



# Structural Studies of DNA End Detection and Resection in Homologous Recombination

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DNA double-strand breaks are repaired by two major pathways, homologous recombination or nonhomologous end joining. The commitment to one or the other pathway proceeds via different steps of resection of the DNA ends, which is controlled and executed by a set of DNA double-strand break sensors, endo- and exonucleases, helicases, and DNA damage response factors. The molecular choreography of the underlying protein machinery is beginning to emerge. In this review, we discuss the early steps of genetic recombination and double-strand break sensing with an emphasis on structural and molecular studies.

All domains of life maintain genomes and ensure genetic diversity through homologous recombination (HR) or homology directed repair. HR is initiated by single unprotected DNA ends, which arise at collapsed replication forks and unprotected telomeres, or by DNA double-strand breaks (DSBs), which are products of ionizing radiation, reactive oxygen species, genotoxic chemicals, or abortive topoisomerase reactions (Sutherland et al. 2000; Aguilera and Gomez-Gonzalez 2008; Cadet et al. 2012; Mehta and Haber 2014). In special cellular states, programmed DSBs are introduced by endonucleases to initiate the generation of genetic variability by processes such as meiotic recombination of homologous chromosomes (Lam and Keeney 2014; Zickler and Kleckner 2014), V(D)J and class switch recombination

to generate antibody diversity and yeast-mating-type switching (Gapud and Sleckman 2011; Haber 2012; Xu et al. 2012b). Failure to repair DSBs can lead to cell death or gross chromosomal aberrations, which in humans are a hallmark of cancer (Myung et al. 2001a,b; Hanahan and Weinberg 2011).

Beside HR, DSBs can also be repaired by nonhomologous end joining (NHEJ). Although HR requires a template such as a sister chromatid or a homologous chromosome and is limited to S and G<sub>2</sub> phases of the cell cycle, NHEJ is template-independent and can occur in all cell cycle states. Indeed, the choice of pathways is to a significant extent not stochastic but a function of the cell cycle (Ferretti et al. 2013), with NHEJ being the predominant pathway in mammals outside of S phase. NHEJ is basically a

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ligation reaction of two DNA ends that are only minimally processed. Derivatives of NHEJ such as microhomology-mediated end joining (MMEJ) or alternative NHEJ (alt-NHEJ) require more substantial processing and may lead to the loss of genetic information. For recent reviews of NHEJ, which is not covered in detail here, please refer to, for example, Thompson (2012) and Chiruvella et al. (2013).

HR has multiple steps and requires extensive processing of DNA ends (Symington 2014). First, the free DNA ends are recognized by DSB sensors, followed by 5'-3' resection of the DNA ends. In eukaryotes and archaea, this step may be divided into initial short-range resection, after which MMEJ/alt-NHEJ can still occur, followed by processive long-range resection that commits the pathway to HR. The 3' single-stranded DNA (ssDNA) filament, bound by the DNA strand exchange protein RecA/Rad51, pairs with the homologous sequence on the template and thus forms a D-loop. The 3' tail serves as a primer for a repair polymerase and is extended by using the homologous strand as template, a process that “restores” the disrupted genetic information. Various pathways involve the displacement of the free strand, the capture of the second strand to form Holliday junctions, or the cleavage of the D-loop (Mehta and Haber 2014).

In this review, we focus on structural aspects of the early steps in homologous recombination. Of particular interest is the Mre11-Rad50-Nbs1 (MRN) complex, which recognizes DSBs, performs initial resection, and sets off a DNA damage response (DDR) signaling network. We further discuss the nucleases and helicases that are involved in long-range resection. Recent reviews of later steps in HR, which are not covered here, have been published elsewhere (Amunugama and Fishel 2012; Chiruvella et al. 2013; Jasin and Rothstein 2013).

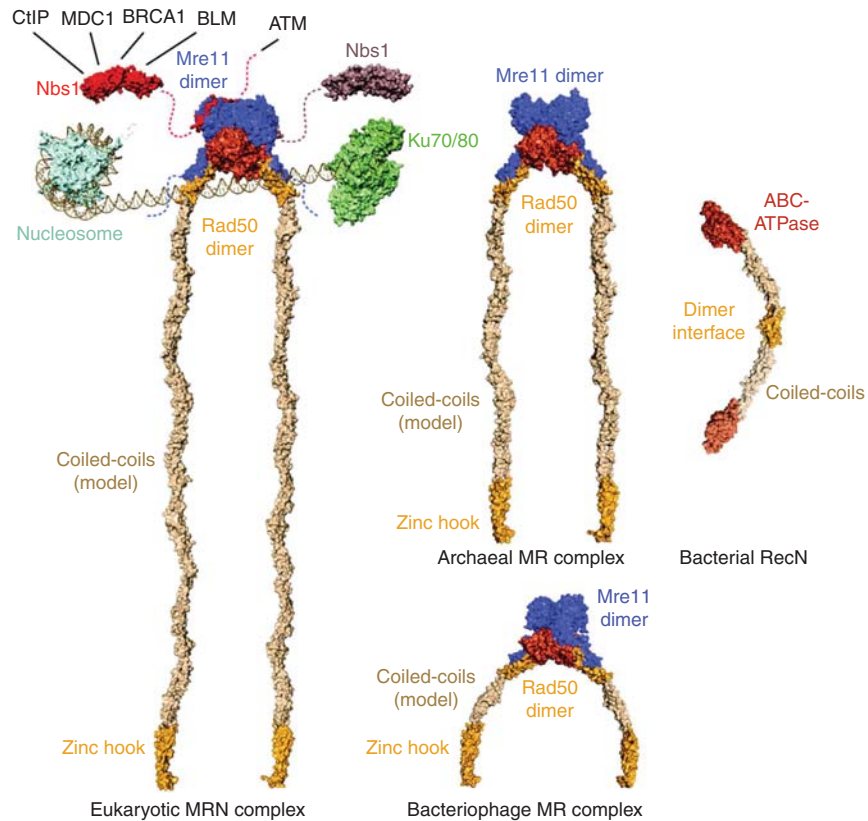
## DSB END RECOGNITION

### The Mre11-Rad50-Nbs1 Complex

Among the early and central players in DNA end metabolism are Ku and the Mre11-Rad50-Nbs1 (MRN) complex, which are considered

“sensors” for DSBs. Ku binds to DNA ends as a ring-shaped heterodimer (Fig. 1) consisting of Ku70/Ku80 and initiates NHEJ (Walker et al. 2001; Chiruvella et al. 2013). The *Saccharomyces cerevisiae* MRN homolog, Mre11-Rad50-Xrs2 (MRX), has been shown to be one of the first complexes that are recruited to DSBs (Lisby et al. 2004). MRN is involved in the selection of DSB repair pathways that require end resection (HR, MMEJ, alt-NHEJ) as opposed to NHEJ (Truong et al. 2013). Homologs of Mre11 and Rad50 (MR) are present in all domains of life and may be fused into a single peptide chain (Yoshida et al. 2011).

MRN is a multifunctional ATP-regulated nuclease with endo- and exonuclease activity and long structural tails. In vitro, the MR(N) complex is able to partially melt and unwind DNA and displays both 3' to 5' exonuclease and ssDNA endonuclease activity to process DSBs (Connelly et al. 1997, 1999; Furuse et al. 1998; Paull and Gellert 1998; Trujillo et al. 1998; Hopfner et al. 2000a, 2001; Trujillo and Sung 2001; Lobachev et al. 2002; Hopkins and Paull 2008; Cannon et al. 2013). Bacteriophage T4 also possesses homologs of Mre11 and Rad50 (gp46/gp47), which play an essential role in initiation of recombination-dependent replication at later stages of infection (Kreuzer and Brister 2010; Almond et al. 2013). In bacteria, MR (denoted SbcCD) degrades hairpin structures in the wake of replication forks and protects the cell against inverted chromosome duplication together with RecA (Zahra et al. 2007; Eykelenboom et al. 2008; Darmon et al. 2010). In archaea, like in eukaryotes, MR(N) is recruited to and repairs DSBs that are induced using ionizing radiation or genotoxic agents and that arise at stalled replication forks (Costanzo et al. 2001; Neale et al. 2005; Trenz et al. 2006; Frols et al. 2007; Quaiser et al. 2008; Delmas et al. 2009, 2013). In eukaryotes, MRN also processes newly replicated telomeres and DSBs that are blocked by DNA hairpin structures or by proteins, such as Ku and the meiotic recombination factor Spo11 (Lobachev et al. 2002; Connelly et al. 2003; Neale et al. 2005; Bonetti et al. 2010; Mimitou and Symington 2010; Langerak et al. 2011).



