

MRN and the race to the break

Agnieszka Rupnik · Noel F. Lowndes · Muriel Grenon

Received: 14 July 2009 / Revised: 12 September 2009 / Accepted: 21 September 2009 / Published online: 28 October 2009
© Springer-Verlag 2009

Abstract In all living cells, DNA is constantly threatened by both endogenous and exogenous agents. In order to protect genetic information, all cells have developed a sophisticated network of proteins, which constantly monitor genomic integrity. This network, termed the DNA damage response, senses and signals the presence of DNA damage to effect numerous biological responses, including DNA repair, transient cell cycle arrests (“checkpoints”) and apoptosis. The MRN complex (MRX in yeast), composed of Mre11, Rad50 and Nbs1 (Xrs2), is a key component of the immediate early response to DNA damage, involved in a cross-talk between the repair and checkpoint machinery. Using its ability to bind DNA ends, it is ideally placed to sense and signal the presence of double strand breaks and plays an important role in DNA repair and cellular survival. Here, we summarise recent observation on MRN structure, function, regulation and emerging mechanisms by which the MRN nano-machinery protects genomic integrity. Finally, we discuss the biological significance of the unique MRN structure and summarise the emerging sequence of early events of the response to double strand breaks orchestrated by the MRN complex.

During the preparation of these proofs, two articles have been published describing the NBS1 crystal structure of both FHA domain and BRCT repeats of human and *S. pombe* cells. The results presented in these publications suggest an important role in phosphopeptide binding for both of these domains. Scott Williams et al. *Cell*, 139 (1) 87–99, Nbs1 Flexibly Tethers Ctp1 and Mre11-Rad50 to Coordinate DNA Double-Strand Break Processing and Repair Lloyd et al. *Cell*, 139 (1) 100–111, A Supramodular FHA/BRCT-Repeat Architecture Mediates Nbs1 Adaptor Function in Response to DNA Damage.

A. Rupnik · N. F. Lowndes (✉) · M. Grenon (✉)
Centre for Chromosome Biology, School of Natural Science,
National University of Ireland Galway,
University Road,
Galway, Ireland
e-mail: noel.lowndes@nuigalway.ie
e-mail: muriel.grenon@nuigalway.ie

Introduction

In somatic cells, the consequences of DNA damage and the resulting accumulation of genetic changes can be severe as they are associated with cellular ageing and cancer. Thus, sophisticated machineries have evolved that constantly monitor DNA structure, sense damage, repair these lesions and induce specific cellular responses including cell cycle delays, specific transcriptional programmes, apoptosis and senescence (reviewed in (Bartek et al. 2007; Harper and Elledge 2007; Kanaar et al. 2008; Fig. 1).

The Mre11-Rad50-Nbs1 (MRN) complex is a key player that functions early in the response to DNA damage, and lack of any of its components leads to embryonic lethality in mammals. MRN has a spectrum of biochemical talents: DNA binding by multiple subunits and tethering of DNA molecules through interactions between MRN complexes, incision of the DNA phosphodiester backbone through its single-stranded endonuclease activities and the ATP hydrolysis. Genes that encode components of the Mre11/Rad50/Xrs2 (MRX) complex were originally isolated in *Saccharomyces cerevisiae* during genetic screens for mutants hypersensitive to DNA damaging agents (Rad50: Radiation sensitive 50 (Game and Mortimer 1974), Xrs2/Ngs1: X-ray sensitive 2 or Nitroguanidine sensitive 1 (Ivanov et al. 1992) and for mutants defective in meiotic recombination (Mre11: meiotic recombination 1 (Ajimura et al. 1993). Hypomorphic mutations in the components of the human MRN complex lead to human genetic disorders characterised by hypersensitivity to ionising radiation, genome instability and immunodeficiency. Cancer predisposition has been associated with Nijmegen breakage syndrome (NBS; Dzikiewicz-Krawczyk 2008; The International Nijmegen Breakage Syndrome Study Group 2000) caused by mutations in NBS1 gene. In contrast, mutations in MRE11 have only been found in sporadic tumours (Wang

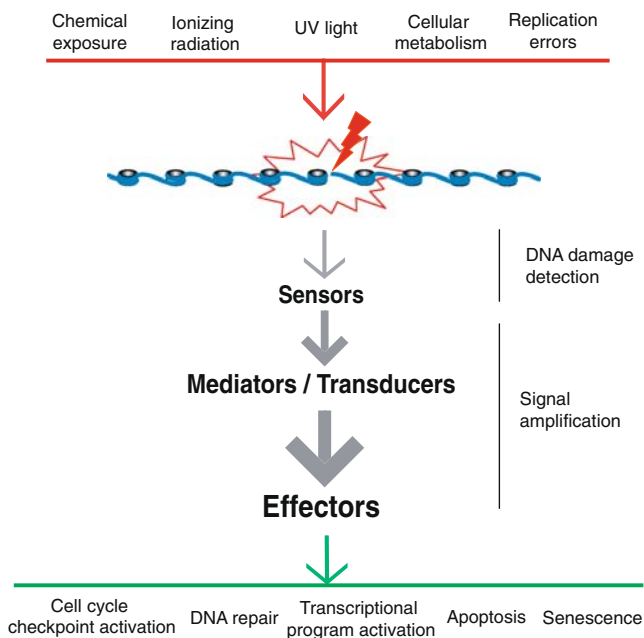


Fig. 1 The DNA damage response cascade. DNA damage caused by endogenous or exogenous sources is recognised by sensor proteins, which activate a signalling cascade. The signal is amplified by mediators/transducers and finally reaches the effector proteins, which are involved in triggering the cellular responses to DNA damage

et al. 2004). Only a few cases of patients with mutations in the *RAD50* gene have been reported to date and have not yet been associated with a defined human phenotype (Heikkinen et al. 2006; Tommiska et al. 2006). Ataxia-telangiectasia-like disorder (ATLD) and NBS caused by mutations in *MRE11* and *NBS1*, respectively, illustrate the importance of the MRN complex in the cellular response to DNA damage in humans (Stewart et al. 1999; Varon et al. 1998).

A multifunctional complex essential for vertebrate life

The MRN complex has been implicated in multiple eukaryotic functions, all key for maintenance of genome integrity and mainly related to the response to double strand breaks (DSBs). These functions include: both principal pathways of DSB repair, telomere maintenance, checkpoint signalling, meiotic recombination and, during DNA replication, responding to stalled replication forks and resolution of DNA hairpins (recently reviewed in Borde 2007; Slijepcevic 2006; Williams et al. 2007). The role in meiotic recombination was first identified in budding yeast and is conserved in *Schizosaccharomyces pombe*, *Coprinus cinereus*, *Caenorhabditis elegans* and *Arabidopsis thaliana* and likely to be conserved in mammals (reviewed in Borde 2007). Budding yeast Mre11 is required both for meiotic DSB formation as well as their subsequent processing, as its endonuclease activity is required to remove covalently bound Spo11 (a topoisomerase-like

enzyme) from the induced DSB. The former function is not always conserved and seems specific to budding yeast and *C. elegans*. Mammalian Mre11 is also believed to be required for meiotic DSB processing as the meiotic Spo11-dependent step of DSB processing is conserved in mammals (Baudat and de Massy 2007). Despite several meiotic defects reported in MRN hypomorphic animal models, the essential nature of this complex in higher cells will require specific knock out of this complex in meiotic tissue to assess the precise role of MRN in meiotic recombination.

Although the MRX complex is not required for viability in yeast, the essentiality of MRN function in mammals complicates study of its role in the DSB response. Note that targeted disruptions of the *Mre11* (Yamaguchi-Iwai et al. 1999), *Rad50* or *Nbs1* (S. Takeda, personal communication) genes are also lethal in the DT40 chicken B-lymphocytic cell line suggesting that the importance of MRN for cellular viability might extend to all vertebrates. Mice defective in *Mre11*, *Rad50* or *Nbs1* function die early in embryonic development, demonstrating that these proteins are essential for mammalian development and viability (Buis et al. 2008; Dumon-Jones et al. 2003; Luo et al. 1999; Xiao and Weaver 1997; Zhu et al. 2001). Hypomorphic mutations in the human *MRE11* and *NBS1* genes causes ATLD and NBS, respectively, and results in genome instability (Taylor et al. 2004; The International Nijmegen Breakage Syndrome Study Group 2000). Two patients have been reported with defects in *RAD50* (Heikkinen et al. 2006; Tommiska et al. 2006). In an elegant study using conditional mouse cell lines, the essential function of MRN has recently been defined as its nuclease activity (Buis et al. 2008). This study, together with that of Williams et al. (2008), also demonstrates that the nuclease activity of the complex is only required for DNA repair and not for DSB signalling in checkpoints nor for telomere metabolism, a point that was ambiguous from budding yeast studies (see below). This discovery suggests that mammalian cells, particularly embryonic, absolutely require MRN nuclease activity in order to cope with spontaneous DSBs that occur during DNA replication, a requirement that would be particularly important for the rapid cycling that occurs during early stages of development.

Structure, functions and post-translational regulation of the three players

Our understanding of the structure of the MRN complex has dramatically increased during the last decade. We now have clearer insights into how MRN interacts with DSBs and how it manipulates broken DNA. In addition, emerging data suggest an important role for post-translational modifications in regulating the activities (and their cell cycle timing) of the complex.

The MRE11 nuclease

The MRE11 protein is known to dimerize (via a hydrophobic cluster localised between two alpha helices of each MRE11 units that is reinforced by a salt bridge) to form a U-shaped dimer (Fig. 2; Hopfner et al. 2001; Williams et al. 2008). This formation of homodimer is needed for basic MRN function, and the conserved residues, allowing for dimer-

ization, are required for MRE11 DNA binding in vitro, and MRE11 repair activity in vivo (Williams et al. 2008). MRE11 dimer interacts with RAD50 via a domain localised in the C-terminal third of protein, flanked by two DNA binding regions (residues 407–421 and residues 643–692; Fig. 2a). It also interacts with NBS1, via an N-terminally located interaction domain. Interestingly, mutations that impair NBS1 binding to MRE11 and cause ATLD cluster to

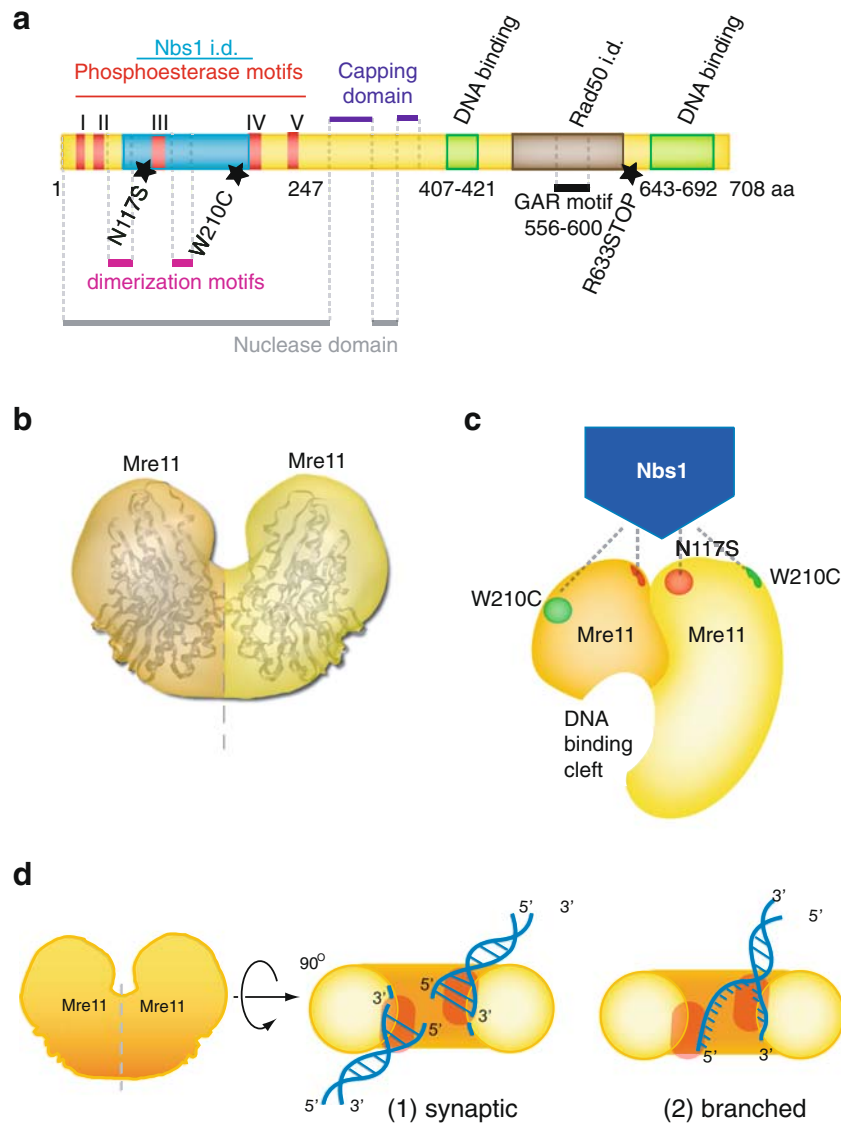


Fig. 2 Structure of Mre11. **a** The domain structure of Mre11: the amino-terminal region of Mre11 contains the phosphoesterase motifs (marked with red rectangles) within the nuclease domain. This region also interacts with Nbs1 (blue rectangles). Located at the carboxyl-terminal region is a Rad50 interacting domain (Rad50 i.d. indicated as a brown rectangle) containing the GAR motif. It is flanked by two DNA-binding motifs (green rectangles). The “capping domain” in the centre of the protein is responsible for Mre11 conformational changes upon DNA binding and partially overlaps with the “nuclease domain”. Indicated with stars are mutations found in ataxia-telangiectasia-like disorder (ATLD). Finally, the Mre11 molecule can dimerise via

indicated dimerisation motifs. **b** The ribbon structure of Mre11 homodimer (adapted from Williams et al. 2008): two Mre11 molecules (indicated using two shades of yellow) interact via the dimerisation motifs and form a U shaped dimer. **c** The Nbs1 protein interacts with the Mre11 dimer via a surface opposite to the DNA binding cleft (see text for further description). Indicated on the schematic are residues causing ATLD, which also abrogate the Nbs1-Mre11 interaction. **d** The Mre11 dimer can interact with (1) two dsDNA ends forming a “synaptic” complex or (2) a single ssDNA/dsDNA end forming a “branched” complex. See main text for a detailed description

a single MRE11 dimer surface opposite the DNA binding cleft (see Fig. 2c and Hopfner et al. 2001; Williams et al. 2008). For example, the N117S mutation in human MRE11 (leading to ATLD) and its analogous yeast mutation N113S (causing sensitivity to IR) both fail to interact with NBS1 and Xrs2, respectively (Williams et al. 2008).

Five conserved motifs in MRE11 found in its amino-terminal region are responsible for its nuclease activity (Fig. 2a; Moreau et al. 1999; Stracker et al. 2004; Williams et al. 2008). In vitro studies have demonstrated that MRE11 has ssDNA endonuclease, 3'→5' ssDNA exonuclease, dsDNA exonuclease and hairpin opening activities (Furuse et al. 1998; Paull and Gellert 1998; Trujillo and Sung 2001; Trujillo et al. 1998; Usui et al. 1998). Mutations in the second or fourth nuclease motif of yeast Mre11 result in defective recombinational DNA repair (Bressan et al. 1998). The nuclease activities of human MRE11 are dependent on the presence of ATP, manganese and NBS1 (Paull and Gellert 1999). In addition, RAD50 was found to stimulate the nuclease activity of human MRE11, but this was not the case for budding yeast Rad50 (Paull and Gellert 1998, 1999; Trujillo and Sung 2001). The in vivo relevance of Mre11's nuclease activities for DSB signalling is an interesting question as, although MRN is required for signalling, in vivo DSBs are processed with the opposite polarity (5'-3', resulting in ssDNA tails with 3' ends) to the 3'-5' exonuclease activity of Mre11 in vitro (Hopkins and Paull 2008; Nakada et al. 2004). This conundrum has been resolved recently by two major observations, firstly, the demonstration that the role of MRN in initial checkpoint signalling does not depend on its nuclease activity and, secondly, by the identification of exonucleases of the correct polarity involved in DSB resection in vivo (see next section).

