence of partially redundant activities[109] and the preservation of stoichiometry and intracellular localization provided by the *in vivo* setting may provide a more nuanced assessment of function. We favour the view that the NBS1 C terminus is dispensable for ATM activation but is required to permit ATM access to certain substrates (fig. 4b). The limited phenotypic outcomes observed in $Nbs1^{\Delta C}$ and $Nbs1^{tr735}$ are consistent with this. In this regard, it is notable that the C terminus of NBS1 is sufficient to promote apoptosis in $Nbs1^{\Delta B/\Delta B}$ mice, despite a marked reduction in levels of the $NBS1^{\Delta B}$ protein[110].

NBS1 is phosphorylated by ATM in response to damage, and this has been proposed to be a prerequisite for checkpoint activation by the MRE11 complex[111,112]. However, mice

expressing a humanized allele lacking both of the prominent ATM phosphorylation sites at Ser278 and Ser343 showed normal checkpoint responses, suggesting that these sites are not essential for checkpoint activation in mice[104]. As a result, the role of ATM phosphorylation in regulating MRE11 complex function remains unclear. Additional phosphorylation sites exist and it is possible that compound mutations inactivating them en masse are required to see an effect.

RAD50 mouse models

The MRE11 complex is required for the completion of DNA replication, presumably reflecting the importance of HDR during this process[113,114]. Data obtained from a conditional knockout of *Rad50* (*Rad50*$^{\Delta lox}$) lend strong support for this idea. As is the case with *Mre11* and *Nbs1* genes, inactivation of the *Rad50* gene in cultured cells or proliferative tissue leads to precipitous cell death associated with dramatic genome instability. Telomere dysfunction is not seen, indicating that RAD50 function at telomeres is not acutely required for viability[8,115]. By contrast, deletion of *Rad50* in postmitotic Purkinje cells of the *Rad50*$^{\Delta lox}$ mouse had no effect, even for 1-year-old mice. Similarly, *Rad50* deletion in quiescent liver cells was completely innocuous. However, partial resection of the liver to induce division of RAD50-deficient hepatocytes was associated with widespread DNA damage, indicating that RAD50 deficiency had a profound effect even in this single round of replication. Collectively, these and other data indicate that the essential function of the MRE11 complex is in the HDR-dependent resolution of DNA replication-associated DSBs[115] (box 1). By extension, these data suggest that HDR itself is dispensable in non-dividing cells.

MRE11 mouse models

The nuclease domain of MRE11 is among the most highly conserved components of DDR factors, with easily recognizable orthologues from bacterio phage T4 to humans[4]. However, the consequences of nuclease deficiency differ widely according to phylo-genetic context, which may be due to redundant activities in some settings or species-specific substrates for MRE11 in others. for example, in *S. cerevisiae*, nuclease-dead *mre11* mutants have a relatively mild phenotype in vegetatively growing cells. Conversely, nuclease-dead *S. pombe rad32* mutants (Rad32 is the *S. pombe* orthologue of Mre11) nearly phenocopy the clastogen sensitivity of *rad32*$^{\Delta}$ mutants, although nuclease deficiency blocks the initiation of meiotic recombination in both yeasts[17,60–62,116]. In mammalian systems, the MRE11 nuclease activity has been implicated in the activation of ATM through several approaches and phenotypic outcomes, including effects on the stability of replication forks and the initiation of checkpoints and repair processes[54,117–120].

A nuclease-dead allele of *Mre11*, *Mre11*^{H129N}, leads to embryonic lethality when homozygous in mice, indicating that the nuclease activity is essential during development[89]. *Mre11*^{H129N} cells rapidly senesced and showed a similar spectrum of spontaneous and damage-induced chromosomal aberrations as seen in cells deficient for RAD50, NBS1 or MRE11, suggesting a severe defect in the repair of spontaneous DNA lesions[8]. These defects in DNA repair correlated with defects in the accumulation of RPA and RAD51 foci, suggesting that the resection of DSB ends is impaired. Notably, the presumptive effect on DSB resection was not associated with defects in cell-cycle checkpoint activation; ATM activation and downstream effects on targets such as CHK2 were normal, as was G2/M arrest following radiation treatment. Similarly, the *Rad50*^{S} mouse, which may also exhibit impaired MRE11 nuclease function, did not display checkpoint deficiencies[121]. Together, these data suggest that the role of MRE11 nuclease activity in DSB resection is not strictly required for the activation of ATM or many aspects of the DDR.