**Figure 1.** The Mre11-Rad50-Nbs1 complex and phylogenetic orthologs. Structural model of MR(N) complexes together with a nucleosome, the Ku-DNA complex and RecN. Nbs1 interaction partners are indicated. The eukaryotic MRN model was built from *Schizosaccharomyces pombe* MN and Nbs1 (PDB code 4FBW, Schiller et al. 2012), *Methanocaldococcus jannaschii* MR, *Pyrococcus furiosus* Zn-hook and a coiled-coil model. The archaeal model is based on the *M. jannaschii* MR structure and the *P. furiosus* Zn-hook. Bacteriophage MR is modeled on the *Thermotoga maritima* MR complex together with the *P. furiosus* Zn-hook and a coiled-coil model. PDB codes are 1AOI (nucleosome, Luger et al. 1997), 1JEY (Ku-DNA complex, Walker et al. 2001), 4AD8 and 4ABX (RecN, Pellegrino et al. 2012), 4FBW (MN complex, Schiller et al. 2012), 3HUE (Nbs1, Williams et al. 2009), 3AVO (MR complex, Lim et al. 2011), and 1L8D (Zn-hook, Hopfner et al. 2002).

How MR(N) functions as a DNA end sensor and processing factor is still poorly understood. Although Ku forms a ring structure with DSB-binding affinity in the nanomolar range (Fig. 1) (Blier et al. 1993; Walker et al. 2001), readily explaining how it acts as a DSB sensor, we have not yet arrived at a model that explains the mechanism of DSB detection by MR(N). Many bulk biochemistry experiments on MRN or MR homologs show a relatively moderate DNA-binding affinity in the high nanomolar to micromolar range and, in general, no clear binding specificity for DNA ends (e.g., Lee et al.

2003; Möckel et al. 2012). However, recent single-molecule fluorescence resonance energy transfer (FRET) analysis of human MRN determined an extraordinarily high DNA-binding affinity in the picomolar range (Cannon et al. 2013). This discrepancy may be caused by differing experimental conditions. MR(N) is intrinsically able to form large macromolecular assemblies in vitro (de Jager et al. 2001), and the ratio of higher-order to lower-order multimers of MR(N) might influence its affinity to DNA. This relationship may partly explain the apparent involvement of the Rad50 coiled-



coil domain in high affinity DNA binding, as this domain mediates MR(N) multimerization (Lee et al. 2013).

During the last decade, a substantial number of high- and low-resolution structural studies of MR and MRN components have led to plausible models for MR and MRN complexes from different domains of life (Fig. 1). MR or MRN form large bipolar complexes with globular heads that harbor the nucleotide-binding domains (NBDs) of Rad50 and the nuclease domain of Mre11 (Connelly et al. 1998; Anderson et al. 2001; de Jager et al. 2001; Hopfner et al. 2001). The Mre11 nuclease dimerizes and forms the center of the head module (Hopfner et al. 2001; Williams et al. 2008; Das et al. 2010; Park et al. 2011). Each Mre11 protomer binds one Rad50 coiled-coil domain near the Rad50 NBD, generating a conserved  $M_2R_2$  architecture (Hopfner et al. 2001; Lammens et al. 2011; Lim et al. 2011; Limbo et al. 2012). Prokaryotic Mre11 binds to Rad50 through a carboxy-terminal helix-loop-helix motif (Fig. 2A) (Lammens et al. 2011; Lim et al. 2011; Möckel et al. 2012). The interaction of eukaryotic Mre11 and Rad50 has not been described on a structural level yet. However, structural information is available for the interaction of *S. pombe* Mre11 with Nbs1, which binds to the Mre11 nuclease dimer through a conserved motif near the carboxyl terminus of Nbs1 (Schiller et al. 2012).

### The Mre11 Nuclease

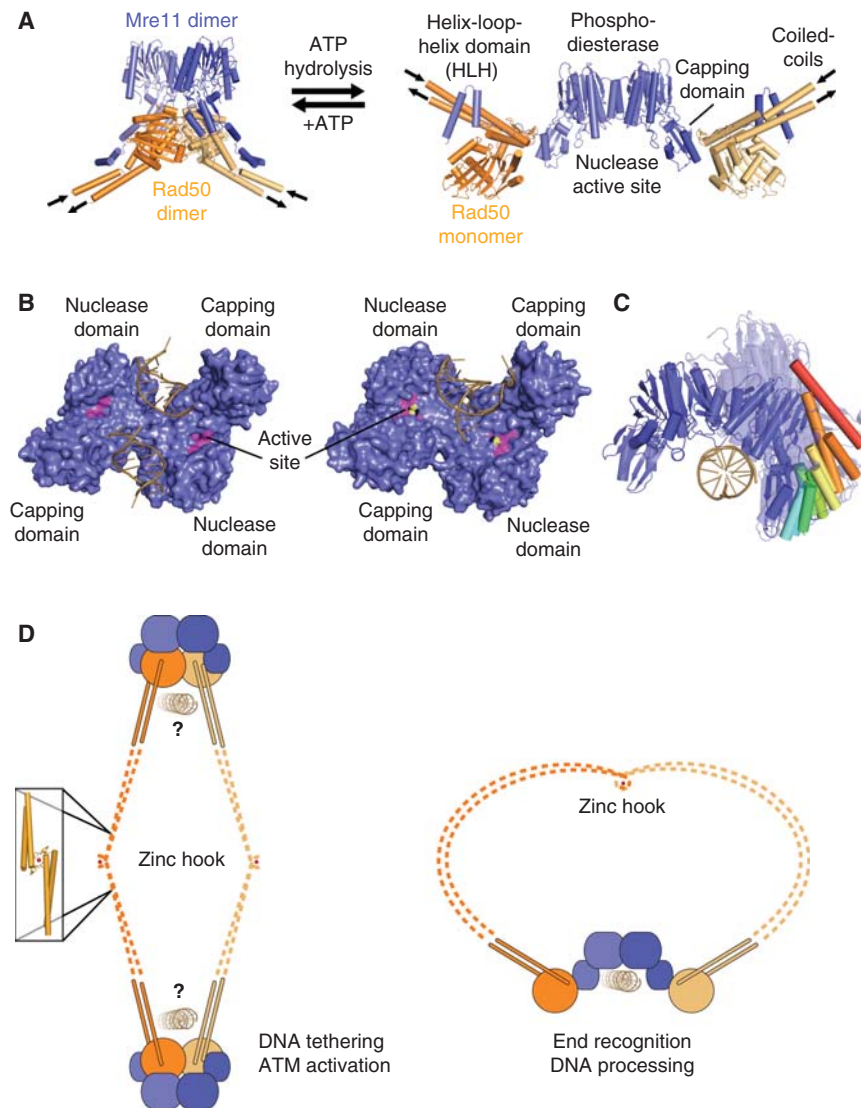
Mre11 interacts with both Rad50 and Nbs1 and can be envisioned as the core of the MRN complex. Crystal structures of Mre11 homologs from all three domains of life emphasize the high structural conservation of the amino-terminal Mre11 domain and a universally conserved dimer architecture (Fig. 2B,C) (Hopfner et al. 2001; Arthur et al. 2004; Williams et al. 2008; Das et al. 2010; Lammens et al. 2011; Lim et al. 2011; Limbo et al. 2012; Möckel et al. 2012; Schiller et al. 2012; Liu et al. 2014). The functional importance of Mre11 dimerization is highlighted by findings that mutations of the yeast Mre11 dimer interface phenocopy an *mre11* knockout (Williams et al. 2008; Schiller

et al. 2012). The conserved amino-terminal domain of Mre11 consists of a phosphoesterase domain and an adjacent capping domain (Fig. 2B). The phosphoesterase active site coordinates two manganese ions, which are essential for exonuclease and ssDNA endonuclease activities (Trujillo et al. 1998; Hopfner et al. 2001).

The Mre11 dimer can directly bind and bridge two DNA ends in vitro (Fig. 2B) (Chen et al. 2001; Williams et al. 2008; Ghodke and Muniyappa 2013), a function that could be important in the context of HR and end-joining reactions (Reis et al. 2012). It is also known that the carboxyl terminus of eukaryotic Mre11 contains additional DNA-binding sites. One site maps to a region adjacent to the capping domain and is crucial for DSB-repair functions. A second DNA-binding motif at the carboxyl terminus of Mre11 was shown to be essential for DSB formation and spore viability in meiosis in *S. cerevisiae* (Furuse et al. 1998; Usui et al. 1998). Metazoan Mre11 homologs contain, in addition, a glycine/arginine-rich (GAR) motif, which is important for DNA binding and nuclease activity in vitro and localization to DSBs in vivo (Dery et al. 2008).