Two key residues, neither of which impair Mn²⁺ binding, have been identified that separate the endonuclease and exonuclease activities of MRE11. The *S. pombe* *Mre11-H68S* mutant (H68, located in the second nuclease motif, is required for phosphate rotation before cleavage and is homologous to *furiosus* H52) lacks the 3'-5' exonuclease activity; whereas, H134S (H134, located in nuclease motif III, is required for interaction with an intermediate DNA substrate and is homologous to *furiosus* H84) lacks both the 3'-5' exonuclease and endonuclease activities (Williams et al. 2008). Analysis of these mutants demonstrates that in mitotic cells, the exonuclease defective mutant is proficient for DSB repair, whereas the mutation that additionally affects endonuclease activity is repair defective. Importantly, the lack of these MRE11 enzymatic activities does not affect the role of MRE11 in DSB-specific checkpoint signalling. Furthermore, this recent study ends the controversy resulting from the usage of several yeast mutants that have only recently been discovered affecting both complex integrity and its nuclease function (Krogh et al. 2005). In addition, the data obtained by

Williams et al. are in agreement with the phenotype of budding yeast H125S (equivalent to *S. pombe* H134S) which is proficient for complex formation and nuclease deficient but can still initiate checkpoint activation in response to DNA damage (MG and NL unpublished data; Clerici et al. 2006; Krogh et al. 2005).

The Mre11 dimer is able to form two types of complexes with DNA, depending on the DNA structure (see Fig. 2b–d). The first type, termed a “*synaptic DNA complex*”, is formed between Mre11 and the ends of oligonucleotides containing mostly double-stranded DNA with short 3' overhangs that mimic the DSB. The second type, termed a “*branched DNA complex*”, is created between Mre11 and ‘Y-shaped’ oligonucleotides containing regions of both double- and single-stranded DNA oligonucleotides that resemble a stalled replication fork. Binding to dsDNA appears to be mediated primarily by the minor groove of DNA, which has less potential for sequence specific interactions. Surprisingly, once bound, the DNA is sculpted to a different shape helping to accommodate the DNA backbone as required for the exonuclease activity of Mre11. The phosphodiester backbone is distorted by a wedge-like structure formed by amino acid residues located on a loop that recognises the DNA. Thus, the minor groove of the DNA double helix is widened by 2Å and bent towards the major groove by 10°. Furthermore, the DNA is translocated toward the active site of the Mre11 dimer by approximately two third base pairs. This is achieved by rotation of a part of Mre11 molecule, including the so-called capping domain, which would otherwise occlude access to the active site (see Fig. 2a). Another part of each Mre11 subunit, containing the phosphoesterase motifs, forms an active site termed the “*nuclease domain*”, which accesses individual nucleotides. The endonuclease activity allows for opening of the DNA double helix, whereas the 3'-5' exonuclease activity facilitates the production of ssDNA substrates suitable for RPA and hSSB1 proteins (Williams et al. 2008 and see below).

The RAD50 coiled-coil protein

RAD50 belongs to the structural maintenance of chromosome (SMC) family of proteins, involved in chromosome condensation and sister-chromatid cohesion in eukaryotes. The N- and C-termini contains Walker A and B motifs required for ATPase activity as well as being responsible for DNA binding and partial unwinding (Fig. 3a and Chen et al. 2005a; de Jager et al. 2002; Hopfner et al. 2000a, b; Raymond and Kleckner 1993). In *S. cerevisiae*, replacement of the conserved lysine residue within the Walker A motif with alanine, glutamate or arginine results in the same DNA damage sensitivity as well as DSB repair (Chen et al. 2005a) and meiosis (Alani et al. 1990) defects observed in *rad50Δ* deletion mutants. In vitro yeast Rad50 proteins harbouring

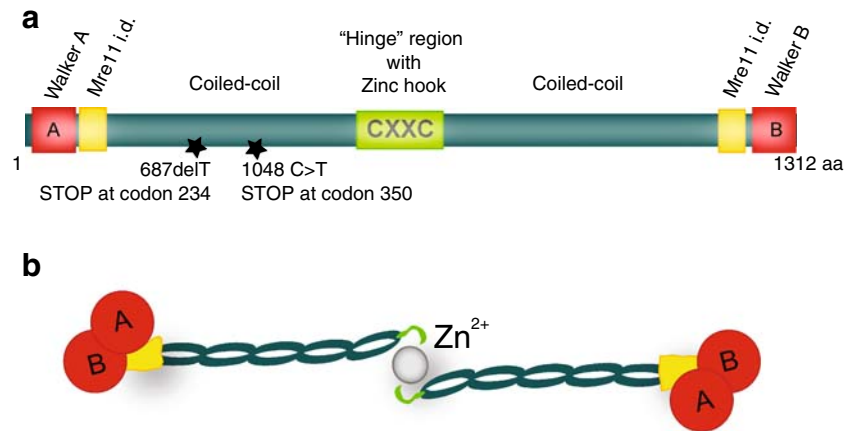


Fig. 3 Structure of Rad50. **a** The domain structure of Rad50: Walker A and B motifs, responsible for Rad50 ATPase activity, are located at both ends of protein (*red squares*). The Mre11 protein interacts with Rad50 via a region localised in close proximity to the Walker motifs (Mre11 i.d. indicated as *yellow rectangles*). The centre of Rad50 is formed by two coiled-coil regions (*dark green*) that are linked by a “hinge” containing a Zinc-hook (*light green rectangle*). **b** Schematic showing dimerisation of Rad50: the Rad50 protein folds back on itself

Walker A and B mutations are defective in ATPase, DNA binding, ATP-dependent DNA unwinding and ATP-stimulated endonuclease activities (Chen et al. 2005a).

Outside of the Walker A and B motifs, the central region of RAD50 is composed of a large coiled-coil structure that can fold back on itself, via a “hinge” region (Hopfner et al. 2001). This intra-molecular interaction of the RAD50 molecule allows both Walker motifs to directly interact, forming a globular ABC transporter-type ATPase domain. Furthermore, co-expression and electron microscopy revealed that MRE11 binds to the coiled-coil region near the ATPase domain (Fig. 3a, b). The “hinge” region at the end of the coiled-coil arm contains a zinc hook, composed of the CXXC motif, which allows RAD50 molecules to dimerize (Hopfner et al. 2002). Mutations of Cysteine 690 of the yeast Rad50 hook motif confers radiation, MMS and hydroxyurea sensitivity, telomere shortening and impaired interaction with itself and also with Mre11 (Hopfner et al. 2002; Wiltzius et al. 2005). However, interaction between Rad50 molecules appears to involve more than a functional hook region as disruption of this region in human RAD50 does not fully abrogate self-interaction (Cahill and Carney 2007). Together, these results suggest an architectural role for the Rad50 coiled-coils in forming metal-mediated bridging complexes between two DNA-binding heads. Importantly, the resulting assemblies have appropriate lengths and conformational properties to link sister chromatids in homologous recombination and DNA ends in non-homologous end-joining (Fig. 5c).

The globular ABC transporter-type ATPase domain is found mainly in integral membrane proteins (e.g. the cystic fibrosis transmembrane regulator (CFTR)) that gate ions or

via a “hinge” region resulting in the formation of a long flexible arm by the coiled-coil regions. The Rad50 molecules can interact with each other, forming dimers, using the Zinc hook ending the hinge region. The interaction between two Rad50 molecules occurs via the “hinge” regions in the presence of a Zn^{2+} ion. On the opposite end of the Zinc hook, a globular head is formed by the Walker motifs A and B and Mre11 interacting domains

small molecules across membranes (Holland and Blight 1999; Lewis et al. 2004). Similarly to other ABC transported superfamily members, RAD50 has recently been shown to possess an additional activity, the reversible transfer of a phosphate group from ATP to AMP to generate two ADP molecules (Bhaskara et al. 2007). Specific RAD50 mutants (equivalent to a mutation found in CFTR that causes severe cystic fibrosis) defective in this adenylate kinase (AK) activity, but proficient in its ATPase activity, form the MRN complex normally but are severely defective in RAD50 functions. It is believed that the ATPase and the adenylate kinase (AK) activities of RAD50, which act competitively, both regulate conformational (allosteric) changes in the RAD50 protein important for various activities (DNA binding, unwinding and/or tethering) of the MRN complex in vivo. However, the precise details of how each nucleotide bound state (ATP or AMP) affect the catalytic domain and coiled-coil domains of RAD50 in multimeric MRN complexes remains to be determined.

The NBS1 regulator protein

Although NBS1 does not have any known enzymatic activities, it clearly regulates MRN functions. Firstly, it is essential for the nuclear localisation of Mre11 and Rad50 (Carney et al. 1998; Shima et al. 2005; Tsukamoto et al. 2005; Cerosaletti et al. 2000; Desai-Mehta et al. 2001), and the sensitivity of *xrs2Δ* cells to MMS and HU can be rescued by fusing a nuclear localisation signal to Mre11 (Tsukamoto et al. 2005). Secondly, NBS1 is also needed for the rapid focal assembly of MRN complexes at sites of DNA damage (Horejsi et al. 2004; Kobayashi et al. 2002; Lee and

Paull 2004). Thirdly, Nbs1 stimulates the activities of Rad50 and Mre11 as it is required for nucleotide-dependent DNA binding by the MRN complex and the ATP-dependent DNA unwinding (Lee et al. 2003; Paull and Gellert 1999). Several Nbs1 regions have been identified that are implicated in the response of cells to DSBs (Fig. 4a).

The fork head-associated (FHA) domain (first identified in nuclear kinases and transcription factors; Hofmann and Bucher 1995) is present at the N-terminus of Nbs1 and is followed by two tandemly repeated BRCA1 carboxyl-terminus motifs (BRCT; first identified in the BRCA1 tumour suppressor, Bork et al. 1997; Fig. 4a, c). Interest-

ingly, until recently, only one BRCT domain was thought to be present in NBS1. Becker et al. suggested the presence of a second BRCT domain, in NBS1, as a result of a series of sequence alignments and protein structure prediction (Becker et al. 2006). The presence of a pair of BRCT domains is not surprising. In fact, many proteins involved in DNA damage checkpoint control and DNA repair have been shown to contain, typically, multiple copies of the tandem BRCT motif (Callebaut and Morion 1997). For example: BRCA1, mediator of the DNA damage checkpoint 1 (MDC1), 53BP1, CtIP and budding yeast Rad9 contain a tandem pair of BRCT motifs. Other proteins

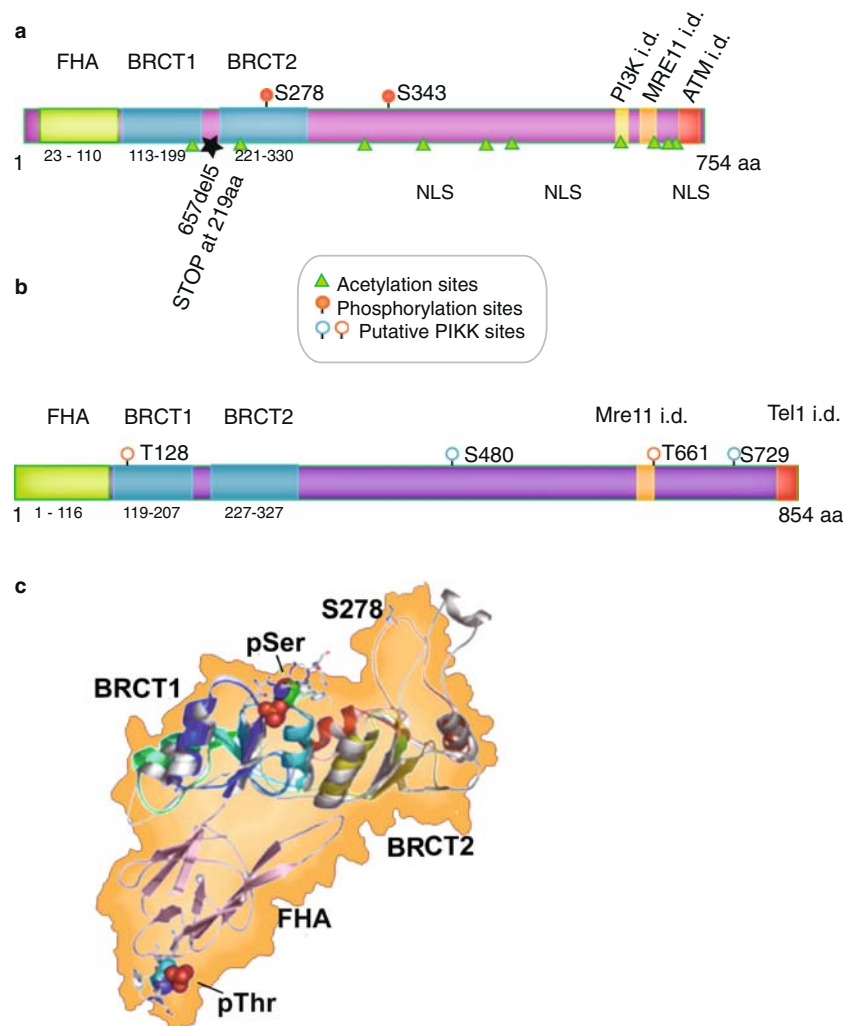


Fig. 4 Structure of Nbs1/Xrs2. **a** The domain structure of Nbs1: the phosphopeptide binding fork head-associated domain (*green rectangle*) is followed by a tandem BRCT motif (*blue rectangles*). Three short motifs responsible for Nbs1 interactions with PI3K, Mre11 and ATM kinase are localised at the carboxyl end of the protein (*respectively indicated by yellow, orange and red rectangles*). Indicated with *stars* are mutations found to cause Nijmegen Breakage Syndrome (NBS). Two serine residues (indicated with *red circles*) become phosphorylated in response to DNA damage. Nuclear localisation signals are indicated by NLS. **b** The domain structure of Xrs2:

the domain architecture of Xrs2 resembles that of Nbs1. At the amino terminus, a FHA domain is followed by a newly identified tandem BRCT motif. Xrs2 interacts with Mre11 and Tel1 via short motifs localised at the carboxyl-terminus. Four putative PIKK phosphorylation sites are indicated (*red and blue circles*). **c** Three dimensional structure of the FHA-BRCT1-BRCT2 module of Nbs1: the *ribbon representation of the module structure* indicates regions that interact with phospho-Serine and phospho-Threonine containing peptides (shown as *sticks*). Adapted from Becker et al. 2006

contain multiple pairs (e.g. fission yeast Rad4/Cut5, which has four motifs arranged in two pairs and TOPBP1 which has eight, arranged in four pairs (reviewed in Callebaut and Mornon 1997; Rodriguez and Songyang 2008). Both the FHA and tandem BRCT domains have been shown to have domains specific for phospho-peptides that mediate interaction with phosphorylated DNA damage response (DDR) proteins (Bork et al. 1997; Durocher et al. 1999; Hofmann and Bucher 1995; Li et al. 2000; Mahajan et al. 2008; Yu et al. 2003).

