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The MRE11–RAD50–NBS1 Complex Conducts the Orchestration of Damage Signaling and Outcomes to Stress in DNA Replication and Repair

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Abstract

Genomic instability in disease and its fidelity in health depend on the DNA damage response (DDR), regulated in part from the complex of meiotic recombination 11 homolog 1 (MRE11), ATP-binding cassette–ATPase (RAD50), and phosphopeptide-binding Nijmegen breakage syndrome protein 1 (NBS1). The MRE11–RAD50–NBS1 (MRN) complex forms a multifunctional DDR machine. Within its network assemblies, MRN is the core conductor for the initial and sustained responses to DNA double-strand breaks, stalled replication forks, dysfunctional telomeres, and viral DNA infection. MRN can interfere with cancer therapy and is an attractive target for precision medicine. Its conformations change the paradigm whereby kinases initiate damage sensing. Delineated results reveal kinase activation, posttranslational targeting, functional scaffolding, conformations storing binding energy and en-abling access, interactions with hub proteins such as replication protein A (RPA), and distinct networks at DNA breaks and forks. MRN biochemistry provides prototypic insights into how it initiates, implements, and regulates multifunctional responses to genomic stress.

Keywords

ATPase; nuclease; FHA domain; forkhead-associated domain; BRCT domains; BRCA1 C-terminal domains; coiled-coil domain; CtIP tetramerization; CtBP-interacting protein tetramerization

INTRODUCTION

At the cellular level, life is constantly challenged by genotoxic stress originating from endogenous and exogenous sources, with stalled replication forks (RFs) and double-strand breaks (DSBs) being among the most deleterious and extreme forms of DNA damage.

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Failure to properly respond to genomic distress can be mutagenic and toxic to cells as well as fatal to the organism. Cells have therefore evolved to counteract genotoxic stress by integrating DNA repair mechanisms and cell cycle regulation through checkpoint signaling (1). In this network of DNA damage responses (DDR), the meiotic recombination 11 homolog 1 (MRE11)–DNA repair protein RAD50–Nijmegen breakage syndrome protein 1 (NBS1) (MRN) complex (**Figure 1*a,b***) holds a key position. It is among the first responders and regulates both signaling and damage responses to at least four types of extreme cellular stress: DNA damage at DSBs, stalled RFs, dysfunctional telomeres, and viral invasion. MRN also has a prime role in kinase [ataxia-telangiectasia mutated (ATM) and ataxia-telangiectasia and RAD3-related (ATR)] signaling that helps orchestrate cell cycle progression and damage responses (2).

MRN functional significance in both signaling and repair is underscored by the observation that the core Mre11–Rad50 (MR) catalytic subcomplex is conserved across all domains of life, encompassing archaea (e.g., *Pyrococcus furiosus*), eubacteria (*Escherichia coli* SbcC/D), some phage (T4 phage gp46/47), and eukaryotes including homologs in *Saccharomyces cerevisiae* and *Schizosac-charomyces pombe* (3). The NBS1 (Xrs2 in *S. cerevisiae*) component is conserved only in eukaryotes. However, mutations in any of the genes encoding MRN components cause severe consequences in humans. Germline mutations (**Figure 1*a***) in *MRE11*, *NBS1*, or *RAD50* cause ataxia-telangiectasia (A-T)–like disorder (ATLD), Nijmegen breakage syndrome (NBS), or NBS-like disorder (4–10). A-T is caused by inherited mutations in *ATM* kinase. Aside from germline mutations, all 3 MRN complex components are mutated in >50 cancers, as assessed from the International Cancer Genome Consortium projects (**Figure 1*c*** and **Supplemental Table 1**). Furthermore, the MRN complex is frequently found absent in patients with epithelial ovarian cancer (11). MRN functional disruption may be a synthetic lethal combination with poly(ADP-ribose) glycohydrolase (PARG) inhibition (12) providing a possible strategy to target MRN-defective cancers by inhibition of PARG, whose structure is defined (13). However, upregulation of RAD50 increases cellular radioresistance, and knockdown sensitizes some cancer cells to radiation, suggesting MRN may be a target for precision medicine (14). Overall, its conservation across all domains of life coupled to the catastrophic consequences of its mutations affirms the role of the MRN complex in maintaining essential aspects of genomic stability for all cells.