Roles in immune system development

A primary feature of human genetic instability diseases is variable immune deficiency. In patients with A-T and patients with NBS, this includes aberrant immunoglobulin isotype profiles in serum, reduced numbers of mature T cells and increased sinopulmonary infections. Although the underlying cause of many of these defects remains unclear, mouse models have provided an excellent system for analysing the roles of the MRE11 complex in DNA repair and the consequences of an impaired DDR for immunological development.

There are mixed reports for whether the MRE11 complex affects class switch recombination (CSR). In ATM-deficient mice, there is a substantial defect in T cell development and CSR[122,123]. furthermore, conditional NBS1 or MRE11 deletion in lymphocytes, which leads to cell death within several cell passages, results in defects in CSR, raising the possibility that the MRE11 complex has a role in CSR[124,125]. The nuclease-deficient *Mre11*^{H129N} allele also resulted in deficient CSR, and it has been suggested that defects in resection may underlie this phenotype[125]. This is at odds with the observation that mice expressing the *Nbs1*$^{\Delta B}$ and *Mre11*^{ATLD1} alleles, with substantial defects in MRE11 complex formation and function, showed normal thymocyte development as well as normal CSR[98,126]. However, trans-rearrangements caused by aberrant V(D)J recombination and increased levels of unrepaired DNA were detected in these and similar animal models, suggesting a more subtle defect in the fidelity of end-joining[126,127]. future identification of alleles that affect CSR without severely affecting cell viability or other functions of the complex may shed light on these contrasting results.

The MRE11 complex also functions in A-NHEJ[6,125,128–132] (fig. 1). In mice lacking the DNA-dependent protein kinase catalytic subunit (DNA-PKcs, encoded by the *Prkdc* gene) or the Artemis (ART) nuclease, hairpin-capped coding joint ends generated by the RAG recombinases, which initiate V(D)J recombination, are unresolved, leading to immunodeficiency[133]. *Prkdc*^{scid} mice, as well as DNA-PKcs knockouts, are synthetically lethal with *Atm*$^{-/-}$ or *Mre11*^{ATLD1} (refs 134–136). These genetic interactions are not simply due to deficiencies in the ability of DNA-PKcs to regulate the ART nuclease, as *Art* knockouts do not show a synthetic interaction with ATM or MRE11 complex alleles[136,137]. *Prkdc*$^{scid/scid}$ *Nbs1*$^{\Delta B\Delta B}$ mice are nearly inviable, but a limited number of double-mutant mice and cells have allowed the role of the MRE11 complex in cells lacking a primary component of NHEJ to be investigated. using a hyperactive RAG protein to initiate DNA breaks, it was demonstrated that an A-NHEJ pathway could be activated in cells lacking DNA-PKcs[138]. This system, together with analysis of the endo genous TCR^{tm} loci in *Nbs1*$^{\Delta B/\Delta B}$*Art*$^{-/-}$ double-mutant mice, revealed that the MRE11 complex is required for A-NHEJ-mediated joining of V(D)J substrates[128]. This A-NHEJ activity of the MRE11 complex was independent of the nuclease activities of MRE11 (ref. 128). Consistent with this, only mild defects in NHEJ were observed in B cells from mice expressing the nuclease-defective allele of MRE11 despite substantially impaired CSR[125]. Additional MRE11 complex alleles that separate the diverse functions of the complex will be essential for elucidating its precise roles in V(D)J recombination and CSR *in vivo*.

Checkpoints, apoptosis and malignancy

Mouse mutants for the MRE11 complex have been invaluable for yielding novel insights into how MRE11 signalling is integrated with the DDR during apoptosis and tumour suppression (fig. 4; table 1; see Supplementary information S1 (table)).

Intercrosses of *Rad50*$^{S/S}$ mice have provided insight into how the MRE11 complex affects ATM activation and DNA damage signalling. *Rad50*$^{S/S}$ mice exhibit precipitous loss of

haematopoietic stem cells and die of anaemia by 4 months of age[139]. The severity of this outcome was reduced by creating $Rad50^{S/\Delta}$ mice, indicating that the $Rad50^{S}$ allele is hypermorphic[140]; and deleting ATM in $Rad50^{S/S}$ mice completely rescued the $Rad50^{S/S}$ pheno-type. Surprisingly, lymphomas, radiation sensitivity and chromosome instability normally associated with ATM deficiency were reduced in $Rad50^{S/S}Atm^{-/-}$ mice[64,140]. This effect of the $Rad50^{S}$ allele was attributed to hyperactivation of ATR pathways and thereby partially compensates for ATM deficiency (for a review of $Rad50^{S}$ function in mice and yeast, see ref. 121).