The FHA and the BRCT domains of NBS1 are required for its interaction with γ H2AX (H2AX phosphorylated on serine 139) and phosphorylated MDC1 important for the DDR (Chapman and Jackson 2008; di Masi et al. 2008; Kobayashi 2004; Kobayashi et al. 2002; Wu et al. 2008). The FHA domain is also been reported to be required for an interaction with ataxia-telangiectasia-related (ATR; Olson et al. 2007; Shimada et al. 2009). In yeast, the FHA domain is necessary for non-homologous end joining (NHEJ) efficiency and specific interaction with Lif1, the cofactor of Ligase IV (Matsuzaki et al. 2008; Palmboos et al. 2005, 2008; see Fig. 4b). This function might not be well conserved as MRN does not seem to be necessary for NHEJ in fission yeast nor in *Xenopus* (Di Virgilio and Gautier 2005; Manolis et al. 2001).

Interestingly, most NBS patients share a five nucleotide deletion 657del5 (also termed the Slavic mutation) causing the premature truncation of the protein and expression of two NBS1 fragments, at lower level than the endogenous full length protein in NBS1 proficient cells (Maser et al. 1997). Thus, the mutant protein is expressed as 26 kDa amino-terminal part, consisting of the FHA domain with the first BRCT motif, and a 70-kDa carboxyl-terminal part, containing the second BRCT motif and the remaining carboxyl region of NBS1. Although the carboxyl terminal part of NBS1 appears to be sufficient for cell viability, the lack of a contiguous amino-terminal FHA domain and BRCT1 motif leads to sensitivity to DNA damaging agents, such as IR, and radio-resistant DNA synthesis. The reduced expression levels of the two expressed NBS1 fragments probably contributes to this phenotype since higher expression level of the C-terminus of NBS1 partially rescues the IR sensitivity of NBS cells (Desai-Mehta et al. 2001). These phenotypes are consistent with those observed in other organisms in which the FHA and BRCT1 have been mutated.

The extreme carboxyl terminus of NBS1 contains short motifs responsible for its interactions with MRE11 (residues 683–691), ataxia-telangiectasia-mutated (ATM; residues 734–754) and a more recently identified interaction with p110 α , the catalytic subunit of PI 3-kinase (residues 653–669; Carney et al. 1998; Chen et al. 2008a; Desai-Mehta et al. 2001; Falck et al. 2005; Matsuura et al. 1998; Varon et al. 1998). Direct physical interaction between NBS1 and MRE11 is required for cell survival after IR. However,

neither nuclear localization nor focal accumulation of NBS1 after IR (ionising radiation-induced foci (IRIF)) required the ability of NBS1 to interact with MRE11 (Desai-Mehta et al. 2001; Tauchi et al. 2001).

Falck et al. identified short motifs at the carboxyl-termini of NBS1, ATRIP and KU80 that serve as motifs capable of docking the relevant phosphoinositide-3 kinase-related (PIK) kinases: ATM, ATR and DNA-PK_{cs}, respectively. Deletion of the conserved ATM-interacting domain (ATM i.d., Fig. 4a) of NBS1 impaired ATM recruitment to the sites of DNA damage and delayed kinase activation and ATM-dependent DNA damage signalling pathways (Falck et al. 2005). Similar observations with the equivalent region of budding yeast Xrs2 (see Fig. 4b) or fission yeast Nbs1 indicate evolutionary conservation of this function (Nakada et al. 2003).

The recent demonstration that NBS1 can interact with and activate PI3-kinase (important in oncogenic signalling) is intriguing, as overexpression of *NBS1*, detected in the advanced stages of neck and head cancers, appears to induce PI3-kinase activation. These findings may implicate NBS1 in a direct role in transformation and tumourigenesis (Chen et al. 2008b). Note that there is no evidence for a similar PI3-kinase binding motif in lower eukaryotes.

Three components, many complexes

The stoichiometry of the MRN complex has been addressed in hydrodynamic experiments. Surprisingly, gel filtration experiments indicated that the size of the MR (MRE11-RAD50 only) complex was greater than 1.3MDa. Moreover, MRE11 alone did not elute in the range of predicted molecular weight of 80 kDa but instead formed a higher order structure of about 350 kDa (Paull and Gellert 1998). These results were in agreement with yeast two-hybrid data and genetic studies supporting Rad50-Mre11 and Mre11-Mre11 interactions (Johzuka and Ogawa 1995; Nairz and Klein 1997) and suggest that Mre11 can form homo-dimers and be present in the MRN complex as either a dimer or tetramer. In addition, the formation of stable Mre11 homodimers, in the absence of Rad50, was later confirmed using analytical ultracentrifugation experiments (Lee et al. 2003). Trujillo et al. have reported that human NBS1 appears to be sub-stoichiometric in regards to MRE11 and RAD50 (both of which eluted from the purification column with 1:1 molar ratios). Therefore, NBS1 either interacts with only a fraction of MR complexes or is of a lower stoichiometry in the MRN complex (Paull and Gellert 1998; Trujillo et al. 1998). Finally, X-ray crystallography of *Pyrococcus furiosus* Rad50 with a nuclease-proficient fragment of Mre11 (residues 1–342 and lacking the C-terminal 82 residues) led to the conclusion that the *P. furiosus* MR complex is formed of two Mre11 and two Rad50 molecules (note that archaean Mre11 complex does not contain an Nbs1 subunit;

Hopfner et al. 2001). Consistent with the above, the purified MRX complex from yeast has a stoichiometry of 2:2:1, resulting in a total size of approximately 560 kDa (Chen et al. 2001; Ghosal and Muniyappa 2007). However, the predicted size for the yeast M2/R2/N is still significantly smaller than the 1.3 MDa suggested by gel filtration experiments performed with recombinant purified proteins (Paull and Gellert 1998).

Observations consistent with a yeast complex lacking Mre11 suggest the possibility of the formation of RN sub-complexes (Ho et al. 2002). This hypothesis has recently been supported by a study using scanning force microscopy. van der Linden et al. (2009) reported that a structure similar to the big globular head and flexible coiled-coil arms of the M2R2 complex can form between RAD50 and NBS1 molecules, and this basic shape is maintained upon addition of MRE11. Their data support the possibility of complexes composed of a single RAD50 molecule (R), a RAD50 dimer (R2) or even RAD50 multimers (Rx, see Fig. 5). Interestingly, NBS1 and MRE11 availability affects the type of complex formed (Fig. 5a). When both MRE11 and NBS1 proteins are present in equal amounts, the complex formed consists of M2R2N or M2RxN (where x is greater than 2). However, in abundance of NBS1 compared to MRE11, a complex containing RAD50 multimers is preferably formed. It is possible that in vivo MRN can also interact pleiotropically with itself forming complex higher order structures, perhaps explaining past hydrodynamic analyses (see above). Thus, the observed ≥ 1.3 MDa complex could be explained, at least in part, by various higher order MRN complexes, in addition to interactions with other proteins (Fig. 5).

Structural data published by Hopfner and subsequently by Moreno-Herrero revealed that the Mre11 dimer binds to Rad50 at the base of globular ABC ATPase domain (Fig. 3 and Hopfner et al. 2001; Moreno-Herrero et al. 2005). The 50-nm long Rad50 arms can adopt two conformations (Fig. 5). The first one is an “open” conformation, in which the arms do not touch each other. The second conformation is called a “closed” conformation, where the zinc hooks of the two Rad50 molecules interlock with one another forming a ring-like structure, which resembles that of SMC (Hopfner and Tainer 2003). Moreno-Herrero et al. (2005) reported that the complex, when not bound to DNA, was able to switch between the open and closed conformation as a function of time. The basic structure of the M2R2 complex, with big globular “head” (formed by Mre11 molecules and the ATPase/AK domains of Rad50) and flexible Rad50 coiled-coil “arms”, remains unchanged when the third complex member, Nbs1, binds to Mre11 (Moreno-Herrero et al. 2005; Fig. 5).

Striking changes to the structure of the MRN complex take place upon DNA binding (Fig. 5c). The flexible Rad50

arms become more rigid and parallel to each other, albeit retaining some flexibility, and protrude away from the DNA-binding globular head domain. The Zinc hook in the hinge region allows the interaction of different MRN complexes together. This interaction is crucial to the physiological roles of MRN mitotic or meiotic recombination by bridging of DNA ends, sister chromatid or chromosomes together. These structural changes to the arms are dynamic, as MRN can dissociate from DNA and return to its flexible open or closed loop conformations (Moreno-Herrero et al. 2005). Interestingly, the DNA-bound complex was observed to move along the DNA following the contour of the DNA molecule. Thus, the MRN complex is able to slide along the DNA, which would allow further MRN molecules to bind DNA ends (Moreno-Herrero et al. 2005), an ability that might also be useful for the initial steps of DNA repair. Note that it remains unclear whether the ability of MRE11 to translocate DNA ends towards the MRE11 active site is involved in MRN sliding along the DNA. In addition, further studies are required to solve the mystery of how the RAD50 molecule (together with MRE11 and possibly NBS1) regulates the complex conformational changes observed between its ATPase/AK head and its 50 nm extending arms.

Post-translational modifications of the complex

Regulation of MRN by post-translational modifications adds still further complexity to the structure and function of the MRN complex. The three members of the complex are phosphorylated after damage (Matsuoka et al. 2007; Mu et al. 2007), but the role of the phosphorylation sites have only been carefully studied in NBS1 (see below). ATM-dependent phosphorylation of NBS1 and Tel1-dependent phosphorylation of Xrs2 and Mre11 has been reported in response to DNA damage (D’Amours and Jackson 2001; Dong et al. 1999; Nakada et al. 2003; Usui et al. 2001; Wu et al. 2000). MRE11 phosphorylation is abrogated in NBS cells, which also lack focal recruitment of MRE11 after ionising radiation (MRE11 IRIFs), indicating that both MRE11 phosphorylation and foci formation are NBS1 dependent (Dong et al. 1999). NBS1 phosphorylation was reduced in ATM-deficient cells after IR and in ATR deficient cells in response to UV, MMS or HU (Costanzo et al. 2001; Wu et al. 2000). Sites of ATM-dependent phosphorylation of NBS1 have been mapped to serine 278 and 343 after IR (see Fig. 4a and Gatei et al. 2000; Lim et al. 2000). These serine residues are well conserved in vertebrates with the exception of mice, where serine is conservatively substituted with threonine (Tsuchi et al. 2001, 2002). They are also crucial for proper activation of the intra-S phase checkpoint in response to DNA damage, pointing to a putative function for the second BRCT motif

in this checkpoint. Recently, S278 and S343 have been shown to be critical residues regulating p53-independent apoptosis via an interaction with Ku proteins (Iijima et al. 2008a). Budding yeast Xrs2 contains four putative PIK kinase sites (S/T-Q), but no sites of phosphorylation have yet been identified in any fungal homologues of Nbs1.

Interestingly, acetylation of NBS1 has been shown to function antagonistically to S343 phosphorylation. Yuan et al.

(2007) have determined that NBS1 can be acetylated by PCAT and p300 histone acetylases (HATs) on ten out of its 70 lysine residues with the histone deacetylase SIRT1 exclusively regulating de-acetylation. SIRT1 interacts with NBS1 (residues 572–754) when it is bound to MR; an interaction that is not affected by IR. Interestingly, hyper-acetylation of Nbs1, observed in response to over-production of HAT or by down regulation of SIRT1, diminished

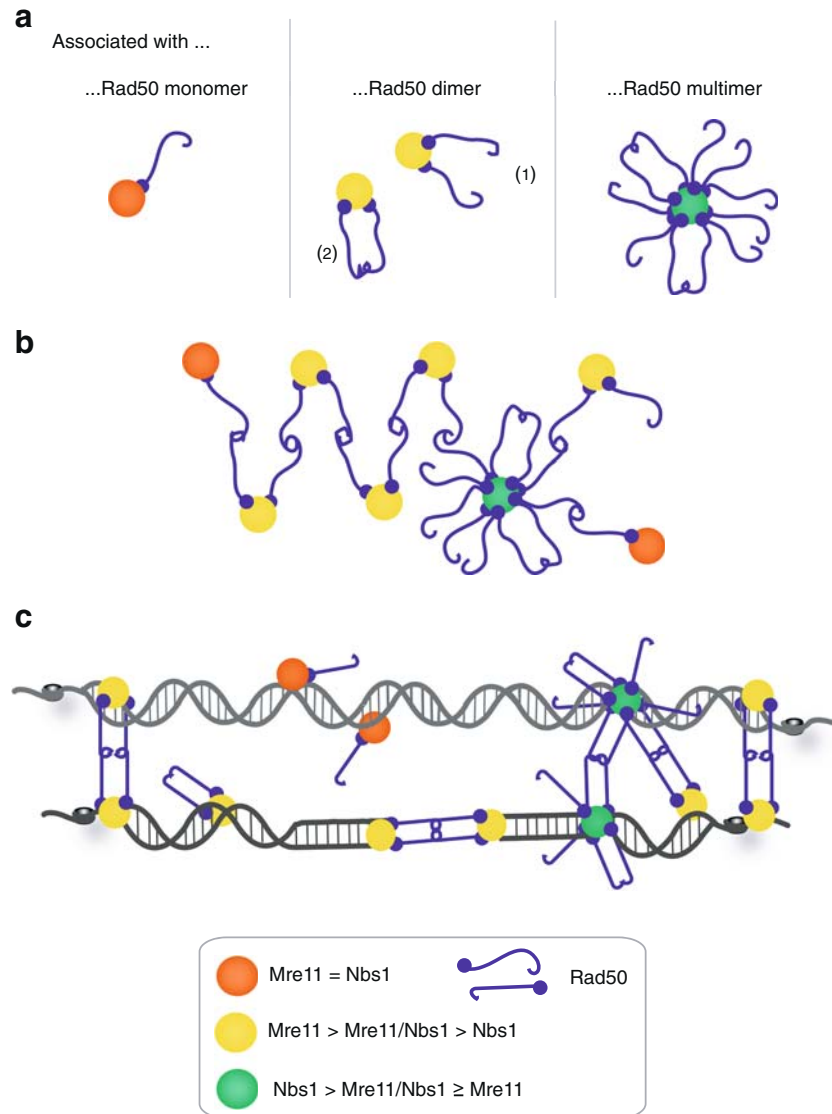


Fig. 5 Formation of the MRN complexes: **a** The MRN complex can be formed with one, two or multiple Rad50 molecules depending on the molar ratio of Nbs1 and Mre11. If both Nbs1 and Mre11 are present in equimolar amounts, then a complex containing a single Rad50 molecule is most likely to form (*red circle*). If Mre11 is more abundant than Nbs1, the complex is most likely to contain two Rad50 molecules (*yellow circle*). Finally, if Nbs1 is more abundant than Mre11, Rad50 multimers are most likely to be found in the MRN complex (*green circle*). An MRN complex with a double-Rad50 molecule exists in two conformations: (1) “open” when the Rad50 coiled-coil arms do not interact (2) “closed” when the Rad50 coiled-coil arms interact via their

hinged region forming a ring-like structure. **b** MRN may form a complex protein network. Interactions between the Rad50 molecules allow for aggregation of the MRN complex. **c** The conformation of the MRN complex changes upon DNA binding: when the MRN complex is not in contact with DNA the Rad50 arms remain flexible (see panel **a** and **b**). Association with DNA triggers conformational changes and Rad50 arms become more rigid and parallel to one another, facilitating the formation of a bridge bringing DNA molecules in close proximity. The Zinc hook ending the hinge region of Rad50 is a key element that contributes to link broken DNA ends and sister chromatids together, facilitating DNA processing and repair

activation of NBS1 by DNA damage-induced, ATM-dependent phosphorylation on Ser 343. Thus, the balance between acetylation and phosphorylation of NBS1 regulates its role in the intra-S checkpoint although the precise mechanism remains poorly understood (Yuan et al. 2007).