In this review, we consider exemplary recent developments and paradigm-shifting results linking MRN structural biochemistry to its biology to complement and build upon prior excellent reviews (2, 4, 15, 16). We integrate recent advances to delineate how MRN's multidomain three protein composition enables its central enzymatic, sensing, signaling, architectural, and scaffolding functions in damage responses. MRN conformations are key to damage signaling through the ATM and ATR kinases, regulating the MRE11 nuclease and opening DNA for nuclease incision. By activating ATM, the MRN complex regulates phosphorylation of >900 sites on >700 proteins, including MRE11, RAD50, and NBS1 (17, 18). NBS1 interacts with ATM kinase plus its N-terminal phosphopeptide-interacting domains that link MRN to other partners including the C-terminal binding protein (CtBP)-interacting protein (CtIP) (19, 20). As it directly binds NBS1, DNA, and RAD50, the

MRE11 nuclease dimer is the core of the MRN complex (21–23). We therefore update the two-step MRE11 nuclease incision model to explain its endo- and exonuclease properties in light of recent structures, including the eukaryotic RAD50 Zn-hook and CtIP N-terminal domain. We describe factors affecting MRN functions at DSBs, stalled RFs, dysfunctional telomeres, and complexes combating exogenous viral DNA infection. We discuss how MRN acts more as a dynamic molecular machine for the expeditious formation of protein–nucleic acid functional scaffolds at DSBs, RFs, dysfunctional telomeres, and viral DNA than as part of linear pathways, as also proposed for nonhomologous end joining (NHEJ) complexes (24). As a multifunctional macromolecular machine, the structural biochemistry of the MRN complex initiates sensing of stalled forks and DSBs, cell cycle checkpoint signaling cascades, incision for and commitment to break repair pathways, reestablishment of posttranslational phosphorylations via ATM kinase, and functional regulation of chromatin remodeling. The collective results outlined here suggest how MRN conformations and interactions enable both its specificity and multiple functions in cells with implications for science and biomedicine.

MRN COMPLEX: STRUCTURAL BIOCHEMISTRY AND BIOLOGY

The MRN complex is an intriguing chemo-mechanical molecular machine that converts energy from binding to DNA, ATP, and protein partners into conformational changes that control nuclease activities and network assemblies (2). MRN binds DNA and acts as a sensor of DNA damage (25). It is furthermore the activator for ATM and ATR kinase cascades (26), a tethering scaffold for recognition and stabilization of protein interactions, a processor of RFs, a licensing factor for homologous recombination (HR), and a facilitator of fork restart and DNA repair processes (2, 27, 28). At the basic level, MRN consists of two RAD50 ATP-binding cassettes (ABC)–ATPase sub-units, two MRE11 subunits forming a DNA structure-specific endo/exo/hairpin nuclease, and the NBS1 regulatory docking protein with phosphopeptide-interacting forkhead associated (FHA) and BRCA1 C-terminal (BRCT) domains flexibly linked to an MRE11 interface and adjacent C-terminal ATM kinase interaction motif (16, 29) (**Figure 1*a*,*b***).

The core MR complex exists as a hetero-tetrameric assembly (M2R2), whose dynamic morphology emerges from shifts in its modular head, coil, and hook domains (**Figure 1*b***). The globular DNA binding head is formed by the two Rad50–ATPase domains and the Mre11 nuclease that bind to the base of the Rad50 helical coiled-coil domains (**Figure 2*a***). The heart of the MR (in prokaryotes) and MRN (in eukaryotes) assembly originates from the Mre11 dimerization interface (29). Yet, RAD50 is the largest subunit: It belongs to the structural maintenance of chromosomes (SMC) protein family (30). Each Rad50 polypeptide assembles into a globular ATPase domain despite a large intramolecular antiparallel coiled-coil sequence insertion. This coiled-coil domain extends far from the head domain (~500 Å for eukaryotic Rad50 homologs) with a central CxxC motif forming a Zn-hook and reverse turn that caps its distal end and mediates Zn-dependent Rad50 subunit interactions (31–33). The resulting Rad50 bipartite ATPase dimerizes in a $Mg^{2+}$and ATP-dependent manner (30). Notably, Rad50 conformation at the dimer interface is controlled by binding two ATP molecules: Each is sandwiched between signature motifs conserved in ABC–ATPases on

one subunit and the Walker A, Walker B, Q-loop, and D-loop motifs from the other subunit (34, 35).

MRE11 key features are an N-terminal core nuclease domain followed by a cap domain that restricts access to the nuclease active site and a flexibly linked RAD50 binding motif (21, 22, 36–39). X-ray structures of *P. furiosus* Mre11 revealed the two-domain architecture for the catalytic core (40). The N-terminal calcineurin-like phosphoesterase domain bears the Mn^{2+}-coordination and nuclease catalytic motifs. The phosphoesterase is further decorated by an alpha-beta fold C-terminal domain positioned to enforce DNA binding specificities (**Figure 1*a***) (40). The ternary complex of *P. furiosus* Mre11 bound to Mn^{2+} and a 5′-dAMP nucleotide hydrolysis product supports the biochemically observed 3′–5′ directionality of the Mre11 exonuclease activity. Yet, as noted below, Mre11 nuclease activity also enables 5′–3′ resection of DNA ends to generate 3′ single-stranded DNA (ssDNA) overhangs for template-mediated HR and homologous repair (27). The partly disordered MRE11 C terminus is regulated by posttranslational modifications (**Figure 1*a***).