Crosses of mutants of the MRE11 complex with mouse models of ATLD and NBS ($Mre11^{ATLD1/ATLD1}$ and $Nbs1^{\Delta B/\Delta B}$, respectively) have revealed that ATM and CHK2 function in parallel pathways to activate apoptosis[141] (fig. 4b). The apoptotic defects of ATM mutants have been attributed in part to impaired phosphorylation of CHK2 and p53. $Mre11^{ATLD1/ATLD1}$ and $Atm^{-/-}$ mice exhibit impaired DNA damage-induced CHK2 hyper-phosphorylation and intermediate defects in thymocyte apoptosis, similar to cells lacking CHK2. Intercrosses of $Atm^{-/-}$ or $Mre11^{ATLD1/ATLD1}$ mice with CHK2-deficient mice resulted in a complete apoptotic defect, approximating that seen in p53-deficient mice[105,141]. This indicates that CHK2-dependent apoptosis operates in the absence of ATM activity and that ATM phosphorylation of CHK2 is not essential for its induction of apoptosis. Supporting this, $Nbs1^{\Delta C}$ mice that show normal ATM-dependent phosphorylation of CHK2 (ref. 105) also synergize with CHK2 deficiency and exhibit profound apoptotic defects (T.H.S. and J.H.J.P., unpublished observations). As thymocytes predominantly reside in G0/G1 phase, we favour the possibility that DNA-PKcs, which can phosphorylate CHK2 *in vitro* and affect apoptosis independently of ATM, is an activator of CHK2 apoptotic activity[142,143].

Apoptosis occurs in the developing brain in response to radiation or defects in DNA repair[144]. Mice lacking DNA ligase 4 ($Lig4^{-/-}$), which catalyses the final ligation step during NHEJ (fig. 1), exhibit apoptosis in postmitotic neurons and die in late embryogenesis[145,146]. Using a conditional knockout of *Lig4* under the control of the nestin promoter that is active in the brain, a similar disparity was observed between the apoptotic responses of $Nbs1^{\Delta B}$ and $Mre11^{ATLD1}$. Radiation or *Lig4* deletion in the brains of $Nbs1^{\Delta B/\Delta B}$ mice led to apoptosis similar to that observed in wild-type cells, whereas either $Atm^{-/-}$ or $Mre11^{ATLD1/ATLD1}$ mice exhibited reduced apoptosis[110]. We proposed that these differences in apoptotic signalling could account for the striking difference in neuronal pathology of NBS compared with ATLD or A-T. It is thought that in a background competent for ATM-dependent apoptosis, microcephaly would predominate, whereas, when apoptosis is impaired, neurodegeneration would arise. However, this idea is questioned by the recent identification of heteroallelic RAD50 alleles in a patient with NBSLD who presented with microcephaly, but also showed defects in p53 signalling, suggestive of defects in apoptosis[92]. Resolving this issue will require the generation of an animal model of NBSLD for in-depth analysis of apoptosis.

Loss of CHK2 in either $Nbs1^{\Delta B/\Delta B}$ or $Mre11^{ATLD1/ATLD1}$ mice leads to increased predisposition to a wide variety of tumour types after a long latency period, in contrast to the rapid lymphomas observed in ATM. This is observed with MRE11 complex alleles that affect the CHK1-dependent S and G2/M transitions or HDR, suggesting that the functions of the complex in monitoring replication-associated DNA damage may affect tumour suppression[141]. Supporting this possibility, increased tumour predisposition is observed in CHK2-null mice that lack functional breast cancer associated 1 (BRCA1) or are heterozygous for CHK1 (refs 147, 148). A more detailed understanding of how the MRE11 complex suppresses replicative damage should provide further insight into its functions in tumour suppression.

Conclusions and perspectives

As our understanding of how the MRE11 complex functions in the DDR has increased, issues such as the precise role of MRE11 nuclease activity, the mechanism by which the eukaryotic MRE11 complex binds DNA, the importance of the RAD50 coiled-coil domains and the mechanisms underlying the effects of the complex on HDR and NHEJ have grown richer and more complicated. The ongoing merger of information from genetic analyses with biochemical and structural analysis of the eukaryotic MRE11 complex will undoubtedly continue to illuminate these issues. As this occurs, it is likely that species-specific and cell-type-specific differences in how the complex influences the DDR will be revealed. Even with these gaps, the importance of the MRE11 complex in driving the successful execution of S phase has been firmly established, as has its role in preserving genomic integrity by activating DNA repair and signal transduction in the DDR. Through these functions, the MRE11 complex sustains the viability of proliferating cells, and suppresses the oncogenic potential of DNA replication-associated DNA damage.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Glossary