Comparison of all published structures reveals that the Mre11 dimer angle is not fixed, but it shows a large pivot angle range of one protomer with respect to the other (Fig. 2C). The observed variation of the dimer angle is not necessarily species specific, as *S. pombe* Mre11, for instance, was crystallized in very different dimer angles in the presence and absence of Nbs1 (Schiller et al. 2012). There might be a correlation between the Mre11 dimer angle and different binding states of Rad50, DNA, and Nbs1. Thus, the observed conformational flexibility might be an important functional aspect that should be addressed in future studies. An exceptional and somewhat surprising case is that of human Mre11, which was crystallized as a dimer cross-linked by an unexpected disulfide bond that leads to an unusual dimer interface and abolishes flexibility (Park et al. 2011).

At present, we have some basic understanding of the interaction of Mre11 with DNA, but important questions remain open. The metal-binding site with its conserved dimetal coordi-



**Figure 2.** The Mre11 nuclease and its regulation by Rad50. (A) Structure of the ATP-bound and ATP-free *T. maritima* MR complex. The PDB codes are 3QG5 and 3THO (Lammens et al. 2011; Möckel et al. 2012). (B) Comparison of Mre11-DNA structures: the surface of the Mre11 dimer (blue) bound to synaptic DNA (left) and branched DNA (right). In the right structure, the active site (magenta) coordinates two manganese ions (yellow). The PDB codes are 3DSC (synaptic DNA) and 3DSD (branched DNA, Williams et al. 2008). (C) Mre11 structure comparison: dimeric crystal structures are aligned onto the left monomer of *P. furiosus* Mre11 (blue) (PDB code is 1S8E, Arthur et al. 2004). For clarity, the overlaid monomers are not depicted, the right monomers are transparent, and the first  $\alpha$ -helix from the capping domain is marked from blue to red to highlight the differences. DNA (sand) indicates the accessible nuclease active site. The PDB codes are 1II7 (Hopfner et al. 2001), 3DSD, 3DSC (Williams et al. 2008), 4HD0 (Limbo et al. 2012), 3AUZ, 3AV0 (Lim et al. 2011), 3THO, 3THN (Möckel et al. 2012), 3QG5 (Lammens et al. 2011), 2Q8U (Das et al. 2010), 4FBQ, 4FBW, 4FBK, and 4FCX (Schiller et al. 2012). (D) MR model for DNA tethering and processing: Mre11 (blue) in complex with Rad50 (orange) forms intercomplex (left) and intracomplex (right) interactions through the zinc hook (zinc ion, red).



nating histidines readily explains the preference for manganese over magnesium for the 3' exonuclease. However, *P. furiosus* Mre11 was also shown to possess magnesium-dependent endonuclease activity that promotes 5' strand resection, the structural features of which remain elusive so far (Hopkins and Paull 2008). Moreover, our understanding of the molecular mechanism of DNA processing by Mre11 is still limited by the lack of a structure of Mre11 bound to a transition state DNA substrate.

### The Rad50 Coiled-Coils

Arguably, the most distinguished yet most poorly understood structural feature of the MRN complex is the long coiled-coil extensions of Rad50. They emerge from the NBDs of Rad50 and carry the universally conserved “zinc-hook” dimerization motif at their apices (Fig. 1) (Hopfner et al. 2002). Two zinc hooks can dimerize by jointly coordinating a zinc ion via four invariant cysteines, two from each zinc hook (Fig. 2D) (Hopfner et al. 2002). In vitro, this dimerization can tether different MRN complexes or help to form supramolecular assemblies to cross-link DNA (de Jager et al. 2001; Hopfner et al. 2002), a feature that may explain the ability of MRN to aggregate DNA in *Xenopus* cell extracts (Costanzo et al. 2004).

Although the lengths of the coiled-coils are rather conserved between more closely related phylogenetic taxa, they can considerably vary between the different domains of life (Fig. 1). Studies in yeast have shown that the zinc hooks are critical for the function of the complex, but can be partly substituted by dimerization domains of a different type (Wiltzius et al. 2005) or can be compensated for by higher concentrations of MRN in the context of ATM activation (Lee et al. 2013). However, reduction of the length of the coiled-coil dramatically impairs functionality of the MRN complex (Hohl et al. 2011; Deshpande et al. 2014). It is interesting to note that yeast MRN is impaired when the length of the Rad50 coiled-coils is reduced to that of the bacteriophage protein. These results suggest that the dimensions of the Rad50 coiled-coil regions seem to be functionally relevant, but

the mechanistic requirements differ strongly between phylogenetic kingdoms and phages. However, care should be taken in the interpretation of these results and the design of such studies, as it is difficult to alter the length of coiled-coil domains without affecting their proper assembly or the orientation of the zinc hooks because of the helical nature of coiled-coils.

Scanning force microscopy (SFM) shows that the coiled-coil domains of Rad50 are organized into segments with flexible hinges that seem to coincide with regions of lower coiled-coil propensity (van Noort et al. 2003; de Jager et al. 2004). Because of this flexibility, two coiled-coil domains can form both inter- and intracomplex interactions, mediated by the dimerization of two zinc-hook motifs (Fig. 2D) (de Jager et al. 2001; Hopfner et al. 2001, 2002; Moreno-Herrero et al. 2005). Importantly, the recent structure of a small, Rad50-like prokaryotic DSB repair factor, RecN, described, for the first time, an atomic model for a full Rad50/SMC/RecN-type structure, assembled from overlapping, crystallographically resolved fragments (Fig. 1) (Pellegrino et al. 2012). This RecN dimer model illustrates the segmental nature of the coiled-coils, but at the same time, it suggests that the coiled-coil domain is overall rather stiff (Fig. 1).

### Integrative Model for MR Mechanism

The ATP-binding and hydrolysis motifs of Rad50 are functionally critical elements of MRN. The NBDs of Rad50 dimerize in response to ATP binding, and studies with isolated NBDs show that Rad50 binds DNA in this ATP-engaged conformation (Hopfner et al. 2000b). ATP binding to the NBDs is also important for other functions of the complex such as activation of DNA damage checkpoint regulator ATM (Lee et al. 2013; Deshpande et al. 2014). Recent structural analysis on Mre11-Rad50<sup>NBD</sup> head complexes revealed that the NBDs of Rad50 are far apart in the absence of ATP, allowing DNA to access the Mre11 nuclease active sites (Fig. 2A) (Lammens et al. 2011). In the presence of ATP, however, the two NBDs dimerize and bind into the DNA-binding/nuclease

cleft of the Mre11 dimer (Lim et al. 2011; Möckel et al. 2012; Deshpande et al. 2014). In this conformation, the two active sites of the Mre11 dimer are blocked, at least for binding of double-stranded DNA (dsDNA). These structural studies are consistent with reports that ATP binding to Rad50 negatively regulates the processive 3' dsDNA exonuclease and dsDNA endonuclease activity (but not the ssDNA endonuclease activity) of Mre11 (Herdendorf et al. 2011; Lim et al. 2011; Majka et al. 2012; Deshpande et al. 2014). The closed, ATP-bound conformation is also the conformation that activates ATM (Lee et al. 2013; Deshpande et al. 2014). Thus, a model may be formulated that was confirmed in a very recent study (Deshpande et al. 2014): The closed MR(N) complex is involved in ATM activation and DSB recognition or tethering, whereas the open complex after ATP hydrolysis is involved in DNA processing (Fig. 2D). It is yet unclear, however, how MRN binds DNA in the closed conformation, in which the Mre11 dsDNA-binding sites are blocked. We also do not know how Rad50 interacts with DNA.

The nature of supramolecular structures of MR and MRN that involve additional interactions mediated by the coiled-coils still needs to be resolved. Several different architectures are conceivable and may play roles in recombination and end joining. Using scanning force microscopic analysis of human MRN, DNA binding was shown to cause a shift from intra-MRN to inter-MRN hook–hook interactions through a mesoscale conformational change (Fig. 2D) (Moreno-Herrero et al. 2005). Therefore, the formation of higher-order structures could be directly coupled to DNA binding. The situation may be different for the rather short coiled-coil structures of the bacteriophage Rad50 orthologs, which leave little room for intramolecular coiled-coil interactions; thus, more work is needed to functionally dissect and validate different superstructures.

### Nbs1

The eukaryote-specific subunit of the MRN complex, Nbs1 (or Xrs2 in *S. cerevisiae*), has multiple functions. It was found to stimulate

DNA binding and unwinding of MRN (Paull and Gellert 1999; Trujillo et al. 2003) and is necessary for the nuclear localization of Mre11 and Rad50 (Carney et al. 1998; Desai-Mehta et al. 2001; Tsukamoto et al. 2005). Nbs1 recruits and helps to activate the DNA damage checkpoint regulator ATM/Tel1p (Nakada et al. 2003; Falck et al. 2005; You et al. 2005; Berkovich et al. 2007). Although MR alone seems to be able to interact with ATM in vitro (Costanzo et al. 2004; Lee and Paull 2004; Lee and Paull 2005), the Nbs1 carboxyl terminus was shown to interact with and activate ATM through an acidic patch and a FXF/Y motif (Falck et al. 2005; You et al. 2005). A carboxy-terminal 147-amino-acid fragment of Nbs1 carrying these two motifs was sufficient to restore ATM activation in an Nbs1-depleted *Xenopus* egg extract (You et al. 2005). In addition, the carboxyl terminus of Nbs1 was found to be necessary for control of cell cycle arrest and apoptosis signals in a mouse model (Stracker et al. 2007).