Methylation of Mre11 has also been shown to regulate MRN intra-S checkpoint function (Boisvert et al. 2005b). Mass spectrometric analyses identified nine methylated arginines in a glycine- and arginine-rich region (GAR domain, residues 566–600) of Mre11 (Boisvert et al. 2005b). The first six arginines of the motif are the most important for the functionality of this region (Dery et al. 2008). Additionally, western blot analysis shows that this modification is constitutive. In mouse, protein arginine methyl transferase 1 (Prmt1) is required for Mre11 methylation, as modification is absent in Prmt1^{-/-} ES cells (Boisvert et al. 2005a). Interestingly, both Prmt1 and Nbs1 proteins localise in PML nuclear bodies, structures involved in several cellular mechanisms including DNA repair (Bernardi and Pandolfi 2007). Methylation of human MRE11 is likely to occur before MRN complex formation, as PRMT1 does not interact with RAD50 and NBS1 (Dery et al. 2008). MRE11 that cannot be methylated purified from baculovirus interacts normally with RAD50 and NBS1 but displayed defective DNA binding that impacted on MRN nuclease activity in vitro (Boisvert et al. 2005b; Dery et al. 2008). Furthermore, inhibition of PRMT activities resulted in a radio-resistant synthesis phenotype and defective formation of γ H2AX and MRE11 damage induced foci (Boisvert et al. 2005a). Note that the intra-S checkpoint defect could be rescued by overexpression of Mre11 (Boisvert et al. 2005a, b), and DNA damage-induced MRE11 binding to chromatin is diminished in the absence of the GAR domain (Dery et al. 2008). These data suggests that methylation of MRE11 on its C terminal GAR domain contributes to the regulation of its recruitment at sites of DNA damage and lack of methylation affects DSB processing and the integrity of the intra-S phase checkpoint.

Important interaction partners: novel members of the MRN complex?

Recently, two proteins have been shown to interact with MRN and to play important functions in the response to DSBs. Their role in MRN function suggests that they might be novel components of the MRN complex.

The CtIP/Ctp1/Sae2 protein

Yeast Sae2 (also termed Com1) was first identified as necessary, together with MRX, for processing of meiotic DSB with covalently attached Spo11 (Borde 2007). Sae2

orthologues have recently been found in all eukaryotes, and its meiotic functions appear to be conserved (Limbo et al. 2007; Penkner et al. 2007; Sartori et al. 2007; Uanschou et al. 2007). The human orthologue, CtIP, was first identified as a binding protein for Rb and CtBP1 (reviewed in Wu and Lee 2006). Similarly to *S. cerevisiae* Sae2 and fission yeast Ctp1, CtIP is necessary for DSB survival, in particular, after topoisomerase inhibitor-induced damage (Hartsuiker et al. 2009a, b; Huertas et al. 2008; Limbo et al. 2007; Sartori et al. 2007). CtIP/Ctp1/Sae2 also has a role in mitotic DSB resection as it is required for RPA accumulation at DSBs (Huertas et al. 2008; Limbo et al. 2007; Sartori et al. 2007) and for processing and DNA end bridging of an HO-induced DSB (Clerici et al. 2005). Its role in HR has been demonstrated in plasmid based assays, gene conversion at a unique I-SceI break and in sister chromatid recombination (Huertas et al. 2008; Limbo et al. 2007; Sartori et al. 2007). The recombinant Sae2 protein has endonuclease activity in vitro and can cleave hairpin-capped structures cooperatively with MRX (Lengsfeld et al. 2007). However, human CtIP has no associated nuclease activity but can stimulate MRN endonuclease activity in an ATP-independent manner (Sartori et al. 2007). Importantly, CtIP behaves like a novel member of the MRN complex as it binds MRN constitutively (i.e. independently of DNA damage) and is recruited to DSBs in an MRN-dependent manner in fission yeast (Limbo et al. 2007). However, it is not yet known if interactions between MRN and CtIP/Sae2 are conserved in all organisms. Note that inactivation of CtIP, similarly to MRN, leads to embryonic lethality (Chen et al. 2005b). In agreement with its role in DSB processing, Sae2/CtIP is not required for the initial step of checkpoint activation as depletion of CtIP has no effect on ATM-dependent phosphorylation of H2AX, CHK2 nor SMC1, but does affect resection-dependent ATR-signalling as detected by CHK1 and RPA2 phosphorylation (Sartori et al. 2007). Additionally, yeast cells harbouring a *sae2* null mutation display normal activation of Rad53 (similar to *mre11* nuclease defective cells) but fail to inactivate the checkpoint in response to DSB as detected by persistent Rad53 phosphorylation (Baroni et al. 2004; Clerici et al. 2006). In agreement with this, cells that overproduce Sae2 exhibit the opposite phenotype showing Rad53 defective activation like *mre11*Δ cells, and this is dependent on Mec1/Tel1 phosphorylation sites (Baroni et al. 2004; Clerici et al. 2006). These data suggest a role for Sae2/CtIP in regulating MRX/MRN nuclease activity and in terminating checkpoint activation in a PIKK-dependent manner.

Interestingly, Sae2 is the target of additional post-translational modification that regulates its abundance (Yu and Baer 2000) as well as its function during cell cycle. CtIP interacts with BRCA1, and this interaction depends on phosphorylation of Ser273 in G2 (Chen et al. 2008a;

Greenberg et al. 2006; Yu and Baer 2000; Yu and Chen 2004; Yu et al. 2006). In response to DNA damage, BRCA1 can then ubiquitinylates CtIP, which is dependent upon the prior phospho-mediated interaction. The ubiquitinylated form of CtIP binds chromatin dependently of S373 (Yu et al. 2006). Sae2 is also cell cycle phosphorylated by Cdc28 (Baroni et al. 2004; Huertas et al. 2008), and this modification seems to control Sae2 DNA repair function, more precisely its role, together with MRX, in regulating the balance between HR and NHEJ (see below).

The SOSS complexes

Recently, two novel, heterotrimeric ssDNA-binding complexes, termed SOSS1 and SOSS2 (sensors of ssDNA 1 and 2) have been identified (Huang et al. 2009; Li et al. 2009). Both complexes exist preformed in undamaged cells and share two interacting components, SOSS-A (also called INTS3) and SOSS-C (also called hSSBIP1 or C9ORF80). The distinct, although related proteins, human single-strand DNA binding proteins 1 or 2 (hSSB1 or hSSB2; also renamed SOSSB1 and SOSSB2), define the third component of these complexes, with hSSB1 being specific for SOSS1 and hSSB2 for SOSS2 (Huang et al. 2009; Li et al. 2009). Interestingly, the SOSS-A component interacts with Nbs1 (although not Mre11 or Rad50), indicating that the SOSS1 and SOSS2 are likely to be MRN interacting complexes.

Like the loss of MRN, the loss of any component of the SOSS complex resulted in abrogated or reduced activation of ATM, as well as phosphorylation of some of its targets (including CHK1 and NBS1). This suggests that SOSS may be another factor required for efficient activation of ATM and the ATM-dependent signalling cascade (Huang et al. 2009; Li et al. 2009; Richard et al. 2008). Indeed, hSSB1 deficiency has been shown to result in defective G1 and G2/M checkpoints (Richard et al. 2008). More recently, abrogation of the G2/M checkpoint has also been reported in cells lacking the common SOSS complex component, SOSS-A (Huang et al. 2009).

Similarly to the more widely studied RPA complex, the hSSB1 and hSSB2 proteins bind to ssDNA substrates (Huang et al. 2009; Richard et al. 2008) and appear to function in DNA repair. Depletion of components of the SOSS complex resulted in increased cellular sensitivity to IR (Huang et al. 2009; Li et al. 2009; Richard et al. 2008) and camptothecin (Huang et al. 2009). This suggests that these novel protein complexes could be involved in the repair of various types of DNA damage and contribute specifically to the HR-mediated pathway of DSB repair. In agreement with such a possibility, increased chromosomal aberrations, reduced homology-directed DSB repair and deficient Rad51 foci formation were observed in cells defective in components of SOSS complexes (Huang et al.

2009; Li et al. 2009; Richard et al. 2008). Interestingly, SOSS complexes form IR-induced foci in G1, S and G2; however, these foci are MRN-dependent only in S and G2 (Huang et al. 2009). Therefore, in these phases, MRN either acts together with SOSS, via the SOSS-A component to guide SOSS complexes to IR-induced foci, or the MRN complex must act upstream.

Even though both hSSB1 and 2 bind to ssDNA, like RPA, hSSB1 does not precisely co-localise with RPA foci (Richard et al. 2008). However, both types of foci were detected in close proximity, suggesting that both proteins may function within the same larger site of ongoing DNA repair, were they are likely performing distinct functions (Richard et al. 2008). Unlike RPA, the role of the SOSS complexes is specific for the DDR, as they do not appear to function in DNA replication. Thus, hSSB1 does not form foci in normal S phase (Richard et al. 2008). Also, unlike RPA (which does not form foci in G1 cells), hSSB1 forms IR-dependent foci independently of cell cycle phase. This suggests that the recruitment of SOSS1 and 2 might be independent of the MRN/CtIP-dependent resection of DSBs. In support of this hypothesis, SOSS-A, hSSB1 and hSSB2 foci formation is independent of CtIP (Huang et al. 2009). These results, together with the observation that RPA foci are independent of SOSS complexes (Huang et al. 2009), suggest the existence of two parallel pathways, MRN-SOSS and MRN-CtIP-RPA. Indeed, it is possible that these two different pathways might contribute in two distinct mechanisms of DSB signalling and repair (Nam and Cortez 2009).

Assigned functions in the DNA damage response

Cell cycle checkpoint control

The phenotype of MRN/MRX-deficient cells suggests a role in DSB signalling necessary for checkpoint activation and DNA repair. The Mre11 complex harbours a suite of enzymatic activities, and its striking structure makes it potentially extremely versatile while dealing with DNA damage in living cells. Since the first studies implicating its role in the DDR, a greater understanding on the molecular function of MRN in DSB signalling, particularly its role in activating ATM, has been acquired (reviewed in Williams et al. 2007). Cell lines harbouring hypomorphic mutations in components of the MRN complex and yeast cells harbouring null mutations of MRX components have been reported to present several deficiencies in DNA damage-induced cell cycle delays at the G1, intra-S and G2/M checkpoints (reviewed in Zhang et al. 2006). Evidence indicates that MRN acts very early in the DDR, most likely as a DSB sensor. However, it also acts as a target of the

checkpoint contributing to preservation of genomic integrity by preventing DNA replication forks collapse and facilitating DSB repair (reviewed in Borde and Cobb 2009). We review here recent advances enlightening the molecular role of the complex in the early sensing events of the DDR.

DSB sensing and ATM activation

The MRN complex has been shown to act upstream of ATM and serve as a DNA damage sensor specific for DSBs (Rupnik et al. 2008). In foci forming assays after IR, the yeast and higher eukaryotic cells MRX/MRN complexes are among the earliest to be recruited to sites of damage (Lisby et al. 2004; Maser et al. 1997). As the earliest events in the DDR occur so rapidly, including both recruitment of MRN to the sites of damage and ATM activation (Bakkenist and Kastan 2003), it is difficult to discern the exact sequence of events. However, it has been demonstrated that neither ATM nor its yeast homologue Tel1 is required for MRN/MRX recruitment into IRIF (Lisby et al. 2004; Mirzoeva and Petrini 2001).

Interestingly, MRN/MRX-dependent checkpoint regulation is independent of its nuclease activity, implicating functions other than DNA end processing in this function (Buis et al. 2008; Clerici et al. 2006, MG and NL unpublished data). The ability of NBS1 to bind to chromatin after the induction of endonuclease-generated DSBs is required for ATM autophosphorylation on serine 1981 (Berkovich et al. 2007). Soutoglou et al. expressed MRN components fused to a DNA-binding domain specific for an introduced lac operator array to demonstrate the importance of DNA recruitment, without a requirement for DNA end processing, for MRN-dependent activation of ATM *in vivo* (measured by autophosphorylation of S1981 and γ H2AX formation). Thus, checkpoint activation can occur without DNA damage if MRN is tethered to DNA by another mechanism (Soutoglou and Misteli 2008). NBS1 is the subunit of the complex that recruits and activates ATM, and this mechanism is conserved from yeast to human (Falck et al. 2005; Nakada et al. 2003; You et al. 2005). The carboxyl-terminus of NBS1 contains a PIK kinase-interacting motif specific for ATM. This motif is also conserved in Ku80 and ATRIP where it regulates interaction with DNA PKcs or ATR, respectively (Falck et al. 2005). The NBS1 PIK kinase interacting motif is necessary not only for ATM recruitment to DNA damage (assayed by IRIF) but also for its activation as CHK2, SMC1 and NBS1 itself, are not activated when this NBS1 motif is absent. Note that acetylation of ATM by the Tip60 histone acetyltransferase is also important for early ATM activation (reviewed in Iijima et al. 2008b; Squatrito et al. 2006), but a direct connection between Tip60 and MRN has not been established yet.

In addition to NBS1, further ATM activation leading to the generation of ssDNA coated with RPA requires the ATP-dependent DNA unwinding activity of RAD50, hSSB1 (see above) and the endonucleolytic activity of MRE11 possibly enhanced by CtIP (Moncalian et al. 2004; Richard et al. 2008; Sartori et al. 2007; Takeda et al. 2007; Uziel et al. 2003). Other *in vivo* events that are not yet well connected to MRN, such as acetylation of Lys3016 in the FATC region of ATM (Sun et al. 2007), autophosphorylation of ATM residues S367 and S1893 (Kozlov et al. 2006) and, even more recently, the nucleosome-binding protein HMGN1 (Kim et al. 2009), has been implicated in activation of ATM. Furthermore, Jazayeri et al. have reported that single-stranded oligonucleotides, generated as by product of the endonucleolytic processing of DNA ends, can associate with the MRN complex and stimulate ATM activity (Jazayeri et al. 2008). This is consistent with *in vitro* results obtained with purified ATM, where both the MRN complex and linear DNA were shown to be required for activation of ATM (Lee and Paull 2005). In an elegant extension of this *in vitro* work, Shiotani and Zhou (2009) have provided data consistent with distinct roles for different DNA substrates on ATM activity. They also suggest that processing of DSBs into ssDNA ultimately inhibits ATM and contributes to the switch from ATM to ATR activation, a hypothesis that is likely to be true for Tel1 and Mec1 in yeast (Mantiero et al. 2007). Thus, MRN may function to regulate ATM activity during ongoing DSB signalling by controlling the production of suitable DNA substrates at distinct stages of the DSB response. Clearly, determination of the detailed sequence of MRN/MRX-dependent events required for ATM activation and the likely hand over to ATR activation requires further study.

Indeed, the exact mechanism of how the damage is initially sensed and the role, if any, played by MRN remains unclear. One possibility for this “*immediate early*” sensing step is that under physiological conditions, the MRN complex transiently interacts with chromatin. Dynamic but weak MRN interactions that occur with undamaged chromatin might be reinforced in the vicinity of DSBs, initially due to break-induced alterations in chromatin structure but further strengthened by the ability of NBS1 to interact with other DDR proteins upon their subsequent modification or recruitment. For example, the FHA and BRCT domains of NBS1 are known to mediate interaction with phosphorylated amino acid residues on surface of other proteins such as MDC1 and H2AX. A reinforcement loop needed for MRN retention at sites of DNA damage would no doubt involve interaction with many other proteins, both dependent upon and independent of post-translational modifications. Consistent with this possibility, it is worth noting that although after damage NBS1 interacts with phosphorylated H2AX (Kobayashi et

al. 2002), this modification (γ H2AX) is not required for the initial recognition of DSBs by DDR proteins (Celeste et al. 2003). NBS1 recruitment to the sites of damage induced by microbeam radiation was not impaired in H2ax^{-/-} mice cells, although a slight defect in the formation of late foci formation was reported, consistent with defective retention of the MRN complex. It is likely that many events are required for the progressive recruitment of MRN to sites of DNA damage, and these remain to be elucidated.