Cytotoxic response	A cellular response to stimuli leading to cell death.
Cytostatic response	A cellular response to stimuli leading to a suppression of cell growth.
Topoisomerase poison	A class of drugs used in cancer therapy that trap covalent intermediates of topoisomerases, causing DNA damage that is exacerbated by DNA replication.
Clastogenic cancer therapy	A type of cancer therapy that relies on a clastogen, or DNA break-inducing agent, to target proliferating cells in tumours.
Ataxia	A neurological condition characterized by loss of motor control. This often results from defects in the development or degeneration of the cerebellum.
Homology directed repair	A major double-strand break repair pathway that is template-mediated and therefore considered to be highly accurate. Particularly important for sister chromatid regulation in S/G2 phase.
Non-homologous end-joining	A major double-strand break repair pathway that involves the ligation of free ends, sometimes after processing that leads to the loss or gain of sequence. This pathway is particularly well studied in the context of V(D)J recombination.

Alternative non-homologous end-joining	A poorly characterized end-joining pathway (or pathways) that is not dependent on the core non-homologous end-joining components. This pathway frequently uses short microhomologies (5–25 nucleotides) and is thought to be resection dependent.
Sister chromatids	Identical chromatids that are joined by a centromere and generated during S phase DNA replication.
Resection	The process of converting double-stranded DNA to single-stranded DNA by the exonucleolytic removal of one strand. Resection is often performed in conjunction with the action of a helicase and is implicated in both the checkpoint activation and multiple repair pathways.
Scanning force microscopy	A type of microscopy that uses a physical probe to scan the surfaces of a specimen and provide high-resolution images at a nanoscale level. Also known as atomic force microscopy.
Endonuclease	An enzyme that cleaves the phosphodiester bond of DNA within a polynucleotide chain.
Exonuclease	An enzyme that cleaves the phosphodiester bond of DNA from the end of a polynucleotide chain.
Zinc finger domain	A protein structural motif that coordinates zinc ions via Cys and His residues to stabilize folds involved in nucleic acid or protein binding.
FKBP domain	A domain originally found in the FK506-binding protein (FKBP) that mediates its interactions with the immunosuppressant FK506. Binding of FK506 or analogues leads to dimerization and is used as an inducible artificial dimerization domain in fusion proteins.
Isosteric mutant	An amino acid substitution that approximates the spatial and chemical properties of the residue that it replaces.
BRCA1 C-terminal domain	A phosphopeptide-binding domain first identified in the carboxyl terminus of the breast cancer associated 1 (BRCA1) protein. These domains are usually found in tandem.
Methyl methanesulphonate	A carcinogenic alkylating agent that generates strand breaks and is used in cancer therapy.
Microcephaly	A neurodevelopmental disorder characterized by reduced head circumference and often accompanied by neurological problems including mental retardation and delayed development of motor functions.
Hypomorphic allele	An allele that results in a partial loss of function.
Hypermorphic allele	An allele that results in a partial gain of function or increased activity.
Class switch recombination	The process by which B cells change the production of antibody from one class (or isotype) to another. This involves the exchange of constant and variable regions and involves the

	induction and repair of DNA double-strand breaks. Also known as isotype switching.
V(D)J recombination	A process that assembles diverse immunoglobulin and T-cell receptor genes from existing variable (V), diversity (D) and joining (J) gene segments. V(D)J recombination is initiated by the RAG1–RAG2 recombinase in a sequence-specific manner. Also known as antigen receptor gene rearrangement.

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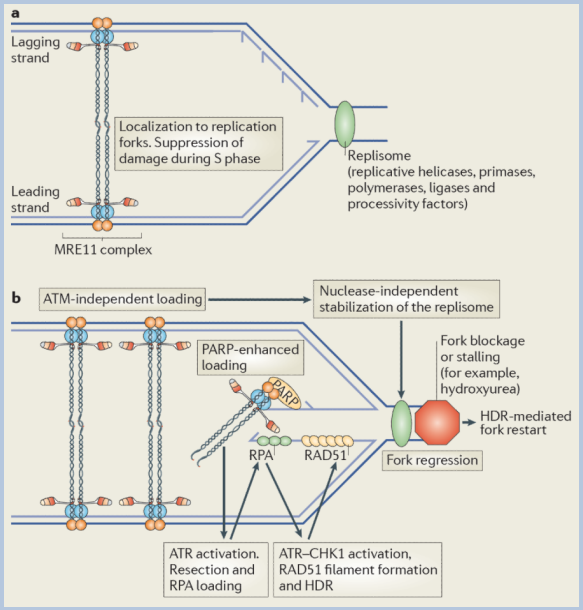
Box 1 | The MRE11 complex promotes HDR and replication fork stability