Nbs1 comprises a folded amino-terminal region and a carboxy-terminal part predicted to be of low structural order (Williams et al. 2009). Crystal structures of the amino-terminal folded region revealed a rigid structure that consists of a fork-head-associated (FHA) domain and tandem BRCA1 carboxy-terminal (BRCT) domains (Lloyd et al. 2009; Williams et al. 2009). FHA and BRCT domains have been shown to recognize phosphoproteins (Durocher and Jackson 2002; Yu et al. 2003). In Nbs1, these domains serve as a recruitment platform for various DSB repair factors such as mediator of DNA damage checkpoint protein 1 (MDC1), Bloom syndrome mutated (BLM), breast cancer 1 (BRCA1), CtBP-interacting protein (CtIP), and phosphorylated histone H2AX (via MDC1) (Fig. 1) (Wang et al. 2000; Burma et al. 2001; Kobayashi et al. 2002; Chapman and Jackson 2008; Chen et al. 2008; Melander et al. 2008; Spycher et al. 2008; Wu et al. 2008). At least in the case of MDC1, both FHA and BRCT domains participate in an interdependent fashion (Lloyd et al. 2009; Hari et al. 2010).

Because of its flexible nature, only limited structural information is available for the carboxy-terminal region of Nbs1. Nbs1 binds to

Mre11 through a conserved NFKxFxK motif in this carboxy-terminal region (Desai-Mehta et al. 2001; Tauchi et al. 2001; You et al. 2005; Schiller et al. 2012). Significantly, the crystal structure of *S. pombe* Mre11 in complex with a carboxy-terminal fragment of Nbs1 showed that this peptide binds across the Mre11 dimer and breaks its symmetry (Schiller et al. 2012). Whether this binding has only the function to tether Mre11 to Nbs1 or—as the peculiar interaction at the Mre11 dimer axis may indicate—is functionally linked to Mre11-Rad50 conformations should be subject of future studies. It also remains to be clarified how this apparently asymmetric binding translates into the stoichiometry of the MRN complex (2:2:2 or 2:2:1).

#### Mutations in Mre11-Rad50-Nbs1 in Human Disease

Although knockouts of *MRE11*, *RAD50*, and *NBS1* are lethal in mice (Luo et al. 1999; Zhu et al. 2001; Buis et al. 2008), there are hypomorphic mutations of these genes that are associated with a set of related but phenotypically distinct syndromes such as ataxia-telangiectasia-like disease (ATLD), Nijmegen breakage syndrome (NBS), and NBS-like disorder (NBSLD). These diseases are related to ataxia telangiectasia (A-T), which is caused by mutations in *ATM* (Savitsky et al. 1995). All three MRN-associated syndromes and A-T share phenotypes on a cellular level, but patients differ with respect to the extent of neurological, immunological, and cancer predisposition disorders. Whereas NBS and NBSLD lead to microcephaly, A-T and ATLD are associated with neurodegeneration (Carney et al. 1998; Varon et al. 1998; Stewart et al. 1999; Maser et al. 2001; Waltes et al. 2009; Matsumoto et al. 2011).

Presently, the literature describes 18 cases of ATLD and one case of NBSLD that were all linked to mutations in the *MRE11* gene and one NBSLD patient with two *RAD50* mutations (Hernandez et al. 1993; Stewart et al. 1999; Pitts et al. 2001; Delia et al. 2004; Fernet et al. 2005; Uchisaka et al. 2009; Matsumoto et al. 2011; Palmeri et al. 2013). The availability of atomic structures of eukaryotic Mre11 and Nbs1 and

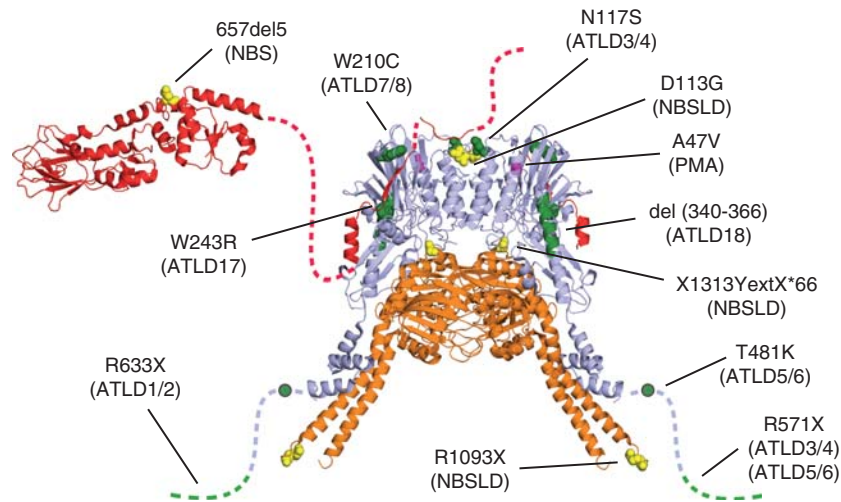
prokaryotic Rad50 and the high degree of conservation of MRN allow us to map the underlying mutations onto a structural model of the MRN complex (Fig. 3). Most mutations described so far, apart from truncation mutants, map to the interface between Nbs1 and Mre11. As this interface is quite extended, point mutations reduce, but do not abolish, the interaction between Nbs1 and Mre11, explaining their hypomorphic nature. Functional analysis of some mutations by mutating corresponding conserved residues in *S. cerevisiae* MRX showed that an ATLD-mimicking mutation did impair mitotic repair functions solely by lowering the nuclear concentration of MRX (Schiller et al. 2012). In addition, telomere maintenance was affected, suggesting a defect in Tel1/ATM activation. For another ATLD-mimicking mutation, a study in *S. pombe* showed that DSB repair was affected, but not Tel1/ATM activation (Limbo et al. 2012). This situation is somewhat surprising because ATLD is similar to A-T, which is caused by inactivation of ATM. Very recently, progressive myoclonic ataxia (PMA) was also linked to an *MRE11* mutation that maps to the surroundings of the Nbs1–Mre11 interface (Miyamoto et al. 2013).

Further work is thus necessary to correlate the molecular defects in MRN with the observed disease phenotypes. However, the structural studies on the conformational and functional states of MRN will now allow a more detailed structure–function correlation. The mutations may affect these distinct states of MRN and may lead to partial separation of function, which may explain how different disease phenotypes such as NBS and ATLD can result from mutations in a single complex.

#### RESECTION

Once a DNA DSB has been recognized, 5′-3′ resection of the DNA ends may proceed, which requires a 5′-3′ nuclease and, in most pathways, a helicase. Although this principle holds true for all three domains of life, resection and the initiation thereof are governed by different machineries with conservation limited to single domains. In bacteria, the multisubunit com-





**Figure 3.** MRN and human disease. Mapping of MRN mutations found in human disorders onto a model of MRN (model and color code from Fig. 1). ATLD, NBS/-LD, and PMA mutations are indicated in green, yellow, and lilac, respectively.

plexes RecBCD and AddAB are stand-alone machineries that recognize DSBs, initiate resection, and perform long-range resection in a highly processive way (for an excellent recent review, see Wigley 2013). Under certain circumstances, an alternative resection pathway may take over that involves the nuclease RecJ and the helicase RecQ (Handa et al. 2009). In archaea, the MR complex identifies DSBs and initiates resection, but a complex comprising the nuclease NurA and the helicase HerA executes long-range resection (Hopkins and Paull 2008; Blackwood et al. 2012). There is evidence, however, that NurA-HerA may form a larger resection complex together with MR (Quaiser et al. 2008). In eukaryotes, the MRN complex initiates resection together with the protein CtIP (Limbo et al. 2007; Mimitou and Symington 2008). Eukaryotic long-range resection has been found to follow partly redundant pathways that involve either the processive nuclease Exo1 or the complex of the nuclease/helicase DNA2 and the RecQ-like helicase Bloom syndrome mutated (BLM, Sgs1 in yeast) (Gravel et al. 2008; Mimitou and Symington 2008; Nimonkar et al. 2008; Zhu et al. 2008). In the following section, we will describe the initiation of resection in eukaryotes, followed by a discussion of the re-

cent advances of our structural understanding of the Exo1, DNA2/BLM, and NurA/HerA pathways.