Generation of ssDNA: the end of the DNA end processing paradox

How do cells process DSBs to intermediates that can be used for both DNA repair and checkpoint signalling? In yeast, a role for MRX has been known for many years (Lee et al. 1998), but the details of the process have only recently been elucidated (Gravel et al. 2008; Mimitou and Symington 2008; Zhu et al. 2008). In addition to MRX, DSB processing was known to require other nucleases and the exonuclease, Exo1, has been implicated in this process (Llorente and Symington 2004; Nakada et al. 2004; Tsubouchi and Ogawa 2000). However, DSB processing is not fully abolished in *exo1* cells or even cells doubly defective in EXO1 and a member of the MRX complex (Llorente and Symington 2004; Moreau et al. 2001). In *Escherichia coli*, in the absence of the RecBCD exonuclease activity, both the RecJ exonuclease and the RecQ helicase are required for DSB processing. Similarly in yeast, DSB processing is now known to require a RecQ family member, the Sgs1 helicase, as well as an additional exonuclease, Dna2 (Gravel et al. 2008; Mimitou and Symington 2008; Zhu et al. 2008). When mutated, EXO1 and SGS1 have synergistic negative effects on the efficiency of single strand annealing, gene conversion at the MAT locus and ssDNA accumulation at an HO DSB.

Thus, Exo1 and Sgs1 act in parallel pathways to generate resected DSB. In addition, Zhu et al. (2008) demonstrated that a *dna2* mutant has similar defects in DSB processing to *sgs1*.

Detailed analysis of DSB processing also demonstrated that it requires two steps. Firstly, Mimitou and Symington (2008) demonstrated that Sae2 is required to remove 50 to 100 nucleotides from the 5' ends of the break, leaving a short ssDNA tail with a 3' end, which is also in agreement with the detection of short oligonucleotides generated during MRE11-dependent processing of DSBs (Jazayeri et al. 2008). Secondly, the generation of longer region of ssDNA requires both Exo1 and Sgs1. Interestingly, the *exo1 sgs1* double mutant has a severe defect in checkpoint signalling as judged by defective Rad53 activation (Gravel et al. 2008), lack of Ddc2 foci formation and defective accumulation of damaged cells at the G2/M transition in response to unprocessed DSB (Zhu et al. 2008).

These studies explain the apparent contradiction between the polarity of the MRX exonuclease in vitro, which is in the 3' to 5' direction, and the requirement of the MRX complex for resecting DSBs which occurs by removal of one strand with 5' to 3' polarity leaving a ssDNA tail with a 3' end required for homology direct repair. In higher eukaryotes, DSB processing appears to be similar to the process in budding yeast. Indeed, in mice, Exo1 is necessary for ssDNA accumulation and DNA damage signalling (Schaetzlein et al. 2007). Furthermore, Exo1 together with a Sgs1 homologue, BLM (which is mutated in Bloom's syndrome), is required for survival, ssDNA accumulation and efficient ATR-dependent checkpoint signalling (Gravel et al. 2008; Jazayeri et al. 2006). Finally, BLM can directly stimulate EXO1 resecting activity in vitro (Nimonkar et al. 2008). Interestingly, the archaeal helicase HerA and nuclease NurA cooperate with Mre11/Rad50 in *P. furiosus* to generate ssDNA and have been found to be the functional homologues of eukaryotic BLM and EXO1 (Hopkins and Paull 2008).

In conclusion, the generation of ssDNA tails with 3' ends at DSBs involves two distinct steps of processing: the Sae2-dependent endonucleolytic generation of a short tail, followed by the exonuclease (Exo1 and Dna2) and helicase (Sgs1)-dependent extension of this ssDNA tail. These enzymes are absolutely required for signalling, repair and, as a consequence, viability of eukaryotic cells. The fact that similar enzymes are required in bacteria to process DSB demonstrates once again the extraordinary conservation of the basic mechanisms essential for cell survival (reviewed in Niu et al. 2009).

Deciding between homologous recombination and non-homologous end joining

Given its ability to recognise and to process broken chromosome ends, it is not surprising that the MRN complex is involved in homologous recombination. Specifically, its role in resecting DNA is required for Rad51 nucleofilament formation during the presynapsis step of recombination (Li and Heyer 2008). Thus, MRN mutants display defects in meiosis, IR-induced recombination, plasmid based HR assays and recruitment of homologous recombination (HR) proteins in both yeast and higher cells (Bressan et al. 1999; Buis et al. 2008; Tauchi et al. 2002; Yang et al. 2006). DNA end recognition and processing are also consistent with a role for the complex during the early stage of NHEJ, and this has been established in budding yeast (Daley et al. 2005; Milne et al. 1996; Moore and Haber 1996). However, MRN mutants are not deficient in NHEJ repair in a plasmid assays in fission yeast where the complex appears to be mostly involved in homologous directed repair (Akamatsu et al. 2008; Manolis et al. 2001; Tomita et al. 2003). Similarly, depletion of MRN in *Xenopus* extract does not significantly affect NHEJ effi-

ciency (Di Virgilio and Gautier 2005). Until recently, results obtained from mammalian systems have not been consistent with a significant role for the MRN complex in repair of DSBs by NHEJ. Indeed, MRN is not thought to be part of the core NHEJ machinery in mammals (Weterings and Chen 2008). NHEJ is composed of two pathways called the classical or C-NHEJ pathway, which is error-free and dependent on *Ku* and *Xrcc4*, and an alternative or A-NHEJ error prone pathway, that is independent on *Ku* and *Xrcc4* (Zha et al. 2009). A biological model often used for these two pathways is the generation of immunoglobulin diversity, as two important steps in this mechanism are V(D)J recombination, which is dependent on C-NHEJ, and class switch recombination (CSR), which depends equally on C-NHEJ and A-NHEJ. In early 2009, two groups demonstrated that Mre11 and Nbs1 have a role in V(D)J recombination either in C-NHEJ (Helmink et al. 2009) or A-NHEJ (Deriano et al. 2009). The role of Mre11 and Nbs1 in A-NHEJ was observed when the V(D)J Rag2 nuclease is defective. More recently, MRN was shown to be involved in NHEJ during CSR in mice (Dinkelmann et al. 2009). In fact, as recent evidence has emerged a more general role for MRN in NHEJ, separate from its roles in V(D)J recombination, repair has arisen. Studies of repair of I-SceI endonuclease-induced DSBs demonstrated that loss of active MRN (achieved using either siRNA-mediated knockdown of Mre11, Rad50, Nbs1 or CtIP, or by the inhibitor of the MRN termed MIRIN) lead to defects in both C-NHEJ and A-NHEJ in both human or murine cell lines (Rass et al. 2009; Xie et al. 2009). Note that studies of the repair of dysfunctional telomeres also demonstrated a role for MRN in NHEJ mechanisms (Deng et al. 2009; Dimitrova and de Lange 2009). This role could be restricted to G1 cells as MRN could also inhibit NHEJ in G2 cells (Dimitrova and de Lange 2009). Taken together, these recent findings clearly demonstrate the function of the Mre11 complex in NHEJ as previously reported in lower organisms (Zha et al. 2009).

The early function of the MRN complex, immediately upon the generation of DSBs, places this complex at the interface of choice between DSB repair pathways. This choice also depends upon other factors, including the presence of a suitable template for HR (e.g. a sister chromatid), the cell type and the specifics of regulation of the HR and NHEJ core machinery (reviewed in Shrivastav et al. 2008). Importantly, the balance between NHEJ and HR is also cell cycle controlled by the activity of cyclin-dependent kinases (CDKs), with the former being favoured in G1 and the later in S/G2 in both budding and fission yeast (Aylon et al. 2004; Ferreira and Cooper 2004; Ira et al. 2004).

The basis of cell cycle specific regulation of DSB repair pathway is DNA processing as DSB resection is inefficient when yeast cells are in the G1 phase of the cell cycle compared to the G2 phase (Frank-Vaillant and Marcand

2002; Ira et al. 2004). Indeed, CDK activity is known to control the initial endonuclease step of DSB processing as when CDK activity is inactivated in yeast cells in G2, DSB processing is inhibited, and the MRX complex remains bound to the break. In G1 phase yeast cells, when CDK activity is low, DSB processing is inhibited by the NHEJ machinery. In this case, NHEJ deficiency, particularly lack of a functional *Ku* complex, allowed increased activation of the G1 checkpoint in response to a single endonuclease-induced DSB, and this correlated with the accumulation of ssDNA (Barlow et al. 2008; Clerici et al. 2008). Bacterial *Ku* has been recently shown to protect DSB from resection by the nuclease AdnAB, an alternate resecting complex to RecBCD in *Mycobacteria* (Sinha et al. 2009). Significantly, the fact that resection and checkpoint activation observed in the absence of the yeast *Ku* complex is not CDK-dependent anymore suggests that *Ku* proteins might be directly regulated by CDK to control DSB processing (Clerici et al. 2008). Interestingly, in G1 yeast cells, IR generates “ragged” DSB ends that presumably cannot bind the *Ku* complex and that unlike endonuclease-induced DSB can be resected although still less efficiently than in G2 cells. However, Rad52 can only be recruited when cells enter S phase, pointing at CDK regulation of HR proteins activity as well (Barlow et al. 2008).

In yeast cells in G2, the Sae2 protein that modulates MRX activity is tightly controlled. Remarkably, cell cycle regulation of Sae2 and its orthologues, Ctp1/CtIP, is evolutionarily very well conserved, being absent from G1 cells but present in the S and G2 phases (Baroni et al. 2004; Limbo et al. 2007; Yu and Baer 2000). Noticeably, Ser267 of Sae2 has been identified as a CDK target site that is necessary for Sae2 function as when mutated, cells display defects in meiosis and HO resection (Huertas et al. 2008). This site is also conserved in human CtIP and mutation of the conserved site results in sensitivity to camptothecin and impact on DSB resection during cell cycle in both yeast and human cells (Huertas et al. 2008; Huertas and Jackson 2009). Importantly, as CDK inhibition has very little effect on DSB resection when S267 of Sae2 is mutated indicates that Sae2 is the main target regulating cell cycle-dependent DSB resection. Finally, *sae2*Δ cells have decreased HR and enhanced NHEJ, whereas a Sae2 S267 phospho-mimic mutant has the opposite phenotype (Huertas et al. 2008). This suggests a major role for Sae2 in controlling MRX activity in order to regulate the balance between HR and NHEJ (Huertas et al. 2008). The role of CtIP in regulating the balance between DSB repair pathways is conserved in higher cells. Importantly, study in chicken cells has demonstrated that two CDK phosphorylation events regulate this key function: one on S847 regulating DSB repair and resection and the other on Ser 327 regulating the interaction with BRCA1 (Yun and Hiom 2009).

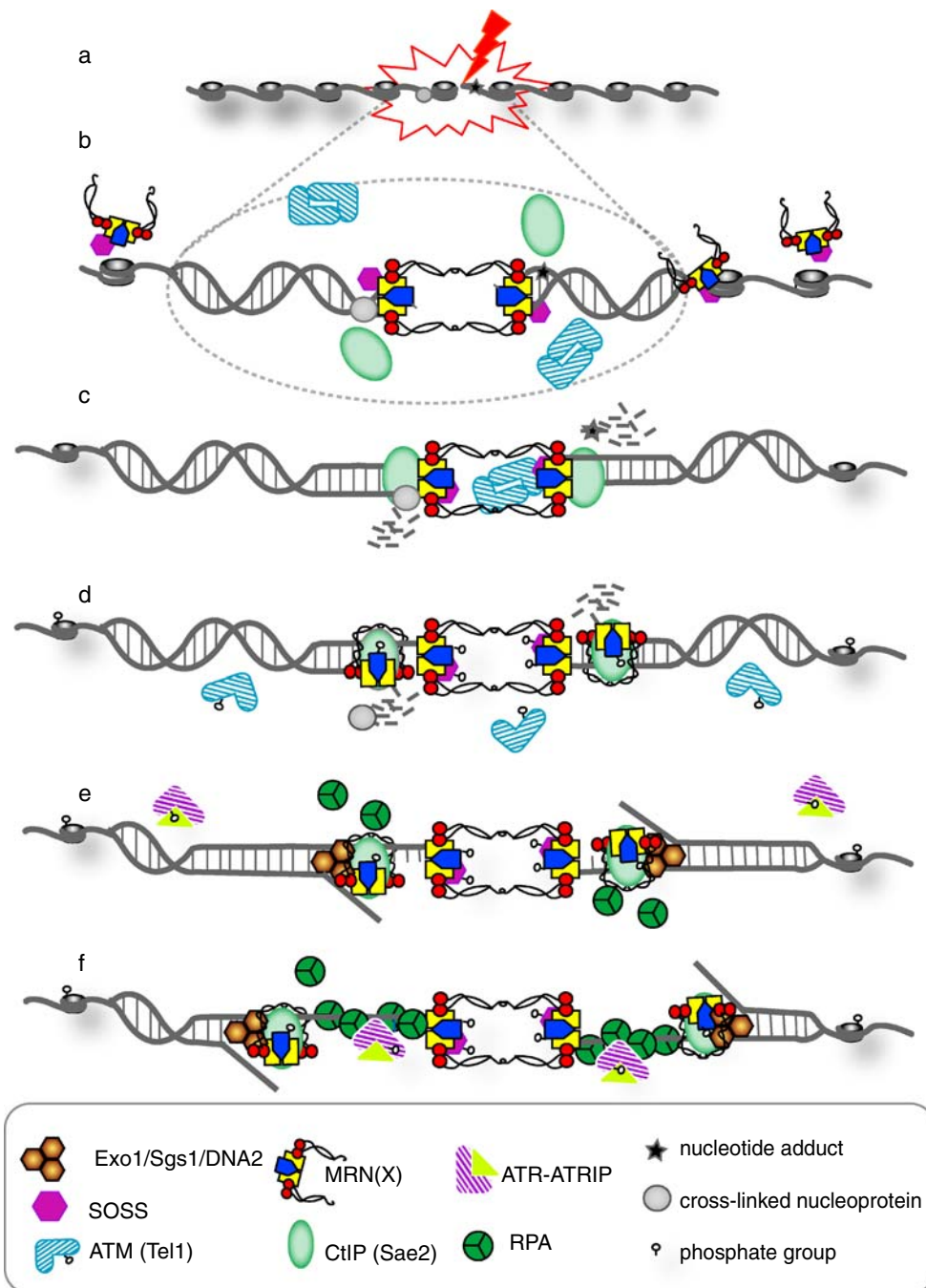
Current model for DSB signalling and conclusion

An updated simplified model presenting the early events of the DDR involving the MRN complex is emerging from the recent discoveries described in this review (Fig. 6).