Yeast lacking components of the Mre11 complex are highly sensitive to DNA damage in S/G2 phase, and show profound defects in homology-directed repair (HDR)[7]. Cells from mouse models expressing hypomorphic alleles of MRE11 complex members exhibit checkpoint defects in S and G2 phases of the cell cycle and display increased chromatid breaks and fragments, consistent with damage occurring in regions of replicating DNA[98,126]. *In vivo*, the MRE11 complex is required for the viability of cycling but not post-mitotic cells[115], and colocalizes with proliferating cell nuclear antigen (PCNA) at replication foci during S phase, when levels of the MRE11 complex on chromatin are most abundant[149,150]. Depletion of the MRE11 complex during DNA replication results in enhanced DNA breakage[151] (see the figure, panel **a**). The MRE11 complex is also recruited to stalled replication forks following hydroxyurea treatment[150,152], a process that is enhanced by poly(ADP-ribose) polymerase (PARP) activity[153] but does not require ataxia-telangiectasia mutated (ATM)[150] (see the figure, panel **b**). DNA double-strand breaks (DSBs) can arise at stalled forks owing to single-strand breaks or as a result of replication fork regression. The MRE11 complex and ATM promote resection at DSBs, allowing replication protein A (RPA) binding and activation of the ATR and CHK1 kinases that potentiate checkpoint responses, promote RAD51 filament formation and HDR, and allow replication to resume (see the figure, panel **b**)[154–157]. Independently of MRE11 nuclease activity, the MRE11 complex stabilizes components of the replisome[152], a complex that includes the replicative helicase, polymerases and processivity factors, and promotes fork restart through HDR pathways in conjunction with ATM or ATR[158] and RAD51 (ref. 120) (see the figure, panel **b**). Together, these data are consistent with the MRE11 complex having a primary role in the maintenance of the replication fork during DNA replication. The precise roles of the MRE11 complex's structural and enzymatic activities in these processes remain to be clarified.