### Initiation of Resection in Eukaryotes

In eukaryotes, initial resection of DSBs requires the MRN complex and CtIP. The precise biochemical function of CtIP is still controversial. CtIP was first characterized as an interaction factor of the transcriptional repressor CtBP, RB1, and the DNA repair and checkpoint protein BRCA1 (Fusco et al. 1998; Schaeper et al. 1998; Wong et al. 1998; Yu et al. 1998). Putative orthologs of CtIP are found in most eukaryotic species, although sequence identity is limited to small regions at the amino and carboxyl termini. CtIP orthologs (Sae2 in *S. cerevisiae* and Ctp1 in *S. pombe*) also vary considerably in length and may have diverged in their exact function, for example, with regard to the interaction with other proteins. Nonetheless, both Sae2 and Ctp1 were reported to play roles in initial DNA end resection similar to vertebrate CtIP (McKee and Kleckner 1997; Prinz et al. 1997; Lengsfeld et al. 2007; Limbo et al. 2007; Akamatsu et al. 2008; Nicolette et al. 2010).

Two sequence motifs are conserved between CtIP orthologs from most species. One motif is a predicted coiled-coil region at the amino terminus, which appears to mediate dimerization of CtIP and Sae2, a prerequisite to its functionality (Dubin et al. 2004; Kim et al. 2008; Wang et al. 2012). The second conserved region maps to the carboxyl terminus. It harbors a phosphorylation site (T847 in human and S267 in *S. cerevisiae*, but absent in *S. pombe* Ctp1 and some other fungi) that is phosphorylated by CDK to initiate resection (Huertas et al. 2008; Huertas and Jackson 2009). The carboxyl terminus also contains a functionally important CxxC motif (absent in *S. cerevisiae* Sae2) (Limbo et al. 2007; Akamatsu et al. 2008), mutations of which lead to defects in fission yeast DSB repair almost as severe as a *ctp1* knockout. However, the biochemical function of this motif remains to be characterized.

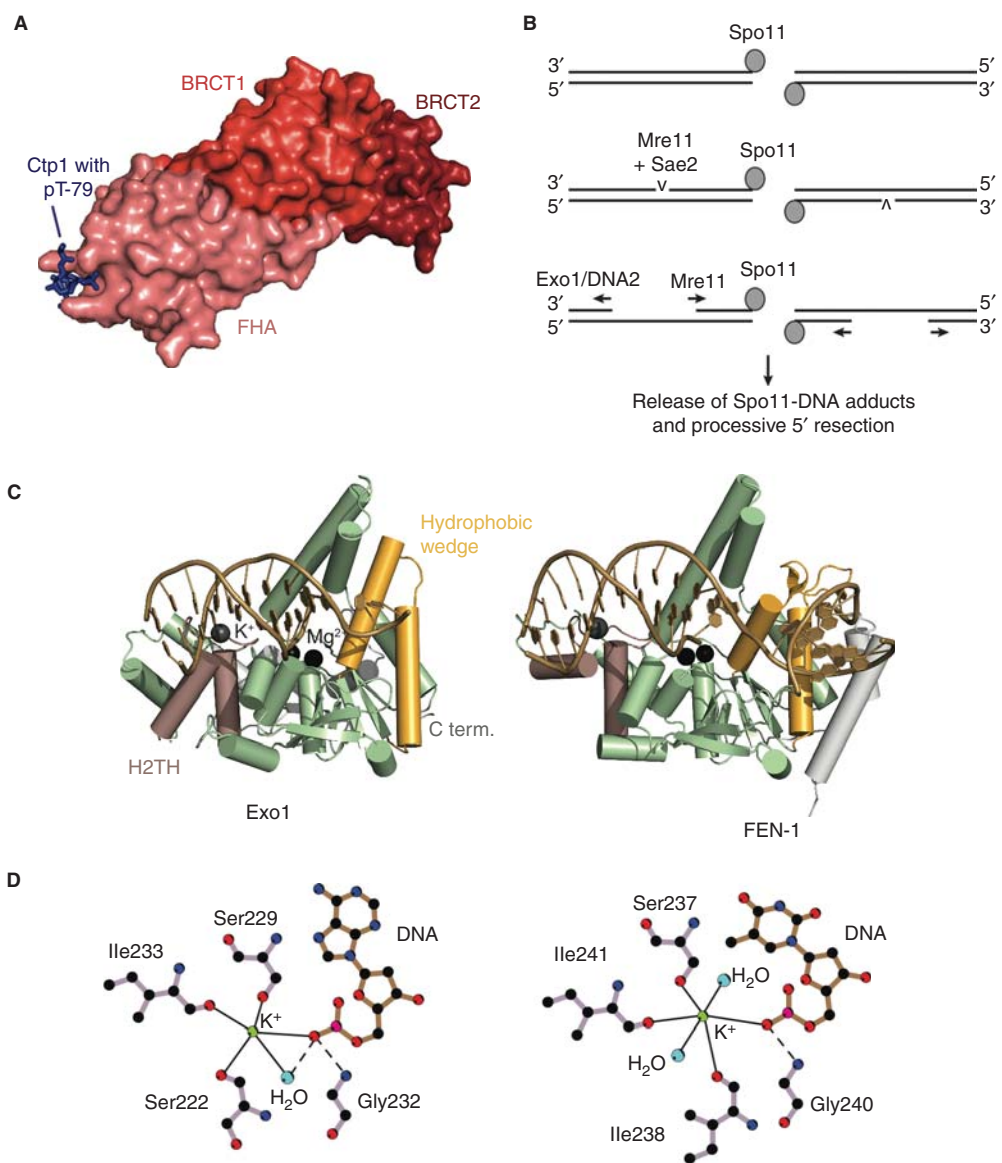
Mammalian CtIP was shown in several studies to physically interact with MRN (Sartori et al. 2007; Chen et al. 2008; Yuan and Chen 2009). In the case of *S. pombe* Ctp1, two crystal structures illustrate how the amino-terminal FHA domain of Nbs1 binds to a phosphorylated Thr-Asp motif in Ctp1 (Fig. 4A) (Lloyd et al. 2009; Williams et al. 2009). This motif in Ctp1 is phosphorylated in a cell cycle-dependent manner by kinase CK2 (Dodson et al. 2010). Recently, a direct interaction was also reported for recombinant *S. cerevisiae* MRX and Sae2 (Ghodke and Muniyappa 2013).

On the basis of studies in budding yeast, Sae2/Ctp1/CtIP has been suggested to initiate resection at DSB ends together with MRN by removing a short stretch of 50 to 100 bp from the 5' strand. Then, processive nucleases and nuclease-helicase complexes like Exo1 and Sgs1-Dna2 take over to resect the 5' strand up to the level of several kilobases (Mimitou and Symington 2008; Zhu et al. 2008). It is unclear, however, what defines the number of nucleotides to be removed by MRN and CtIP.

Priming endonucleolytic cleavage by MRN and CtIP may help to process DNA ends blocked by chemical modifications or by proteins and also offers a way to prevent uncontrolled resection and hyperrecombination. Blocked DNA

ends occur in meiosis or during abortive topoisomerase reactions (Hartsuiker et al. 2009; Longhese et al. 2009). The MRN(X) complex and Sae2/CtIP may indeed be dispensable for the resection of “clean” DNA ends, as shown for HO-endonuclease and I-SceI-induced DSBs in yeast (Llorente and Symington 2004; Westmoreland and Resnick 2013). In contrast to a model, in which MRN(X) and Sae2/CtIP start resection directly at a DNA end, newer studies provide an alternative model, in which MRN(X) incises DNA away from the DNA end (Fig. 4B). For yeast meiotic DNA breaks that are covalently bound by Spo11 (Keeney et al. 1997), it was shown that the MRX complex and Sae2 incise this blocked DNA up to 300 bases downstream from the DSB and resect the DNA strand in a 3'-5' direction toward the break (Fig. 4B) (Garcia et al. 2011). This model reconciles the discrepancy between the 3'-5' exonuclease activity of Mre11 observed in vitro and the 5'-3' resection observed in vivo. DNA incision away from the DSB was found for HR also in mitotic mammalian cells (Shibata et al. 2014), suggesting that this mode of action is not limited to meiosis. However, some issues remain unclear. What defines the distance between the meiotic or mitotic break and the endonucleolytic incision? The observed distance could be particular to the experiment, but may also reflect the influence of nucleosomes or the tethering function of the Rad50 coiled-coils. Another open question is how MRN distinguishes the two DNA strands during the endonucleolytic cut so that it processes toward and not away from the break.