Immediately after damage, MRN, possibly in association with SOSS, is recruited to the sites of damage. The DNA end binding activity of MRE11 and the ability of SOSS to recognise short regions of unwound DNA presumably both contribute to binding broken chromosome ends (Fig. 6b). It is possible that the MRN complex may also dynamically

interact with chromatin independently of DSBs in a mechanism that is enhanced upon DSB-induced changes in chromatin structure. Direct DNA end binding is probably independent of this chromatin-dependent recruitment of MRN to the vicinity of DSBs and is further enhanced by sliding of the MRN complex along the broken DNA. This allows multiple molecules to be bound directly at the DSBs and through Zn^{2+} hook mediated Rad50-Rad50 interactions bridges the broken chromosome arm together. Inactive ATM is then recruited via NBS1 (Fig. 6c). Note that in undamaged cells ATM appears to exist in an inactive

Fig. 6 The early events of DNA damage response: When DNA double strand break (DSB) form, they are often “dirty” with nucleoprotein and nucleotide adducts cross-linked to the DNA strands (a). The MRN (possibly with SOSS) complex is immediately recruited to the sites of damage and secures DNA ends (b). The MRN/CtIP is able to perform the initial cleaning of the ends, which generates short oligonucleotides. Inactive ATM kinase is recruited and activated (c). Active ATM phosphorylates its downstream substrates such as MRN and hSSB. The signalling cascade is activated. The MRN complex secures the processed DNA (d). Further end processing is performed by nucleases and helicases (e). RPA-ssDNA filament is formed, and ATR-ATRIP are recruited to the site of break (f)



multimeric form that can be activated by alterations to chromatin structure resulting from DNA damage or other treatments. Meanwhile, the MRN complexes directly at the broken ends of the chromosome, together with Sae2/CtIP, are involved in processing (unwinding, resection, removal of adducts and resolving DNA structures such as hairpins) the DNA ends to form an early intermediate product, with short, 3' ended ssDNA tails. The short oligonucleotides generated contribute to ATM activation by mechanisms that require further clarification. Activated ATM can now phosphorylate its proximal targets (i.e. at the DSB), which include NBS1 and H2AX (Fig. 6d). Phosphorylated H2AX (γ H2AX) results in the recruitment of additional DDR factors including MDC1 and additional MRN complexes into large focal structures, which appear to facilitate, rather than be required for an efficient damage response.

The early intermediate generated by the MRN/CtIP-dependent processing step is the substrate for recruitment of EXO1, DNA2 and Sgs1/BLM, which rapidly process the broken chromosome ends to generate long 3' ended ssDNA tails (Fig. 6e). These are initially bound by RPA (Fig. 6f), which recruits ATR for a second round of checkpoint signalling, but subsequently, RPA is replaced by RAD51 (not shown) which initiates the search for homologous DNA, usually on the sister chromatid, and commences recombination. As resection proceeds, ATM gets inactivated by the increased production of ssDNA and loses its affinity for this increasingly resected DSB. ATM is then replaced by ATR at the resected break via interaction with RPA-ssDNA. The switch between ATM and ATR may also involve TopBP1 and MRN (Yoo et al. 2009). Activated ATR phosphorylates its downstream substrates, including, importantly, Chk1. Together, these effectors regulate transient cell cycle delays and other biological response to DNA damage.

We have learnt a lot about the structure and functions of the MRN complex during the last decade. Fascinating observations have enhanced our understanding of how this structurally intriguing complex is involved in sensing DNA damage, bridging broken chromosome ends and facilitating DNA damage-dependent signalling and repair. Many questions have been answered and many remain to be addressed. Possibly, the most interesting and challenging for future studies will be the integration of all recent discoveries into coherent mechanisms of MRN function. In addition, understanding the precise molecular details of how this important, multifunctional player in the cellular responses to broken DNA is regulated will remain challenging.

Acknowledgements The authors thank Sunichi Takeda for his authorisation to cite unpublished results, Serge Gravel and Karl Peter Hopfner for critical reading of the manuscript and John Eykelboom for his comments. The work in Noel Lowndes laboratory is supported by Cancer Research Ireland under grant CR105GRE, European

Commission Integrated project “DNA Repair” contract number 512113 and Science Foundation Ireland Principal Investigator award 07/IN1/B958.

References

- Ajimura M, Leem SH, Ogawa H (1993) Identification of new genes required for meiotic recombination in *Saccharomyces cerevisiae*. *Genetics* 133:51–66
- Akamatsu Y, Murayama Y, Yamada T, Nakazaki T, Tsutsui Y, Ohta K, Iwasaki H (2008) Molecular characterization of the role of the *Schizosaccharomyces pombe nip1⁺/ctp1⁺* gene in DNA double-strand break repair in association with the Mre11-Rad50-Nbs1 complex. *Mol Cell Biol* 28:3639–3651
- Alani E, Padmore R, Kleckner N (1990) Analysis of wild-type and rad50 mutants of yeast suggests an intimate relationship between meiotic chromosome synapsis and recombination. *Cell* 61:419–436
- Aylon Y, Liefshitz B, Kupiec M (2004) The CDK regulates repair of double-strand breaks by homologous recombination during the cell cycle. *EMBO J* 23:4868–4875
- Bakkenist CJ, Kastan MB (2003) DNA damage activates ATM through intermolecular autophosphorylation and dimer dissociation. *Nature* 421:499–506
- Barlow JH, Lisby M, Rothstein R (2008) Differential regulation of the cellular response to DNA double-strand breaks in G1. *Mol Cell* 30:73–85
- Baroni E, Viscardi V, Cartagena-Lirola H, Lucchini G, Longhese MP (2004) The functions of budding yeast Sae2 in the DNA damage response require Mec1- and Tel1-dependent phosphorylation. *Mol Cell Biol* 24:4151–4165
- Bartek J, Bartkova J, Lukas J (2007) DNA damage signalling guards against activated oncogenes and tumour progression. *Oncogene* 26:7773–7779
- Baudat F, de Massy B (2007) Regulating double-stranded DNA break repair towards crossover or non-crossover during mammalian meiosis. *Chromosome Res* 15:565–577
- Becker E, Meyer V, Madaoui H, Guerois R (2006) Detection of a tandem BRCT in Nbs1 and Xrs2 with functional implications in the DNA damage response. *Bioinformatics* 22:1289–1292
- Berkovich E, Monnat RJ Jr, Kastan MB (2007) Roles of ATM and NBS1 in chromatin structure modulation and DNA double-strand break repair. *Nat Cell Biol* 9:683–690
- Bernardi R, Pandolfi PP (2007) Structure, dynamics and functions of promyelocytic leukaemia nuclear bodies. *Nat Rev Mol Cell Biol* 8:1006–1016
- Bhaskara V, Dupre A, Lengsfeld B, Hopkins BB, Chan A, Lee JH, Zhang X, Gautier J, Zakian V, Paull TT (2007) Rad50 adenylate kinase activity regulates DNA tethering by Mre11/Rad50 complexes. *Mol Cell* 25:647–661
- Boisvert FM, Dery U, Masson JY, Richard S (2005a) Arginine methylation of MRE11 by PRMT1 is required for DNA damage checkpoint control. *Genes Dev* 19:671–676
- Boisvert FM, Hendzel MJ, Masson JY, Richard S (2005b) Methylation of MRE11 regulates its nuclear compartmentalization. *Cell Cycle* 4:981–989
- Borde V (2007) The multiple roles of the Mre11 complex for meiotic recombination. *Chromosome Res* 15:551–563
- Borde V, Cobb J (2009) Double functions for the Mre11 complex during DNA double-strand break repair and replication. *Int J Biochem Cell Biol* 41:1249–1253
- Bork P, Hofmann K, Bucher P, Neuwald AF, Altschul SF, Koonin EV (1997) A superfamily of conserved domains in DNA damage-responsive cell cycle checkpoint proteins. *FASEB J* 11:68–76

- Bressan DA, Baxter BK, Petrini JH (1999) The Mre11-Rad50-xrs2 protein complex facilitates homologous recombination-based double-strand break repair in *Saccharomyces cerevisiae*. *Mol Cell Biol* 19:7681–7687
- Bressan DA, Olivares HA, Nelms BE, Petrini JH (1998) Alteration of N-terminal phosphoesterase signature motifs inactivates *Saccharomyces cerevisiae* Mre11. *Genetics* 150:591–600
- Buis J, Wu Y, Deng Y, Leddon J, Westfield G, Eckersdorff M, Sekiguchi JM, Chang S, Ferguson DO (2008) Mre11 nuclease activity has essential roles in DNA repair and genomic stability distinct from ATM activation. *Cell* 135:85–96
- Cahill D, Carney JP (2007) Dimerization of the Rad50 protein is independent of the conserved hook domain. *Mutagenesis* 22:269–274
- Callebaut I, Morion JP (1997) From BRCA1 to RAP1: a widespread BRCT module closely associated with DNA repair. *FEBS Lett* 400:25–30
- Carney JP, Maser RS, Olivares H, Davis EM, Le Beau M, Yates JR 3rd, Hays L, Morgan WF, Petrini JH (1998) The hMre11/hRad50 protein complex and Nijmegen breakage syndrome: linkage of double-strand break repair to the cellular DNA damage response. *Cell* 93:477–486
- Celeste A, Fernandez-Capetillo O, Kruhlak MJ, Pilch DR, Staudt DW, Lee A, Bonner RF, Bonner WM, Nussenzweig A (2003) Histone H2AX phosphorylation is dispensable for the initial recognition of DNA breaks. *Nat Cell Biol* 5:675–679
- Cerosaletti KM, Desai-Mehta A, Yeo TC, Kraakman-Van Der Zwet M, Zdzienicka MZ, Concannon P (2000) Retroviral expression of the NBS1 gene in cultured Nijmegen breakage syndrome cells restores normal radiation sensitivity and nuclear focus formation. *Mutagenesis* 15:281–286
- Chapman JR, Jackson SP (2008) Phospho-dependent interactions between NBS1 and MDC1 mediate chromatin retention of the MRN complex at sites of DNA damage. *EMBO Rep* 9:795–801
- Chen L, Trujillo K, Ramos W, Sung P, Tomkinson AE (2001) Promotion of Dnl4-catalyzed DNA end-joining by the Rad50/Mre11/Xrs2 and Hdf1/Hdf2 complexes. *Mol Cell* 8:1105–1115
- Chen L, Trujillo KM, Van Komen S, Roh DH, Krejci L, Lewis LK, Resnick MA, Sung P, Tomkinson AE (2005a) Effect of amino acid substitutions in the rad50 ATP binding domain on DNA double strand break repair in yeast. *J Biol Chem* 280:2620–2627
- Chen PL, Liu F, Cai S, Lin X, Li A, Chen Y, Gu B, Lee EY, Lee WH (2005b) Inactivation of CtIP leads to early embryonic lethality mediated by G1 restraint and to tumorigenesis by haploid insufficiency. *Mol Cell Biol* 25:3535–3542
- Chen L, Nievera CJ, Lee AY, Wu X (2008a) Cell cycle-dependent complex formation of BRCA1.CtIP.MRN is important for DNA double-strand break repair. *J Biol Chem* 283:7713–7720
- Chen YC, Chiang HY, Yang MH, Chen PM, Chang SY, Teng SC, Vanhaesebroeck B, Wu KJ (2008b) Activation of phosphoinositide 3-kinase by the NBS1 DNA repair protein through a novel activation motif. *J Mol Med* 86:401–412
- Clerici M, Mantiero D, Lucchini G, Longhese MP (2005) The *Saccharomyces cerevisiae* Sae2 protein promotes resection and bridging of double strand break ends. *J Biol Chem* 280:38631–38638
- Clerici M, Mantiero D, Lucchini G, Longhese MP (2006) The *Saccharomyces cerevisiae* Sae2 protein negatively regulates DNA damage checkpoint signalling. *EMBO Rep* 7:212–218
- Clerici M, Mantiero D, Guerini I, Lucchini G, Longhese MP (2008) The Yku70-Yku80 complex contributes to regulate double-strand break processing and checkpoint activation during the cell cycle. *EMBO Rep* 9:810–818
- Costanzo V, Robertson K, Bibikova M, Kim E, Grieco D, Gottesman M, Carroll D, Gautier J (2001) Mre11 protein complex prevents double-strand break accumulation during chromosomal DNA replication. *Mol Cell* 8:137–147
- D’Amours D, Jackson SP (2001) The yeast Xrs2 complex functions in S phase checkpoint regulation. *Genes Dev* 15:2238–2249
- Daley JM, Palmbo PL, Wu D, Wilson TE (2005) Nonhomologous end joining in yeast. *Annu Rev Genet* 39:431–451
- de Jager M, Wyman C, van Gent DC, Kanaar R (2002) DNA end-binding specificity of human Rad50/Mre11 is influenced by ATP. *Nucleic Acids Res* 30:4425–4431
- Deng Y, Guo X, Ferguson DO, Chang S (2009) Multiple roles for MRE11 at uncapped telomeres. *Nature* 460:914–918
- Deriano L, Stracker TH, Baker A, Petrini JH, Roth DB (2009) Roles for NBS1 in alternative nonhomologous end-joining of V(D)J recombination intermediates. *Mol Cell* 34:13–25
- Dery U, Coulombe Y, Rodrigue A, Stasiak A, Richard S, Masson JY (2008) A glycine-arginine domain in control of the human MRE11 DNA repair protein. *Mol Cell Biol* 28:3058–3069
- Desai-Mehta A, Cerosaletti KM, Concannon P (2001) Distinct functional domains of nibrin mediate Mre11 binding, focus formation, and nuclear localization. *Mol Cell Biol* 21:2184–2191
- di Masi A, Viganotti M, Polticelli F, Ascenzi P, Tanzarella C, Antoccia A (2008) The R215W mutation in NBS1 impairs gamma-H2AX binding and affects DNA repair: molecular bases for the severe phenotype of 657del5/R215W Nijmegen breakage syndrome patients. *Biochem Biophys Res Commun* 369:835–840
- Di Virgilio M, Gautier J (2005) Repair of double-strand breaks by nonhomologous end joining in the absence of Mre11. *J Cell Biol* 171:765–771
- Dimitrova N, de Lange T (2009) Cell cycle dependent role of MRN at dysfunctional telomeres: ATM signaling-dependent induction of NHEJ in G1 and resection-mediated inhibition of NHEJ in G2. *Mol Cell Biol* 29(20):5552–5563
- Dinkelmann M, Spehalski E, Stoneham T, Buis J, Wu Y, Sekiguchi JM, Ferguson DO (2009) Multiple functions of MRN in end-joining pathways during isotype class switching. *Nat Struct Mol Biol* 16:808–813
- Dong Z, Zhong Q, Chen PL (1999) The Nijmegen breakage syndrome protein is essential for Mre11 phosphorylation upon DNA damage. *J Biol Chem* 274:19513–19516
- Dumon-Jones V, Frappart PO, Tong WM, Sajithlal G, Hulla W, Schmid G, Herceg Z, Digweed M, Wang ZQ (2003) Nbn heterozygosity renders mice susceptible to tumor formation and ionizing radiation-induced tumorigenesis. *Cancer Res* 63:7263–7269
- Durocher D, Henckel J, Fersht AR, Jackson SP (1999) The FHA domain is a modular phosphopeptide recognition motif. *Mol Cell* 4:387–394
- Dzikiewicz-Krawczyk A (2008) The importance of making ends meet: mutations in genes and altered expression of proteins of the MRN complex and cancer. *Mutat Res* 659:262–273
- Falck J, Coates J, Jackson SP (2005) Conserved modes of recruitment of ATM, ATR and DNA-PKcs to sites of DNA damage. *Nature* 434:605–611
- Ferreira MG, Cooper JP (2004) Two modes of DNA double-strand break repair are reciprocally regulated through the fission yeast cell cycle. *Genes Dev* 18:2249–2254
- Frank-Vaillant M, Marcand S (2002) Transient stability of DNA ends allows nonhomologous end joining to precede homologous recombination. *Mol Cell* 10:1189–1199
- Furuse M, Nagase Y, Tsubouchi H, Murakami-Murofushi K, Shibata T, Ohta K (1998) Distinct roles of two separable in vitro activities of yeast Mre11 in mitotic and meiotic recombination. *EMBO J* 17:6412–6425
- Game JC, Mortimer RK (1974) A genetic study of x-ray sensitive mutants in yeast. *Mutat Res* 24:281–292
- Gatei M, Young D, Cerosaletti KM, Desai-Mehta A, Spring K, Kozlov S, Lavin MF, Gatti RA, Concannon P, Khanna K (2000) ATM-