The NBS1 subunit is specific to eukaryotes. It has one FHA and two BRCT domains in its N terminus (**Figure 1*a***). These FHA and BRCT domains bind multiple phosphorylated proteins to regulate MRN interactions (29). The rest of NBS1 is unstructured except for the functionally critical C-terminal MRE11 and ATM-binding subdomains and motifs (23, 41). The NBS1 ATM-binding motif resembles the C-terminal binding motifs of Ku80 and ATR-interacting protein (ATRIP), which interact with and recruit the DNA-dependent protein kinase catalytic subunit (DNA-PKcs) and ATR, respectively (42), suggesting an interaction conserved across the three kinases (43). A recent DNA-PKcs crystal structure with bound Ku80 C terminus showed how Ku80 binds near DNA-PKcs autophosphorylation sites (the PQR cluster) (44). Interestingly, the NBS1 C terminus may also bind near ATM autophosphorylation sites (S367, S1893, and S1891) (42), suggesting that analogous modes of phosphorylation regulate DNA-PKcs and ATM activities. Yet, this NBS1 C-terminal region may not be critical for ATM-mediated checkpoint activation but helps in proper phosphorylation of some of the ATM substrates such as SMC1 and proapoptotic factor BID (45, 46).

The combination of at least two dimer states for structure-specific nuclease (MRE11) and ATPase enzyme (RAD50) activities, long-range allostery from NBS1 and the Rad50 Zn-hook binding domains, and regulatory phosphorylation plus flexible interface linkages enables multiple MRN conformational states. Together, these functional characteristics make MRN a dynamic machine that not only can sense and process DNA damage but also can participate in different pathways and networks, such as NHEJ, alternative nonhomologous end joining (A-NHEJ), or HR, for DNA damage repair. Owing to its dynamic and extended nature, structural analyses of MRN have employed multiple combined methods and biophysical techniques, including X-ray crystallography, small-angle X-ray scattering, electron microscopy (EM), and atomic or scanning force microscopy. Furthermore, structural information on MR has emerged from prokaryotes that can provide more accessible structures than have been analyzed for their eukaryotic counterparts. In fact, prokaryotic Mre11 is used as a molecular avatar (an embodiment of human MRE11–essential features) for design of inhibitors against human MRE11 (47). Excitingly, high-

resolution structures of eukaryotic MRN protein domains are also being solved (33, 38, 39). For eukaryotes, CtIP is also emerging as a key player in MRN functions in HR on the basis of structural analyses of the CtIP N-terminal domain (48–50). From these collective MRN structural and biochemical results, we propose a testable integrated model for MRN structure–function relationships (Figure 2).

RAD50 forms a unique SMC-type structure in which subunits interact through a Zn-hook at the tip of antiparallel coiled-coil arms to form an $M_2R_2N_2$ intralinked complex (**Figure 1*b***) (30). In the ATP-free or hydrolyzed state seen in the *Thermotoga maritima* MR complex,the Rad50 ATPase subunits are flexible and relatively open with the arms relaxed (51, 52) (**Figure 2*b***). In contrast, upon ATP binding, Rad50 closes into a single, more rigid conformation in which both head domains (N- and C-terminal) are interacting with each other in *trans* and form a central groove that can accommodate double-stranded DNA (dsDNA) (**Figure 2*a***) (21, 38, 51). Notably, an added DNA binding site at the coiled-coil domain on Rad50 subunits is seen for the complex of Rad50 (nucleotide binding domain) and Mre11 (helix-loop-helix domain) with AMPPNP and dsDNA (53). Single-molecule imaging of the human MRN complex provided additional evidence for this DNA-binding mode. Fluorescence imaging of MRN on nucleosome coated DNA suggested that this binding mode facilitates an efficient search for DNA lesions via facilitated diffusion along chromatin (34). Also, as seen in EM images for human and *P. furiosus* MRN complexes, the coiled-coil domains can form interlinked complexes (two intralinked MRN complexes bridged through RAD50 Zn-hooks), where they may tether two distant DNA sites, and in this configuration, the arms can extend to 1,200 Å (32). This observation was supported by the crystal structure of the *P. furiosus* Rad50 Zn-hook domain with a small coiled-coil region, where the Zn^{2+}-coordinating cysteines are arranged such that either intra- or interlinked complex coiled-coil domains can form (32). Recently, however, crystal structure and X-ray scattering analyses of human RAD50 Zn-hook with a portion of coiled-coil domain suggest that RAD50 predominantly forms an intralinked complex (**Figure 1*b***) (33) having a novel interaction interface within the coiled-coil domain that supports a rigid rod-shaped RAD50 dimer.