The biochemical mechanisms by which Sae2/Ctp1/CtIP promote DSB resection are not understood and may differ between species. Although *S. cerevisiae* Sae2 shows in vitro endonuclease activity on ssDNA and degrades hairpin DNA structures cooperatively with MRN (Lengsfeld et al. 2007), human CtIP seems to lack nuclease activity, but stimulates the ssDNA endonuclease activity of Mre11 (Sartori et al. 2007). Structural data for Sae2/Ctp1/CtIP or its interaction with MRN(X) so far remain elusive. Thus, many questions are still unanswered: (1) Where is the active site in *S. cerevisiae* Sae2 located? (2) Does it represent



**Figure 4.** Resection initiation and the Exo1 resection pathway. (A) Structure of an Nbs1-Ctp1 complex from *S. pombe* (PDB code is 3HUE, Williams et al. 2009). Nbs1 surface with highlighted FHA (salmon), BRCT1 (red), and BRCT2 (dark red) domains. Peptide from Ctp1 (blue) with phosphorylated T79 bound to the FHA domain. (B) Model for bidirectional resection at meiotic DSBs by Mre11 and Exo1/Sgs1-Dna2. A study of meiotic resection in *S. cerevisiae* suggests an endonucleotic cleavage of the 5' strand by Mre11/Sae2 at a distance of up to 300 bp away from the Spo11-blocked DNA end. The nicked DNA can then be processed bidirectionally by Mre11 in the 3'-5' direction and by Exo1 or Sgs1-Dna2 in the 5'-3' direction (Garcia et al. 2011). (C) Comparison of human Exo1 and FEN-1. Features discussed in the text are highlighted and labeled for Exo1. The PDB codes are 3QEA (Exo1, Orans et al. 2011) and 3Q8K (FEN-1, Tsutakawa et al. 2011). (D) The helix-two-turn-helix (H2TH)-K<sup>+</sup> motif in Exo1 (left) and FEN-1 (right). Straight lines indicate metal coordination, and dashed lines indicate hydrogen bonds. Only residues involved in K<sup>+</sup>-coordination and binding of the K<sup>+</sup>-coordinating DNA base are shown. The scheme was drawn using LIGPLOT (Laskowski and Swindells 2011). C term., carboxy terminal.

a new class of nuclease domains that is absent in vertebrate CtIP? (3) How can this protein stimulate and regulate the nuclease activities of Mre11 in pathways such as mitotic and meiotic HR, telomere maintenance, and MMEJ?

### Exonuclease 1 Resection Pathway

The long-range resection nuclease exonuclease 1 (Exo1, in humans, also Hex1) was first described as a 5'-3' exonuclease from *S. pombe* (Szankasi and Smith 1992) and then found in *S. cerevisiae* (Fiorentini et al. 1997; Tishkoff et al. 1997) and humans (Schmutte et al. 1998; Tishkoff et al. 1998; Wilson et al. 1998). Besides its involvement in resection (Tsubouchi and Ogawa 2000; Mimitou and Symington 2008; Zhu et al. 2008; Nicolette et al. 2010), Exo1 has major roles in mismatch repair (Szankasi and Smith 1995; Genschel et al. 2002) and telomere maintenance (Wu et al. 2012). During resection, human Exo1 is stimulated by BLM, MRN, and the replication protein A (RPA), as shown by experiments using purified proteins (Nimonkar et al. 2008, 2011). However, this situation may be different in yeast (Cannavo et al. 2013).

Exo1 belongs to the XPG/Rad2 and FEN-1 family of structure-specific nucleases, a class of metalloenzymes (Shen et al. 1997; Lee and Wilson 1999; Orans et al. 2011). All members of this family share a conserved amino-terminal nuclease domain (amino acids 1–350 in human Exo1), whereas the carboxyl terminus (the remaining 500 amino acids) is divergent. Human Exo1 is dependent on  $Mg^{2+}$ , and significantly less active in the presence of  $Mn^{2+}$  (Lee and Wilson 1999). For many years, structural information was limited to crystal structures of the paralog flap endonuclease 1 (FEN-1) (Hosfield et al. 1998; Hwang et al. 1998; Matsui et al. 2002; Chapados et al. 2004; Feng et al. 2004; Sakurai et al. 2005; Doré et al. 2006; Devos et al. 2007), which, among other roles, removes Okazaki fragments during replication (reviewed in Balakrishnan and Bambara 2013). However, these structures were incomplete with regard to the metal center or DNA complexation. Only recently, the crystal structures of human Exo1

and FEN-1, each in complex with a DNA substrate, were reported (Fig. 4C) (Orans et al. 2011; Tsutakawa et al. 2011).

The Exo1 fold comprises a central, twisted  $\beta$ -sheet surrounded by  $\alpha$ -helices and is structurally very similar to FEN-1 and related endonucleases (Orans et al. 2011). In the crystal structure, a bound DNA substrate is simulated using a short DNA duplex with a 3' single-strand extension. The double-stranded part of the DNA interacts with Exo1 only at two points that are set one turn apart. A helix-two-turn-helix (H2TH) motif binding a  $K^+$  makes non-specific bonds with the nonsubstrate strand (Fig. 4C,D). At the active site, two structurally conserved helices form a hydrophobic wedge that drives the nonsubstrate strand into a sharp bend away from the nuclease. The 5' end of the substrate strand is led to the active site, which consists of two divalent cations that are coordinated by five conserved acidic residues and a conserved lysine and arginine. A remarkable feature is the fraying of the duplex DNA. As a consequence, the substrate strand becomes a single strand that exposes its scissile bond to the metal center. It was proposed that one of the metals activates a water molecule that can attack the scissile bond, whereas the other metal stabilizes the leaving group (Beese and Steitz 1991; Steitz and Steitz 1993; Orans et al. 2011).

Many of these structural features are conserved between the paralogs Exo1 and FEN-1 despite different substrate specificities (Fig. 4C) (Orans et al. 2011; Tsutakawa et al. 2011). Exo1 is primarily an exonuclease at DNA nicks, FEN-1 removes DNA flaps, and other family members such as XPG or GEN cut at DNA bubbles or Holliday junctions, respectively (Tsutakawa and Tainer 2012). Common to all these DNA structures is a nick or gap, and the insights gained from the Exo1 and FEN-1 structures allow the formulation of a single, common mechanism for their processing by FEN-1 family members (for an in-depth discussion of the two crystal structures and their implications, see Grasby et al. 2012; Tsutakawa and Tainer 2012). The presence of a DNA nick or gap is required by the hydrophobic wedge that induces a sharp bend into the template DNA strand and





thus prevents the processing of dsDNA. Together with the wedge, the H2TH/K<sup>+</sup> motif (Fig. 4D) orientates the substrate strands toward the active site metal center. This potassium ion is absent in FEN-1 crystal structures that lack DNA (e.g., Chapados et al. 2004). A similar configuration was observed in the DNA polymerase  $\beta$ , where a K<sup>+</sup> is involved in binding of a helix-hairpin-helix motif to DNA and was suggested to support processivity (Pelletier et al. 1996). Access to the active site is restricted to ssDNA, again excluding a continuous dsDNA. The ssDNA is generated by melting of two residues of the substrate strand.

Still under discussion is the interaction between FEN-1 paralogs and the substrate ssDNA upstream of the incision. A threading mechanism has been postulated that requires the threading of the ssDNA through a helical arch that is disordered in the absence of bound DNA (Ceska et al. 1996; Tsutakawa et al. 2011; Tsutakawa and Tainer 2012; Balakrishnan and Bambara 2013). Threading has to be ruled out for a DNA bubble in the case of XPG. Some argue that XPG probably does not have a helical arch (Tsutakawa et al. 2011; Tsutakawa and Tainer 2012), whereas others posit that the ssDNA may circumvent the helical arch and bind on surface grooves, which may extend to all FEN-1-related nucleases, including XPG and GEN (Orans et al. 2011).

### The Sgs1/BLM-DNA2 Resection Pathway

The second main pathway for processive 5'-resection in HR, beside Exo1-mediated resection, depends on the cooperative action of the nuclease DNA2 and the helicase activity of Sgs1 in *S. cerevisiae* or its functional homolog BLM in vertebrates (Gravel et al. 2008; Mimitou and Symington 2008; Nimonkar et al. 2008; Zhu et al. 2008). Sgs1 or BLM unwind duplex DNA by their 3'-5' helicase activity. The ssDNA-binding protein, replication protein-A (RPA), then coats ssDNA unwound by Sgs1 and promotes 5'-3' degradation by Dna2 while inhibiting 3' to 5' degradation (Cejka et al. 2010a; Niu et al. 2010; Nimonkar et al. 2011). Recombinant Dna2 and Sgs1 physically interact even in the absence of a

DNA substrate, and a similar interaction was also reported for human DNA2 and BLM (Cejka et al. 2010a; Nimonkar et al. 2011). In addition, Sgs1 is part of the Sgs1-Top3-Rmi1 (STR) complex, together with the topoisomerase class I enzyme Topoisomerase III and the regulatory protein Rmi1 (Gangloff et al. 1994; Chang et al. 2005; Mullen et al. 2005). This complex is responsible for dissolution of double Holliday junctions in the late stage of homologous recombination (Cejka et al. 2010b). Top3 and Rmi1 are also important for the resection function of Sgs1. Deletion mutants of all three proteins share similar resection defects in vivo (Zhu et al. 2008), and Top3-Rmi1 also stimulates the 5'-resection capacity of Sgs1-Dna2 in vitro (Cejka et al. 2010a; Niu et al. 2010). A very recent crystal structure of the conserved core of the human TopIII $\alpha$ -RMI1 complex illustrates how RMI1 might regulate TopIII $\alpha$  through a long insertion loop that invades the central gate of the toroidal topoisomerase (Bocquet et al. 2014).