- dependent phosphorylation of nibrin in response to radiation exposure. *Nat Genet* 25:115–119
- Ghosal G, Muniyappa K (2007) The characterization of *Saccharomyces cerevisiae* Mre11/Rad50/Xrs2 complex reveals that Rad50 negatively regulates Mre11 endonucleolytic but not the exonucleolytic activity. *J Mol Biol* 372:864–882
- Gravel S, Chapman JR, Magill C, Jackson SP (2008) DNA helicases Sgs1 and BLM promote DNA double-strand break resection. *Genes Dev* 22:2767–2772
- Greenberg RA, Sobhian B, Pathania S, Cantor SB, Nakatani Y, Livingston DM (2006) Multifactorial contributions to an acute DNA damage response by BRCA1/BARD1-containing complexes. *Genes Dev* 20:34–46
- Harper JW, Elledge SJ (2007) The DNA damage response: ten years after. *Mol Cell* 28:739–745
- Hartsuiker E, Mizuno K, Molnar M, Kohli J, Ohta K, Carr AM (2009a) Ctp1/CtIP and Rad32/Mre11 nuclease activity are required for Rec12/Spo11 removal, but Rec12/Spo11 removal is dispensable for other MRN-dependent meiotic functions. *Mol Cell Biol* 29:1671–1681
- Hartsuiker E, Neale MJ, Carr AM (2009b) Distinct requirements for the Rad32(Mre11) nuclease and Ctp1(CtIP) in the removal of covalently bound topoisomerase I and II from DNA. *Mol Cell* 33:117–123
- Heikkinen K, Rapakko K, Karppinen SM, Erkkö H, Knuutila S, Lundan T, Mannermaa A, Borresen-Dale AL, Borg A, Barkardottir RB et al (2006) RAD50 and NBS1 are breast cancer susceptibility genes associated with genomic instability. *Carcinogenesis* 27:1593–1599
- Helmink BA, Bredemeyer AL, Lee BS, Huang CY, Sharma GG, Walker LM, Bednarski JJ, Lee WL, Pandita TK, Bassing CH, Sleckman BP (2009) MRN complex function in the repair of chromosomal Rag-mediated DNA double-strand breaks. *J Exp Med* 206:669–679
- Ho Y, Gruhler A, Heilbut A, Bader GD, Moore L, Adams SL, Millar A, Taylor P, Bennett K, Boutlier K et al (2002) Systematic identification of protein complexes in *Saccharomyces cerevisiae* by mass spectrometry. *Nature* 415:180–183
- Hofmann K, Bucher P (1995) The FHA domain: a putative nuclear signalling domain found in protein kinases and transcription factors. *Trends Biochem Sci* 20:347–349
- Holland IB, Blight MA (1999) ABC-ATPases, adaptable energy generators fuelling transmembrane movement of a variety of molecules in organisms from bacteria to humans. *J Mol Biol* 293:381–399
- Hopfner KP, Tainer JA (2003) Rad50/SMC proteins and ABC transporters: unifying concepts from high-resolution structures. *Curr Opin Struct Biol* 13:249–255
- Hopfner KP, Karcher A, Shin D, Fairley C, Tainer JA, Carney JP (2000a) Mre11 and Rad50 from *Pyrococcus furiosus*: cloning and biochemical characterization reveal an evolutionarily conserved multiprotein machine. *J Bacteriol* 182:6036–6041
- Hopfner KP, Karcher A, Shin DS, Craig L, Arthur LM, Carney JP, Tainer JA (2000b) Structural biology of Rad50 ATPase: ATP-driven conformational control in DNA double-strand break repair and the ABC-ATPase superfamily. *Cell* 101:789–800
- Hopfner KP, Karcher A, Craig L, Woo TT, Carney JP, Tainer JA (2001) Structural biochemistry and interaction architecture of the DNA double-strand break repair Mre11 nuclease and Rad50-ATPase. *Cell* 105:473–485
- Hopfner KP, Craig L, Moncalian G, Zinkel RA, Usui T, Owen BA, Karcher A, Henderson B, Bodmer JL, McMurray CT et al (2002) The Rad50 zinc-hook is a structure joining Mre11 complexes in DNA recombination and repair. *Nature* 418:562–566
- Hopkins BB, Paull TT (2008) The *p. furiosus* mre11/rad50 complex promotes 5' strand resection at a DNA double-strand break. *Cell* 135:250–260
- Horejsi Z, Falck J, Bakkenist CJ, Kastan MB, Lukas J, Bartek J (2004) Distinct functional domains of Nbs1 modulate the timing and magnitude of ATM activation after low doses of ionizing radiation. *Oncogene* 23:3122–3127
- Huang J, Gong Z, Ghosal G, Chen J (2009) SOSS complexes participate in the maintenance of genomic stability. *Mol Cell* 35:384–393
- Huertas P, Jackson SP (2009) Human CtIP mediates cell-cycle control of DNA- end resection and double-strand-break repair. *J Biol Chem* 284(14):9558–9565
- Huertas P, Cortes-Ledesma F, Sartori AA, Aguilera A, Jackson SP (2008) CDK targets Sae2 to control DNA-end resection and homologous recombination. *Nature* 455:689–692
- Iijima K, Muranaka C, Kobayashi J, Sakamoto S, Komatsu K, Matsuura S, Kubota N, Tauchi H (2008a) NBS1 regulates a novel apoptotic pathway through Bax activation. *DNA Repair (Amst)* 7:1705–1716
- Iijima K, Ohara M, Seki R, Tauchi H (2008b) Dancing on damaged chromatin: functions of ATM and the RAD50/MRE11/NBS1 complex in cellular responses to DNA damage. *J Radiat Res (Tokyo)* 49:451–464
- Ira G, Pelliccioli A, Balijja A, Wang X, Fiorani S, Carotenuto W, Liberi G, Bressan D, Wan L, Hollingsworth NM et al (2004) DNA end resection, homologous recombination and DNA damage checkpoint activation require CDK1. *Nature* 431:1011–1017
- Ivanov EL, Korolev VG, Fabre F (1992) XRS2, a DNA repair gene of *Saccharomyces cerevisiae*, is needed for meiotic recombination. *Genetics* 132:651–664
- Jazayeri A, Falck J, Lukas C, Bartek J, Smith GC, Lukas J, Jackson SP (2006) ATM- and cell cycle-dependent regulation of ATR in response to DNA double-strand breaks. *Nat Cell Biol* 8:37–45
- Jazayeri A, Balestrini A, Garner E, Haber JE, Costanzo V (2008) Mre11- Rad50-Nbs1-dependent processing of DNA breaks generates oligonucleotides that stimulate ATM activity. *EMBO J* 27:1953–1962
- Johzuka K, Ogawa H (1995) Interaction of Mre11 and Rad50: two proteins required for DNA repair and meiosis-specific double-strand break formation in *Saccharomyces cerevisiae*. *Genetics* 139:1521–1532
- Kanaar R, Wyman C, Rothstein R (2008) Quality control of DNA break metabolism: in the 'end', it's a good thing. *EMBO J* 27:581–588
- Kim YC, Gerlitz G, Furusawa T, Catez F, Nussenzweig A, Oh KS, Kraemer KH, Shiloh Y, Bustin M (2009) Activation of ATM depends on chromatin interactions occurring before induction of DNA damage. *Nat Cell Biol* 11:92–96
- Kobayashi J (2004) Molecular mechanism of the recruitment of NBS1/hMRE11/hRAD50 complex to DNA double-strand breaks: NBS1 binds to gamma-H2AX through FHA/BRCT domain. *J Radiat Res (Tokyo)* 45:473–478
- Kobayashi J, Tauchi H, Sakamoto S, Nakamura A, Morishima K, Matsuura S, Kobayashi T, Tamai K, Tanimoto K, Komatsu K (2002) NBS1 localizes to gamma-H2AX foci through interaction with the FHA/BRCT domain. *Curr Biol* 12:1846–1851
- Kozlov SV, Graham ME, Peng C, Chen P, Robinson PJ, Lavin MF (2006) Involvement of novel autophosphorylation sites in ATM activation. *EMBO J* 25:3504–3514
- Krogh BO, Llorente B, Lam A, Symington LS (2005) Mutations in Mre11 phosphoesterase motif I that impair *Saccharomyces cerevisiae* Mre11-Rad50-Xrs2 complex stability in addition to nuclease activity. *Genetics* 171:1561–1570
- Lee JH, Paull TT (2004) Direct activation of the ATM protein kinase by the Mre11/Rad50/Nbs1 complex. *Science* 304:93–96
- Lee JH, Paull TT (2005) ATM activation by DNA double-strand breaks through the Mre11-Rad50-Nbs1 complex. *Science* 308:551–554

- Lee SE, Moore JK, Holmes A, Umezu K, Kolodner RD, Haber JE (1998) *Saccharomyces* Ku70, mre11/rad50 and RPA proteins regulate adaptation to G2/M arrest after DNA damage. *Cell* 94:399–409
- Lee JH, Ghirlando R, Bhaskara V, Hoffmeyer MR, Gu J, Paull TT (2003) Regulation of Mre11/Rad50 by Nbs1: effects on nucleotide-dependent DNA binding and association with ataxia-telangiectasia-like disorder mutant complexes. *J Biol Chem* 278:45171–45181
- Lengsfeld BM, Rattray AJ, Bhaskara V, Ghirlando R, Paull TT (2007) Sae2 is an endonuclease that processes hairpin DNA cooperatively with the Mre11/Rad50/Xrs2 complex. *Mol Cell* 28:638–651
- Lewis HA, Buchanan SG, Burley SK, Connors K, Dickey M, Dorwart M, Fowler R, Gao X, Guggino WB, Hendrickson WA et al (2004) Structure of nucleotide-binding domain 1 of the cystic fibrosis transmembrane conductance regulator. *EMBO J* 23:282–293
- Li X, Heyer WD (2008) Homologous recombination in DNA repair and DNA damage tolerance. *Cell Res* 18:99–113
- Li J, Lee GI, Van Doren SR, Walker JC (2000) The FHA domain mediates phosphoprotein interactions. *J Cell Sci* 113(Pt 23):4143–4149
- Li Y, Bolderson E, Kumar R, Muniandy PA, Xue Y, Richard DJ, Seidman M, Pandita TK, Khanna KK, Wang W (2009) hSSB1 and hSSB2 form similar multiprotein complexes that participate in DNA damage response. *J Biol Chem* 284:23525–23531
- Lim DS, Kim ST, Xu B, Maser RS, Lin J, Petrini JH, Kastan MB (2000) ATM phosphorylates p95/nbs1 in an S-phase checkpoint pathway. *Nature* 404:613–617
- Limbo O, Chahwan C, Yamada Y, de Bruin RA, Wittenberg C, Russell P (2007) Ctp1 is a cell-cycle-regulated protein that functions with Mre11 complex to control double-strand break repair by homologous recombination. *Mol Cell* 28:134–146
- Lisby M, Barlow JH, Burgess RC, Rothstein R (2004) Choreography of the DNA damage response: spatiotemporal relationships among checkpoint and repair proteins. *Cell* 118:699–713
- Llorente B, Symington LS (2004) The Mre11 nuclease is not required for 5' to 3' resection at multiple HO-induced double-strand breaks. *Mol Cell Biol* 24:9682–9694
- Luo G, Yao MS, Bender CF, Mills M, Bladl AR, Bradley A, Petrini JH (1999) Disruption of mRad50 causes embryonic stem cell lethality, abnormal embryonic development, and sensitivity to ionizing radiation. *Proc Natl Acad Sci USA* 96:7376–7381
- Mahajan A, Yuan C, Lee H, Chen ES, Wu PY, Tsai MD (2008) Structure and function of the phosphothreonine-specific FHA domain. *Sci Signal* 1:re12
- Manolis KG, Nimmo ER, Hartsuiker E, Carr AM, Jeggo PA, Allshire RC (2001) Novel functional requirements for non-homologous DNA end joining in *Schizosaccharomyces pombe*. *EMBO J* 20:210–221
- Mantiero D, Clerici M, Lucchini G, Longhese MP (2007) Dual role for *Saccharomyces cerevisiae* Tel1 in the checkpoint response to double-strand breaks. *EMBO Rep* 8:380–387
- Maser RS, Monsen KJ, Nelms BE, Petrini JH (1997) hMre11 and hRad50 nuclear foci are induced during the normal cellular response to DNA double-strand breaks. *Mol Cell Biol* 17:6087–6096
- Matsuoka S, Ballif BA, Smogorzewska A, McDonald ER 3rd, Hurov KE, Luo J, Bakalarski CE, Zhao Z, Solimini N, Lerenthal Y et al (2007) ATM and ATR substrate analysis reveals extensive protein networks responsive to DNA damage. *Science* 316:1160–1166
- Matsuura S, Tauchi H, Nakamura A, Kondo N, Sakamoto S, Endo S, Smeets D, Solder B, Belohradsky BH, Der Kaloustian VM et al (1998) Positional cloning of the gene for Nijmegen breakage syndrome. *Nat Genet* 19:179–181
- Matsuzaki K, Shinohara A, Shinohara M (2008) Forkhead-associated domain of yeast Xrs2, a homolog of human Nbs1, promotes nonhomologous end joining through interaction with a ligase IV partner protein, Lif1. *Genetics* 179:213–225
- Milne GT, Jin S, Shannon KB, Weaver DT (1996) Mutations in two Ku homologs define a DNA end-joining repair pathway in *Saccharomyces cerevisiae*. *Mol Cell Biol* 16:4189–4198
- Mimitou EP, Symington LS (2008) Sae2, Exo1 and Sgs1 collaborate in DNA double-strand break processing. *Nature* 455:770–774
- Mirzoeva OK, Petrini JH (2001) DNA damage-dependent nuclear dynamics of the Mre11 complex. *Mol Cell Biol* 21:281–288
- Moncalian G, Lengsfeld B, Bhaskara V, Hopfner KP, Karcher A, Alden E, Tainer JA, Paull TT (2004) The rad50 signature motif: essential to ATP binding and biological function. *J Mol Biol* 335:937–951
- Moore JK, Haber JE (1996) Cell cycle and genetic requirements of two pathways of nonhomologous end-joining repair of double-strand breaks in *Saccharomyces cerevisiae*. *Mol Cell Biol* 16:2164–2173
- Moreau S, Ferguson JR, Symington LS (1999) The nuclease activity of Mre11 is required for meiosis but not for mating type switching, end joining, or telomere maintenance. *Mol Cell Biol* 19:556–566
- Moreau S, Morgan EA, Symington LS (2001) Overlapping functions of the *Saccharomyces cerevisiae* Mre11, Exo1 and Rad27 nucleases in DNA metabolism. *Genetics* 159:1423–1433
- Moreno-Herrero F, de Jager M, Dekker NH, Kanaar R, Wyman C, Dekker C (2005) Mesoscale conformational changes in the DNA-repair complex Rad50/Mre11/Nbs1 upon binding DNA. *Nature* 437:440–443
- Mu JJ, Wang Y, Luo H, Leng M, Zhang J, Yang T, Besusso D, Jung SY, Qin J (2007) A proteomic analysis of ataxia telangiectasia-mutated (ATM)/ATM-Rad3-related (ATR) substrates identifies the ubiquitin-proteasome system as a regulator for DNA damage checkpoints. *J Biol Chem* 282:17330–17334
- Nairz K, Klein F (1997) mre11S—a yeast mutation that blocks double-strand- break processing and permits nonhomologous synapsis in meiosis. *Genes Dev* 11:2272–2290
- Nakada D, Matsumoto K, Sugimoto K (2003) ATM-related Tel1 associates with double-strand breaks through an Xrs2-dependent mechanism. *Genes Dev* 17:1957–1962
- Nakada D, Hirano Y, Sugimoto K (2004) Requirement of the Mre11 complex and exonuclease 1 for activation of the Mec1 signaling pathway. *Mol Cell Biol* 24:10016–10025
- Nam EA, Cortez D (2009) SOSS1/2: sensors of single-stranded DNA at a break. *Mol Cell* 35:258–259
- Nimonkar AV, Ozsoy AZ, Genschel J, Modrich P, Kowalczykowski SC (2008) Human exonuclease 1 and BLM helicase interact to resect DNA and initiate DNA repair. *Proc Natl Acad Sci USA* 105:16906–16911
- Niu H, Raynard S, Sung P (2009) Multiplicity of DNA end resection machineries in chromosome break repair. *Genes Dev* 23:1481–1486
- Olson E, Nievera CJ, Lee AY, Chen L, Wu X (2007) The Mre11-Rad50-Nbs1 complex acts both upstream and downstream of ataxia telangiectasia mutated and Rad3-related protein (ATR) to regulate the S-phase checkpoint following UV treatment. *J Biol Chem* 282:22939–22952
- Palmbo PL, Daley JM, Wilson TE (2005) Mutations of the Yku80 C terminus and Xrs2 FHA domain specifically block yeast nonhomologous end joining. *Mol Cell Biol* 25:10782–10790
- Palmbo PL, Wu D, Daley JM, Wilson TE (2008) Recruitment of *Saccharomyces cerevisiae* Dnl4-Lif1 complex to a double-strand break requires interactions with Yku80 and the Xrs2 FHA domain. *Genetics* 180:1809–1819
- Paull TT, Gellert M (1998) The 3' to 5' exonuclease activity of Mre 11 facilitates repair of DNA double-strand breaks. *Mol Cell* 1:969–979