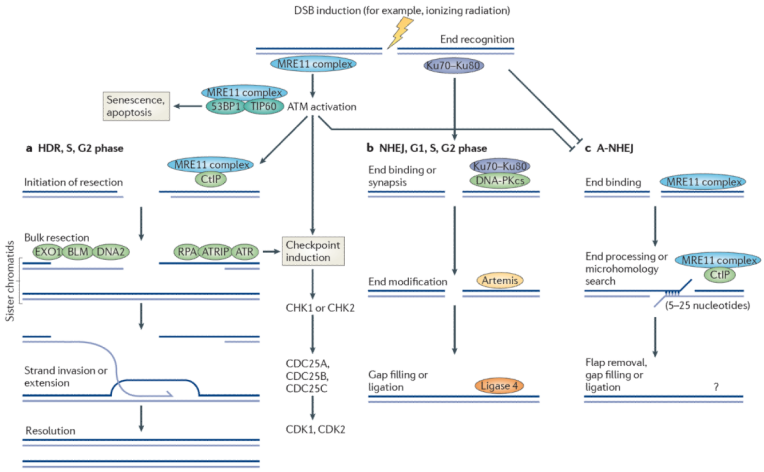


Figure 1. The MRE11 complex regulates the mammalian DNA damage response

Double-stranded DNA (dsDNA) breaks are recognized by the MRE11 complex, which catalyses the activation of ataxia-telangiectasia mutated (ATM) in conjunction with other proteins such as the tat-interactive protein 60 kDa (TIP60; also known as KAT5) acetyltransferase and p53-binding protein 1 (53BP1)[159,160]. ATM activation promotes cell-cycle checkpoint induction, influences DNA repair, and can activate apoptosis and senescence in certain cellular contexts[3]. Depending on the cell-cycle phase and end-binding complexes or end modifications, breaks can be directed into two major repair pathways: homology-directed repair (HDR) or non-homologous end-joining (NHEJ; also known as classical (C)-NHEJ). **a** | HDR requires the 5′–3′ resection of dsDNA to generate single-stranded DNA (ssDNA)–dsDNA junctions. This is initiated by the MRE11 complex and CtBP-interacting protein (CtIP) and further bulk resection is carried out by exonuclease 1 (EXO1), BLM and DNA2 (refs 10,70,161–163). 3′ ssDNA tails generated by resection are bound by replication protein A (RPA), which activates ATR via ATR-interacting protein (ATRIP) binding to influence the checkpoint response[164]. RPA on these 3′ tails is exchanged for RAD51 to promote strand invasion, HDR repair and resolution of repair intermediates. **b** | Ends bound by the Ku70–Ku80 heterodimer can be repaired by NHEJ in conjunction with the DNA-dependent protein kinase catalytic subunit (DNA-PKcs), Artemis nuclease and DNA ligase 4, with the help of additional factors involved in end-modifications, gap filling and ligation[165]. This NHEJ pathway is independent of the MRE11 complex. **c** | The MRE11 complex, in conjunction with CtIP, also regulates the poorly defined alternative NHEJ (A-NHEJ) pathway, which is characterized by large deletions and the frequent use of short microhomologies[128–132,166–169]. This pathway is resection-dependent and requires several enzymatic activities for resection, flap trimming, synthesis and ligation[6]. CDK, cell division protein kinase; DSB, double-strand break.

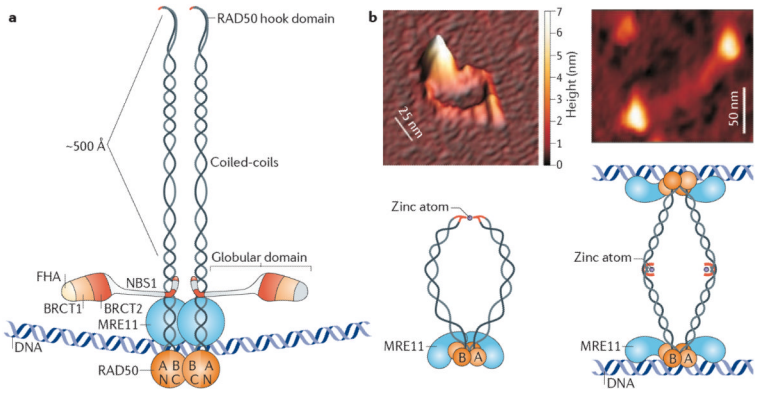


Figure 2. The MRE11 complex consists of a globular domain and extended coiled-coils

a | The MRE11 complex consists of a large globular domain, in which meiotic recombination 11 (MRE11), and Nijmegen breakage syndrome 1 (NBS1; also known as nibrin) associate with RAD50 and DNA, and extended coiled-coil domains of RAD50 in which the amino-terminal and carboxy-terminal regions of the coils associate in an antiparallel manner. At the apex of the RAD50 coils, the N-terminal and C-terminal regions fold back on themselves to form the `RAD50 hook' domain (image is not to scale). The RAD50 hook domain mediates formation of MRE11 complex assemblies. **b** | A dimeric MRE11 complex without DNA is shown by scanning force microscopy and in a schematic (left panel; the white bar represents scale along the horizontal plane, whereas the colour gradient on the right represents scale on the vertical plane). The RAD50 hook domain coordinates binding to a zinc atom. Upon DNA binding, the coiled-coil domains adopt a rigid parallel structure that bridges two DNA strands with a distances of ~1,000 Å (right panel). A, Walker A; B, Walker B; BRCT, BRCA1 C-terminal; FHA, Forkhead-associated. Images in panel **b** are reproduced, with permission, from ref. 37 © (2005) Macmillan Publishers Ltd. All rights reserved.