Sgs1 and BLM both belong to the RecQ family of helicases. Most prokaryotes and yeasts possess only one or two RecQ homologs (like Sgs1 in *S. cerevisiae*), whereas in vertebrates multiple homologs are found. For *Homo sapiens*, five RecQ-like helicases have been described: BLM, WRN, RECQ1, RECQ4, and RECQ5 $\beta$ . All of these helicases play important roles in different pathways of genome maintenance (Chu and Hickson 2009).

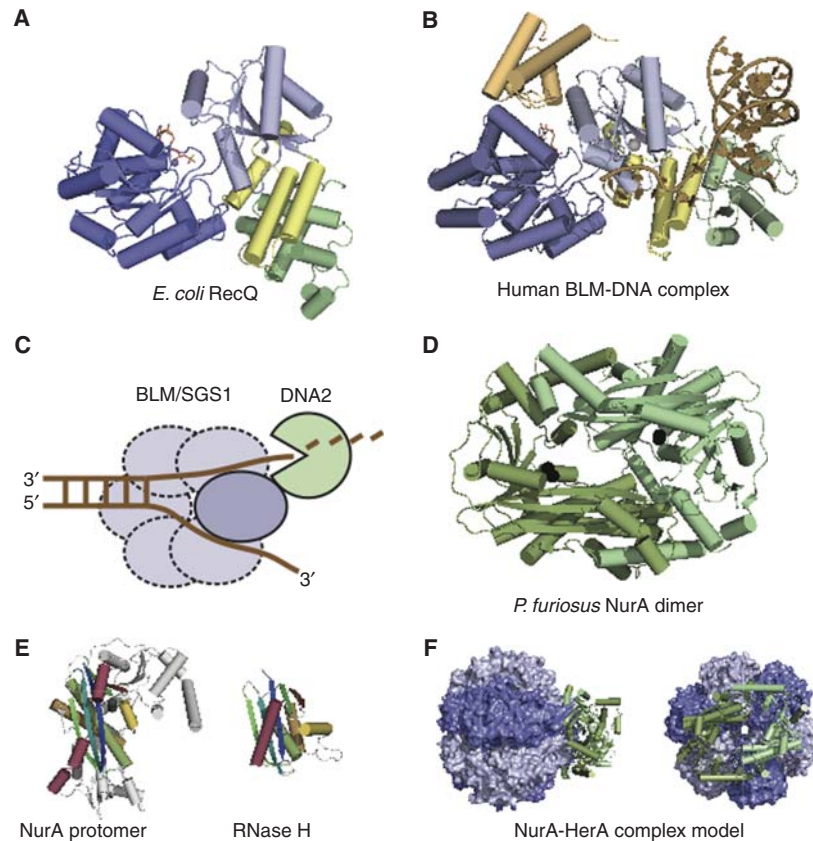
The RecQ-like helicases belong to the SF-2 helicase family and share a conserved core, which consists of two RecA-like domains and a carboxy-terminally adjacent RQC (RecQ carboxy-terminal) region (lacking in RecQ4) (Vindigni et al. 2010; Manthei and Keck 2013). The RQC region is needed for strand separation of DNA substrates (Hu et al. 2005; Pike et al. 2009; Kitano et al. 2010). Many RecQ-like helicases, including bacterial RecQ, yeast Sgs1, and vertebrate BLM also possess a helicase and RNaseD carboxy-terminal (HDRC) domain that is important for DNA substrate recognition and translocation (Liu et al. 1999; Bernstein and Keck 2005; Kocsis et al. 2014).

Several atomic resolution structures are now available and yield insights into the functional



architecture of these helicases. The crystal structure of *Escherichia coli* RecQ revealed the principal architecture of the catalytic core (Fig. 5A) (Bernstein et al. 2003). The RecA-like domains and the RQC region, consisting of a zinc-binding motif and a winged helix domain, compose a compact modular arrangement, which is also

found in the structure of human RecQ1 (Pike et al. 2009). The HRDC domains from *E. coli* RecQ, SGS1, and BLM possess a very similar fold. However, they exhibit different DNA substrate specificities. This is reflected in their differing composition of DNA-interacting residues and distinct surface charge distributions



**Figure 5.** Structures of nuclease–helicase complexes involved in resection. (A) Structure of the helicase catalytic core of *E. coli* RecQ bound to ATP $\gamma$ S. The structure consists of the RecA-like helicase domains (dark and light blue) bound to ATP $\gamma$ S (orange) and the RecQ carboxy-terminal region, consisting of the zinc-binding domain (yellow) and a winged-helix domain (green). The PDB code is 10YY (Bernstein et al. 2003). (B) Structure of human BLM helicase in complex with DNA. The color coding is similar to that in A. The HRDC domain and ADP are drawn in orange, and the DNA in brown. The PDB code is 4CGZ. (C) The RecQ-like helicases BLM in vertebrates or Sgs1 in yeast are both cooperating with DNA2 in DSB resection. Sgs1 or BLM unwind dsDNA by their 3'-5' helicase activity. The ssDNA-binding protein RPA then coats ssDNA unwound by Sgs1 and promotes 5'-3' degradation by Dna2 (Cejka et al. 2010a; Niu et al. 2010; Nimonkar et al. 2011). The potential of BLM and Sgs1 to form multimers is indicated using dashed lines. (D) Structure of the *P. furiosus* NurA dimer. The PDB code is 3TAL (Chae et al. 2012). (E) RNase H fold of NurA. A Comparison of a *P. furiosus* NurA protomer with *E. coli* RNase H (PDB code 1RNH, Yang et al. 1990). Homologous elements are highlighted using the same color. (F) Model of the NurA-HerA complex. The crystal structure of *Sulfolobus solfataricus* NurA (PDB code 2YGK, Blackwood et al. 2012) is fitted to a HerA homolog, the conjugation protein TrwB (PDB code 1E9R, Gomis-Ruth et al. 2001).

(Liu et al. 1999; Bernstein and Keck 2005; Kim and Choi 2010; Sato et al. 2010).

Very recently, structures of human BLM in complex with partially unwound DNA were determined (PDB code 4CGZ, Fig. 5B) (Swan et al. 2014; O Gileadi, pers. comm.). This structure reveals extensive interactions of the winged helix domain with the upstream dsDNA “substrate” and shows that the  $\beta$ -hairpin wing acts as a DNA-splitting element. The zinc-binding insertion domain functions as single-stranded DNA ratchet, whereas the RecA-like domains binds to the ssDNA “product.” The HRDC domain does not make any DNA contacts but is positioned on top of the nucleotide-binding cleft of the RecA-like domains. It will be interesting to see whether the observed position of the HRDC domain is important for the regulation of BLM helicase activity. Bacterial RecQ and BLM structures share high fold conservation, although the winged-helix domain is positioned differently in both structures, indicating flexibility for this element (Fig 5A,B).

Many RecQ-like helicases including BLM are known to form oligomers, at least in vitro (Fig. 5C) (Karow et al. 1999; Xue et al. 2002; Perry et al. 2006; Vindigni and Hickson 2009). However, a more recent study describes that BLM oligomers dissociate into monomers upon ATP hydrolysis and that only monomeric but not oligomeric BLM displays DNA unwinding activity (Xu et al. 2012a). Thus, it remains an open question which functional role oligomerization of Sgs1 or BLM plays in the context of 5'-strand resection in HR.