- Paull TT, Gellert M (1999) Nbs1 potentiates ATP-driven DNA unwinding and endonuclease cleavage by the Mre11/Rad50 complex. *Genes Dev* 13:1276–1288
- Penkner A, Portik-Dobos Z, Tang L, Schnabel R, Novatchkova M, Jantsch V, Loidl J (2007) A conserved function for a *Caenorhabditis elegans* Com1/Sae2/CtIP protein homolog in meiotic recombination. *EMBO J* 26:5071–5082
- Rass E, Grabarz A, Plo I, Gautier J, Bertrand P, Lopez BS (2009) Role of Mre11 in chromosomal nonhomologous end joining in mammalian cells. *Nat Struct Mol Biol* 16:819–824
- Raymond WE, Kleckner N (1993) RAD50 protein of *S.cerevisiae* exhibits ATP-dependent DNA binding. *Nucleic Acids Res* 21:3851–3856
- Richard DJ, Bolderson E, Cubeddu L, Wadsworth RI, Savage K, Sharma GG, Nicolette ML, Tsvetanov S, McIlwraith MJ, Pandita RK et al (2008) Single-stranded DNA-binding protein hSSB1 is critical for genomic stability. *Nature* 453:677–681
- Rodriguez MC, Songyang Z (2008) BRCT domains: phosphopeptide binding and signaling modules. *Front Biosci* 13:5905–5915
- Rupnik A, Grenon M, Lowndes N (2008) The MRN complex. *Curr Biol* 18:R455–R457
- Sartori AA, Lukas C, Coates J, Mistrik M, Fu S, Bartek J, Baer R, Lukas J, Jackson SP (2007) Human CtIP promotes DNA end resection. *Nature* 450:509–514
- Schaetzlein S, Kodandaramireddy NR, Ju Z, Lechel A, Stepczynska A, Lilli DR, Clark AB, Rudolph C, Kuhnel F, Wei K et al (2007) Exonuclease-1 deletion impairs DNA damage signaling and prolongs lifespan of telomere-dysfunctional mice. *Cell* 130:863–877
- Shima H, Suzuki M, Shinohara M (2005) Isolation and characterization of novel xrs2 mutations in *Saccharomyces cerevisiae*. *Genetics* 170:71–85
- Shimada M, Sagae R, Kobayashi J, Habu T, Komatsu K (2009) Inactivation of the Nijmegen breakage syndrome gene leads to excess centrosome duplication via the ATR/BRCA1 pathway. *Cancer Res* 69:1768–1775
- Shiotani B, Zou L (2009) Single-stranded DNA orchestrates an ATM-to-ATR switch at DNA breaks. *Mol Cell* 33:547–558
- Shrivastav M, De Haro LP, Nickoloff JA (2008) Regulation of DNA double-strand break repair pathway choice. *Cell Res* 18:134–147
- Sinha KM, Unciuleac MC, Glickman MS, Shuman S (2009) AdnAB: a new DSB-resecting motor-nuclease from mycobacteria. *Genes Dev* 23:1423–1437
- Slijepcevic P (2006) The role of DNA damage response proteins at telomeres—an “integrative” model. *DNA Repair (Amst)* 5:1299–1306
- Soutoglou E, Misteli T (2008) Activation of the cellular DNA damage response in the absence of DNA lesions. *Science* 320:1507–1510
- Squatrito M, Gorrini C, Amati B (2006) Tip60 in DNA damage response and growth control: many tricks in one HAT. *Trends Cell Biol* 16:433–442
- Stewart GS, Maser RS, Stankovic T, Bressan DA, Kaplan MI, Jaspers NG, Raams A, Byrd PJ, Petrini JH, Taylor AM (1999) The DNA double-strand break repair gene hMRE11 is mutated in individuals with an ataxia-telangiectasia-like disorder. *Cell* 99:577–587
- Stracker TH, Theunissen JW, Morales M, Petrini JH (2004) The Mre11 complex and the metabolism of chromosome breaks: the importance of communicating and holding things together. *DNA Repair (Amst)* 3:845–854
- Sun Y, Xu Y, Roy K, Price BD (2007) DNA damage-induced acetylation of lysine 3016 of ATM activates ATM kinase activity. *Mol Cell Biol* 27:8502–8509
- Takeda S, Nakamura K, Taniguchi Y, Paull TT (2007) Ctp1/CtIP and the MRN complex collaborate in the initial steps of homologous recombination. *Mol Cell* 28:351–352
- Tauchi H, Kobayashi J, Morishima K, Matsuura S, Nakamura A, Shiraishi T, Ito E, Masnada D, Delia D, Komatsu K (2001) The forkhead-associated domain of NBS1 is essential for nuclear foci formation after irradiation but not essential for hRAD50-hMRE11-NBS1 complex DNA repair activity. *J Biol Chem* 276:12–15
- Tauchi H, Kobayashi J, Morishima K, van Gent DC, Shiraishi T, Verkaik NS, vanHeems D, Ito E, Nakamura A, Sonoda E et al (2002) Nbs1 is essential for DNA repair by homologous recombination in higher vertebrate cells. *Nature* 420:93–98
- Taylor AM, Groom A, Byrd PJ (2004) Ataxia-telangiectasia-like disorder (ATLD)-its clinical presentation and molecular basis. *DNA Repair (Amst)* 3:1219–1225
- The International Nijmegen Breakage Syndrome Study Group (2000) Nijmegen Breakage Syndrome. The International Nijmegen Breakage Syndrome Study Group. *Arch Dis Child* 82:400–406
- Tomita K, Matsuura A, Caspari T, Carr AM, Akamatsu Y, Iwasaki H, Mizuno KI, Ohta K, Uritani M, Ushimaru T et al (2003) Competition between the Rad50 complex and the Ku Heterodimer reveals a role for exo1 in processing double-strand breaks but not telomeres. *Mol Cell Biol* 23:5186–5197
- Tommiska J, Seal S, Renwick A, Barfoot R, Baskcomb L, Jayatilake H, Bartkova J, Tallila J, Kaare M, Tamminen A et al (2006) Evaluation of RAD50 in familial breast cancer predisposition. *Int J Cancer* 118:2911–2916
- Trujillo KM, Sung P (2001) DNA structure-specific nuclease activities in the *Saccharomyces cerevisiae* Rad50**Mre11* complex. *J Biol Chem* 276:35458–35464
- Trujillo KM, Yuan SS, Lee EY, Sung P (1998) Nuclease activities in a complex of human recombination and DNA repair factors Rad50, Mre11, and p95. *J Biol Chem* 273:21447–21450
- Tsubouchi H, Ogawa H (2000) Exo1 roles for repair of DNA double-strand breaks and meiotic crossing over in *Saccharomyces cerevisiae*. *Mol Biol Cell* 11:2221–2233
- Tsukamoto Y, Mitsuoka C, Terasawa M, Ogawa H, Ogawa T (2005) Xrs2p regulates Mre11p translocation to the nucleus and plays a role in telomere elongation and meiotic recombination. *Mol Biol Cell* 16:597–608
- Uanschou C, Siwiec T, Pedrosa-Harand A, Kerzendorfer C, Sanchez-Moran E, Novatchkova M, Akimcheva S, Woglar A, Klein F, Schlogelhofer P (2007) A novel plant gene essential for meiosis is related to the human CtIP and the yeast COM1/SAE2 gene. *EMBO J* 26:5061–5070
- Usui T, Ohta T, Oshiumi H, Tomizawa J, Ogawa H, Ogawa T (1998) Complex formation and functional versatility of Mre11 of budding yeast in recombination. *Cell* 95:705–716
- Usui T, Ogawa H, Petrini JH (2001) A DNA damage response pathway controlled by Tel1 and the Mre11 complex. *Mol Cell* 7:1255–1266
- Uziel T, Lerenthal Y, Moyal L, Andegeko Y, Mittelman L, Shiloh Y (2003) Requirement of the MRN complex for ATM activation by DNA damage. *EMBO J* 22:5612–5621
- van der Linden E, Sanchez H, Kinoshita E, Kanaar R, Wyman C (2009) RAD50 and NBS1 form a stable complex functional in DNA binding and tethering. *Nucleic Acids Res* 37(5):1580–1588
- Varon R, Vissinga C, Platzer M, Cerosaletti KM, Chrzanowska KH, Saar K, Beckmann G, Seemanova E, Cooper PR, Nowak NJ et al (1998) Nibrin, a novel DNA double-strand break repair protein, is mutated in Nijmegen breakage syndrome. *Cell* 93:467–476
- Wang Z, Cummins JM, Shen D, Cahill DP, Jallepalli PV, Wang TL, Parsons DW, Traverso G, Awad M, Silliman N et al (2004) Three classes of genes mutated in colorectal cancers with chromosomal instability. *Cancer Res* 64:2998–3001
- Weterings E, Chen DJ (2008) The endless tale of non-homologous end-joining. *Cell Res* 18:114–124
- Williams RS, Williams JS, Tainer JA (2007) Mre11-Rad50-Nbs1 is a keystone complex connecting DNA repair machinery, double-strand break signaling, and the chromatin template. *Biochem Cell Biol* 85:509–520
- Williams RS, Moncalian G, Williams JS, Yamada Y, Limbo O, Shin DS, Grocock LM, Cahill D, Hitomi C, Guenther G et al (2008)

- Mre11 dimers coordinate DNA end bridging and nuclease processing in double-strand-break repair. *Cell* 135:97–109
- Wiltzius JJ, Hohl M, Fleming JC, Petrini JH (2005) The Rad50 hook domain is a critical determinant of Mre11 complex functions. *Nat Struct Mol Biol* 12:403–407
- Wu G, Lee WH (2006) CtIP, a multivalent adaptor connecting transcriptional regulation, checkpoint control and tumor suppression. *Cell Cycle* 5:1592–1596
- Wu X, Ranganathan V, Weisman DS, Heine WF, Ciccone DN, O'Neill TB, Crick KE, Pierce KA, Lane WS, Rathbun G et al (2000) ATM phosphorylation of Nijmegen breakage syndrome protein is required in a DNA damage response. *Nature* 405:477–482
- Wu L, Luo K, Lou Z, Chen J (2008) MDC1 regulates intra-S-phase checkpoint by targeting NBS1 to DNA double-strand breaks. *Proc Natl Acad Sci USA* 105:11200–11205
- Xiao Y, Weaver DT (1997) Conditional gene targeted deletion by Cre recombinase demonstrates the requirement for the double-strand break repair Mre11 protein in murine embryonic stem cells. *Nucleic Acids Res* 25:2985–2991
- Xie A, Kwok A, Scully R (2009) Role of mammalian Mre11 in classical and alternative nonhomologous end joining. *Nat Struct Mol Biol* 16:814–818
- Yamaguchi-Iwai Y, Sonoda E, Sasaki MS, Morrison C, Haraguchi T, Hiraoka Y, Yamashita YM, Yagi T, Takata M, Price C et al (1999) Mre11 is essential for the maintenance of chromosomal DNA in vertebrate cells. *EMBO J* 18:6619–6629
- Yang YG, Saidi A, Frappart PO, Min W, Barrucand C, Dumon-Jones V, Michelon J, Herceg Z, Wang ZQ (2006) Conditional deletion of Nbs1 in murine cells reveals its role in branching repair pathways of DNA double-strand breaks. *EMBO J* 25:5527–5538
- Yoo HY, Kumagai A, Shevchenko A, Shevchenko A, Dunphy WG (2009) The Mre11-Rad50-Nbs1 complex mediates activation of TopBP1 by ATM. *Mol Biol Cell* 20:2351–2360
- You Z, Chahwan C, Bailis J, Hunter T, Russell P (2005) ATM activation and its recruitment to damaged DNA require binding to the C terminus of Nbs1. *Mol Cell Biol* 25:5363–5379
- Yu X, Baer R (2000) Nuclear localization and cell cycle-specific expression of CtIP, a protein that associates with the BRCA1 tumor suppressor. *J Biol Chem* 275:18541–18549
- Yu X, Chen J (2004) DNA damage-induced cell cycle checkpoint control requires CtIP, a phosphorylation-dependent binding partner of BRCA1 C-terminal domains. *Mol Cell Biol* 24:9478–9486
- Yu X, Chini CC, He M, Mer G, Chen J (2003) The BRCT domain is a phospho-protein binding domain. *Science* 302:639–642
- Yu X, Fu S, Lai M, Baer R, Chen J (2006) BRCA1 ubiquitinates its phosphorylation-dependent binding partner CtIP. *Genes Dev* 20:1721–1726
- Yuan Z, Zhang X, Sengupta N, Lane WS, Seto E (2007) SIRT1 regulates the function of the Nijmegen breakage syndrome protein. *Mol Cell* 27:149–162
- Yun MH, Hiom K (2009) CtIP-BRCA1 modulates the choice of DNA double-strand-break repair pathway throughout the cell cycle. *Nature* 459:460–463
- Zha S, Boboila C, Alt FW (2009) Mre11: roles in DNA repair beyond homologous recombination. *Nat Struct Mol Biol* 16:798–800
- Zhang Y, Zhou J, Lim CU (2006) The role of NBS1 in DNA double strand break repair, telomere stability, and cell cycle checkpoint control. *Cell Res* 16:45–54
- Zhu J, Petersen S, Tessarollo L, Nussenzweig A (2001) Targeted disruption of the Nijmegen breakage syndrome gene NBS1 leads to early embryonic lethality in mice. *Curr Biol* 11:105–109
- Zhu Z, Chung WH, Shim EY, Lee SE, Ira G (2008) Sgs1 helicase and two nucleases Dna2 and Exo1 resect DNA double-strand break ends. *Cell* 134:981–994