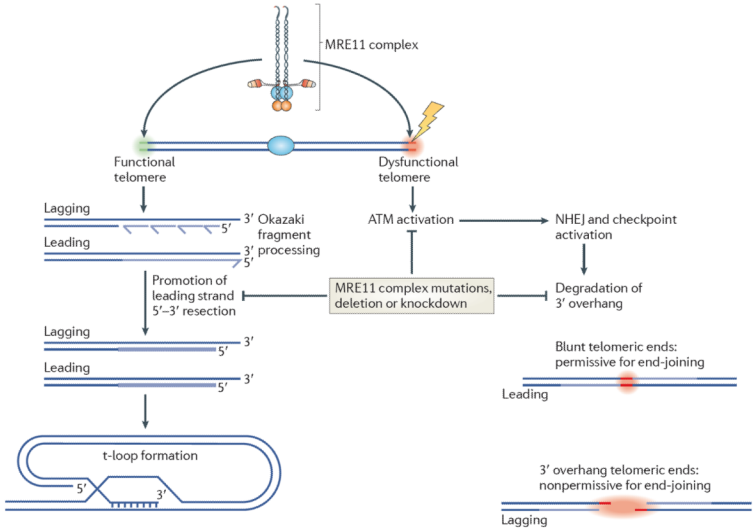


Figure 3. The MRE11 complex controls telomere homeostasis

At a functional telomere (green area, left), the MRE11 complex recognizes the newly synthesized telomeric ends and promotes their resection to create the 3′ overhang, which is a prerequisite for the formation of the t-loop — a DNA structure resembling the d-loop formed by strand invasion during homology directed repair (HDR). The t-loop is critical for normal telomere protection and maintenance[82]. The MRE11 complex also recognizes dysfunctional telomeres (red area, right), leading to activation of ataxia-telangiectasia mutated (ATM) and `repair' (that is, fusion) of the telomere through non-homologous end-joining (NHEJ; also known as classical (C)-NHEJ); this ultimately precludes chromosome segregation and causes cell death. The MRE11 complex may also influence the degradation of the 3′ overhang before, or during, the fusion process. MRE11 complex hypomorphism impairs ATM activation, which sharply reduces the frequency of NHEJ-mediated telomere fusion; this also leads to impaired telomeric end processing on both leading and lagging strands, such that residual fusions are restricted to telomeres that have been replicated by the leading strands and are blunt.

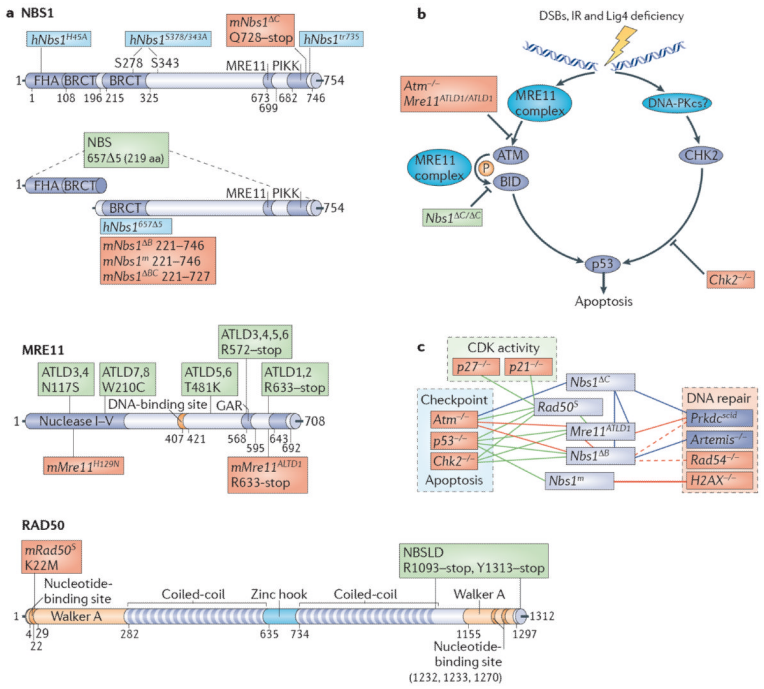


Figure 4. The MRE11 complex in human disease and mouse models

a | Domain structure of the MRE11 complex. The Nijmegen breakage syndrome 1 (NBS1; also known as nibrin), meiotic recombination 11 (MRE11) and RAD50 components of the human MRE11 complex are illustrated. Domains are indicated by name with the corresponding amino acid numbers shown. Human disease mutations are indicated in green. Mouse alleles are indicated in red and `humanized' mouse alleles in blue. This figure is drawn to scale. **b** | The MRE11 complex has multiple roles in activating apoptosis after double-strand break (DSB) exposure. The complex activates ataxia-telangiectasia mutated (ATM) and facilitates the phosphorylation of select ATM substrates, including CHK2 and BH3-interacting domain death agonist (BID), to promote p53-dependent apoptosis through the carboxyl terminus of NBS1. CHK2 signals in parallel, and is possibly activated by the DNA-dependent protein kinase catalytic subunit (DNA-PKcs). Mouse alleles affecting various steps in the signalling pathway are indicated. Alleles in red impair apoptosis in both the haematopoietic and nervous system and alleles in green affect the haematopoietic but not the nervous system. **c** | Genetic interactions between mouse MRE11 complex alleles. MRE11 complex alleles used for genetic analyses are shown in green, components of the non-homologous end-joining (NHEJ; also known as classical (C)-NHEJ) machinery are shown in blue and other DNA damage or cell-cycle regulators are shown in red. Connecting lines indicate that genetic crosses have been analysed. Blue lines indicate that no synthetic interactions were identified, green lines indicate that synthetic interactions were identified, and red lines indicate synthetic lethality. Dashed lines indicate incomplete penetrance of synthetic lethality. Interacting alleles are classified by their major functions of the DNA damage response, although they may affect other aspects of the response. ATLD, ataxia-telangiectasia-like disease; BRCT, BRCA1 C-terminal; FHA, Forkhead-associated; *h*, humanized; *m*, mouse; NBSLD, NBS-like disorder; PIKK, PI3K-related protein kinase.