Homologs of the nuclease-helicase protein DNA2 are found both in archaea and eukarya but are absent in bacteria, although the bacterial AddAB system bears some structural similarity. Although archaeal Dna2 is only poorly characterized (Higashibata et al. 2003), the eukaryotic protein was found to play crucial roles in several genome maintenance processes beside homologous recombination, including Okazaki fragment processing (Kang et al. 2010) and telomere stabilization (Lin et al. 2013). Initial genetic studies in *S. cerevisiae* revealed that the nuclease activity of Dna2 is essential in vivo, whereas a helicase-dead mutant strain is via-

ble at lower growth temperatures (Budd et al. 2000). A later study then clarified that the nuclease of Dna2 is responsible for processive 5' strand resection in DSB repair by HR where it is the second important processive nuclease beside Exo1 (Zhu et al. 2008). Both Exo1 and Dna2 function independently of each other and seem to play redundant roles (Zhu et al. 2008; Cannavo et al. 2013). The nuclease module of Dna2 belongs to the RecB family and maps to the amino terminus of the protein. Remarkably, it was shown to contain an iron-sulfur cluster, which is crucial for both nuclease and ATPase activity (Yeeles et al. 2009; Pokharel and Campbell 2012). This situation is reminiscent of the bacterial DSB resection protein AddB, which possesses a nuclease domain with a 4Fe-4S cluster (Yeeles et al. 2009).

In contrast to its nuclease activity, the helicase activity of Dna2 is dispensable for 5'-strand resection, which was a rather surprising finding. Instead, the unwinding activity of RecQ-like helicases such as Sgs1 or BLM provides the 5'-ssDNA for resection by Dna2 (Cejka et al. 2010a; Niu et al. 2010). The Dna2 helicase exhibits only a weak 5'-3' unwinding activity on dsDNA and depends on the binding to free DNA ends before acting as a helicase (Bae et al. 2002). However, recently, it was shown that Dna2 is a vigorous 5'-3' helicase in an *S. cerevisiae* nuclease dead mutant (Levikova et al. 2013). Apparently, Dna2 depends on the binding to 5'-ssDNA flaps for processive helicase activity, and these flaps are degraded by the Dna2 nuclease domain of the wild-type protein (Levikova et al. 2013). There may be a structural switch in Dna2 that regulates the balance between its nuclease and helicase activities. Atomic resolution structures of Dna2, Sgs1, and their DNA substrates, which are still lacking at the moment, could provide important information to better understand how these key enzymes cooperate to specifically resect 5'-DNA at DSBs.

### Resection in Archaea

Archaea, like eukaryotes, use homologs of Mre11-Rad50 for resection. However, for long-range resection, Mre11-Rad50 are joined by two

proteins unique to archaea, the helicase HerA and the nuclease NurA (Manzan et al. 2004; Hopkins and Paull 2008; Blackwood et al. 2012; Chae et al. 2012). Archaeal resection is thus similar to the eukaryotic process with regard to common players such as Mre11-Rad50 and the overall principle. In contrast, the archaeal resection machinery is completely distinct from bacterial proteins such as RecBCD, AddAB, or AdnAB, although some archaeal species may have taken up AddAB-like proteins by horizontal gene transfer (Cromie 2009).

The genes *nurA* and *herA* are encoded in one operon together with *mre11* and *rad50* in almost all archaea (Constantinesco et al. 2002, 2004; Manzan et al. 2004). NurA is a dimer and has been described as both a 5' and 3' exonuclease for dsDNA and ssDNA and an endonuclease for ssDNA (Constantinesco et al. 2002; Hopkins and Paull 2008; Wei et al. 2008, 2011; Blackwood et al. 2012; Chae et al. 2012). HerA is a hexameric, ATP-dependent DNA helicase that is activated by and unwinds dsDNA in both the 5' and 3' direction (Constantinesco et al. 2004; Manzan et al. 2004; Zhang et al. 2008).

For full activity, NurA and HerA have to form a complex, which is stable in vitro, at least for species such as *P. furiosus* and *S. solfataricus* (Hopkins and Paull 2008; Blackwood et al. 2012; Chae et al. 2012). However, this physical interaction may be less stable or absent in *Sulfolobus acidocaldarius* (Quaiser et al. 2008). The stoichiometry of the complex (if formed) seems to be 2:6, that is, a NurA dimer and a HerA hexamer (Hopkins and Paull 2008; Blackwood et al. 2012; Chae et al. 2012).

The HerA monomers assemble into a hexameric ring, as visualized by electron microscopy analysis (Manzan et al. 2004). Thus, HerA is a typical member of the FtsK class of P-loop ATPases and is likely to have a similar fold (Constantinesco et al. 2004; Iyer et al. 2004). The HerA amino terminus is predicted to comprise a distinct domain that folds into a  $\beta$ -barrel and was called the HAS (HerA-ATP synthase) domain (Iyer et al. 2004).

The crystal structures of two NurA orthologs from *P. furiosus* and *S. solfataricus* were recently reported (Fig. 5D) (Blackwood et al.

2012; Chae et al. 2012). The conserved, active domain is of the RNaseH-like fold (Fig. 5E) with nonconserved extensions that make extensive dimer interactions. The overall shape of the NurA dimer is ring-like, with the active sites of each monomer facing each other within the ring pore (Fig. 5D). Conserved acidic residues bind one or two  $Mn^{2+}$  cations in the active site of *P. furiosus* NurA, depending on the crystallization conditions (Chae et al. 2012). Mutation of these manganese-binding residues completely inactivates NurA (Hopkins and Paull 2008; Wei et al. 2011; Blackwood et al. 2012; Chae et al. 2012). Also,  $Mg^{2+}$  is known to be essential for NurA activity (Constantinesco et al. 2002; Hopkins and Paull 2008; Chae et al. 2012). Thus, the catalytic mechanism of NurA could be similar to that postulated for the RNaseH-like nuclease Argonaute (Wang et al. 2009) or RNase H itself (Nowotny et al. 2005). Structures of these proteins bound to divalent cations and DNA have led to a model in which one cation activates a water for nucleophilic attack on the DNA backbone and the second cation stabilizes the leaving group (Beese and Steitz 1991; Steitz and Steitz 1993; Nowotny et al. 2005; Wang et al. 2009).

The structures of NurA also yielded insight into the cooperative DNA processing of NurA and HerA. The cavity of the NurA ring is positively charged, as expected for a DNA-processing enzyme. However, it holds space only for one or two ssDNA strands, but not for B-form dsDNA (Blackwood et al. 2012; Chae et al. 2012). The interaction interface of NurA and HerA could be mapped to residues on the flat surface of the NurA ring close to the active site, which are bound by the HerA HAS domain (Fig. 5F) (Blackwood et al. 2012). These data imply that the helicase HerA unwinds dsDNA and passes one or both single strands directly on to the NurA dimer.

The NurA structure does not answer the question of whether, in vivo, the NurA/HerA complex digests one or both strands of dsDNA. However, it has been observed that the rate of ATP hydrolysis by NurA/HerA varies with the nature of the DNA substrate. This rate is higher for dsDNA with blunt ends or short overhangs



and lower in the presence of longer overhangs (Blackwood et al. 2012). In consequence, the nature of the DNA end may trigger complete digestion of the DNA double strand or the 5'-3' resection necessary to produce a DNA tail. Blackwood et al. (2012) suggest that the former could be an archaeal defense mechanism against foreign DNA, whereas the latter might rely on the preparation of the DNA by the MR complex. Experimental evidence indeed supports a model in which the MR complex and the NurA/HerA complex cooperate to produce a 3' overhang that is then bound by RadA (archaeal RecA) for homologous recombination (Hopkins and Paull 2008).

### OPEN QUESTIONS AND CONCLUDING REMARKS

The past decade has brought a plethora of new insights into the composition, biochemistry, and regulation of the DSB detection and resection machineries. We now have an inventory of enzymatic activities at DSBs in all three domains of life. Nonetheless, from a mechanistic and also an evolutionary point of view, we are far from understanding the molecular choreography of DSB detection, repair, and resection. Although the bacterial resection machineries, RecBCD and AddAB, are well characterized, the structural nature of the resection machineries in eukaryotes and archaea requires further attention. New developments in electron microscopy and hybrid methods in structural biology may help to better understand the interaction architectures of these complexes and the interplay of different nuclease, helicase, and topoisomerase activities. Likewise, despite progress over the last years, the mechanism of the MR(N) complex in DNA end processing is still unclear. Several fundamental issues remain unsolved, in particular, the mechanism of DSB detection by MRN, the nature of its cryptic endonuclease activity, and the role and mechanism of the cofactor CtIP/Ctp1/Sae2. Other issues such as identifying the site of the initial endonucleolytic cleavage of DNA ends will require the reconstitution of more complete biochemical systems. It will also be important to

mechanistically address the resection in chromatin templates and integrate the activities of chromatin modifying enzymes with resection enzymes. Recent studies have begun to look at exactly that and showed in vitro that Sgs1-Dna2 resection requires some nucleosome-free DNA but can then proceed through nucleosomes. In contrast, nucleosomes provide an obstacle for Exo1-based resection that may be lifted by chromatin-remodeling activities (Adkins et al. 2013).

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