Table 1

Alleles of the MRE11 complex in mice

Allele*	Allele type	Phenotypes	Refs
hNbs1$^{657\Delta5}$	Transgene, N-terminal truncation	S/G2 checkpoint defects, damage sensitivity, chromosomal instability, reduced ATM activity, impaired T cell development, subfertility	99
hNbs1^{H45A}	Transgene, point mutation	S/G2 checkpoint defects, reduced ATM activity	104
hNbs1$^{S278/343A}$	Transgene, point mutations	None described	104
hNbs1^{tr735}	Transgene, C-terminal truncation	Apoptosis defect, reduced ATM activity	104
Mre11$^{\Delta}$ (*Mre11a*$^{tm2.1Dof}$)	Conditional deletion	Embryonic lethality, reduced class switch recombination and DSB repair in B cells (CD19–Cre promoters)	89,125
Mre11^{ATLD1} (*Mre11a*^{tm1Jpt})	C-terminal truncation	S/G2 checkpoint defects, damage sensitivity, chromosomal instability, reduced ATM activation and activity, defective apoptosis, reduced fertility	126
Mre11^{H129N} (*Mre11a*^{tm1Dof})	Conditional allele with point mutation	Embryonic lethality, reduced class switch recombination in B cells (CD19–Cre), DNA repair defects and damage sensitivity	89,125
Nbs1$^{\Delta6}$ (*Nbn*^{tm1Md}) and *Nbs1*$^{-}$ (*Nbn*^{tm1Zqw})	Targeted deletion	Early embryonic lethality	95,170
Nbs1$^{\DeltaB}$ (*Nbn*^{tm1Jpt})	N-terminal truncation	S/G2 checkpoint defects, damage sensitivity, chromosomal instability, reduced ATM activity, subfertility	98
Nbs1$^{\DeltaBC}$ (*Nbn*^{tm3Jpt})	N- and C-terminal truncations	S/G2 checkpoint defects, damage sensitivity, chromosomal instability, reduced ATM activity, subfertility, apoptosis defect	110
Nbs1$^{\DeltaC}$ (*Nbn*$^{tm2.1Jpt}$)	C-terminal truncation	Apoptosis defect, S phase checkpoint defect, reduced ATM activity	105
Nbs1^{F6} (*Nbn*^{tm2Zqw}), *Nbs1*$^{\Delta6}$ (*Nbn*$^{tm1.1Md}$) and *Nbs1*$^{\Delta}$ (*Nbn*^{tm2Nus})	Conditional deletions	Reduced class switch recombination in B cells (CD19–Cre promoter), microcephaly, neuronal apoptosis, cerebellar defects and ataxia (nestin–Cre promoter), lymphopenia, T cell development defects (Lck–Cre promoter)	124, 171–173
Nbs1^{m} (*Nbn*^{tm1Xu})	N-terminal truncation	S/G2 checkpoint defects, damage sensitivity, chromosomal instability, reduced ATM activity, subfertility, cancer	97
Rad50$^{\Delta}$ (*Rad50*^{tm1Jpt})	Targeted deletion	Early embryonic lethality	94
Rad50^{ind} and *Rad50*$^{-}$ (*Rad50*^{tm3Jpt})	Conditional deletion	Chromosomal instability and death in dividing cells (MX1–Cre, PCP2–Cre promoters)	115
Rad50^{S} (*Rad50*^{tm2Jpt})	Knock-in point mutation	Embryonic lethality, bone marrow failure, cancer predisposition, activated DDR, sensitivity to topoisomerase poisons	64,139,140

ATM, ataxia-telangiectasia mutated; C, carboxy; DDR, DNA damage response; DSB, double-strand break; *hNbs1*, humanized *Nbs1; Mre11*, meiotic recombination 11; MX1, myxovirus resistance 1; N, amino; *Nbn*, nibrin; *Nbs1*, Nijmegen breakage syndrome 1; PCP2, Purkinje cell protein 2.

*Allele names are listed from the original publications and are followed by the Mouse Genome Informatics designations, which are hyperlinked to the website.