The Zn-hook and coiled-coil domain are critical for functional RAD50 DNA tethering and ATM activation (54–56). Yet, two Rad50 subunits are also held flexibly by the Mre11 dimer, whose subunits form a flexibly linked helix-bundle interface at the base of two Rad50 subunits that allow the Rad50 dimer to open upon ATP hydrolysis and to expose the Mre11 DNA-binding channel for nuclease excision. Although limited coiled-coil domain–length deletions are tolerated, mutations in the Zn-hook domain share the phenotype of a null mutation in yeast (32, 57), suggesting that long-range allostery connecting the MR core enzymatic activity with the Zn-hook is essential for MRN function in vivo.

Importantly, the closed-state (ATP-bound) Rad50 renders dsDNA inaccessible to the Mre11 nuclease active site (**Figure 2*a***) (21). However, ATP hydrolysis opens dsDNA so that flexible ssDNA may access the Mre11 active site (21). In another ATPase machine, FlaI (58), a structurally defined intermediate with bound phosphate ion (PO_4^{3-}) released from ADP partially opens subunit contacts: This structure suggests that RAD50 conformational intermediates may allow MRE11 ssDNA endonuclease activity while restricting dsDNA and

MRE11 exonuclease excision. By analogy, this as yet unseen RAD50 state would involve binding of arginine on the ABC–ATPase signature helix to the $PO4^{3-}$ released by ATP hydrolysis (35). After ATP hydrolysis, Rad50 forms a structurally defined and more open state that makes DNA accessible to Mre11 for 3′–5′ exonuclease activity. The switch between closed and open Rad50 states orchestrates conformational changes to the MRN complex and its bound DNA (**Figure 2*b***) that result in end protection or processing, as supported by structure-guided mutations (52). Furthermore, functional analyses of the Rad50 mutants show that the correct Rad50 interface with ATP and Mre11 are required for appropriate DSB processing (35, 59). Moreover, RAD50 mutation L1237F resulted in an exceptional patient response to an otherwise failed clinical trial of a checkpoint kinase-1 (CHK1) inhibitor combined with the DNA-damaging agent irinotecan (60). Our analyses suggest that this mutation promotes an open RAD50 that could activate MRE11 nuclease. We argue that matching the biochemistry of this mutation with a structure-based small-molecule inhibitor suggests a strategy to generalize the exceptional clinical response to other patients for precision medicine.

Crystal structures of Mre11 core domain are solved for both the Mre11 alone and in complex with Rad50 from different prokaryotes and eukaryotes (21, 29, 38, 39). All crystal structures, irrespective of source, show dimer formation, yet angles for the protomer arrangement vary. The dimerization surface is in the N terminus of T4 phage Mre11, followed by a capping domain and an acidic linker to the Rad50 binding domain, where the linker can act as an auto-inhibitor when Mre11 is not bound to Rad50 (61). From the X-ray crystal structures of *P. furiosus* Mre11 bound to a two-ended dsDNA and to a one-ended DNA fork (36), MR rearrangements can reshape DNA to access the nuclease active site (62). Existing DNA complex structures suggest that the Mre11 interface helps distinguish two-ended dsDNA breaks (symmetric dimer) from one-ended DNA forks (asymmetric dimer) (36) (**Figure 2*a***). In fact, structure-guided small-molecule inhibitors (27), which bind adjacent to the dimer interface, show that blocking MRE11 endonuclease activity promotes DSB repair through NHEJ. An endonuclease nick distal to the DSB end licenses HR by initiating 3′–5′ MRE11 exonuclease activity back toward the DSB. Furthermore, the MRE11 endonuclease provides an entry point for long-range EXO1- and DNA2-dependent 5′–3′ resection activity away from the DSB. Consequently, inhibition of MRE11 exonuclease activity led to a DNA repair defect owing to a DSB repair defect that could be partially rescued by inhibiting EXO1/BLM resection needed to form extended ssDNA as the committed step for HR (27). Thus, MRN enzyme activities can help determine pathway choice between NHEJ and HR.

Nbs1 structural analyses define the phosphoprotein binding sites and uncover their flexible linkage to the Nbs1 C terminus (19). Furthermore, the crystal structure of Mre11 fused with the Nbs1 C terminus indicates that the Nbs1 C terminus interacts across the Mre11 dimer interface and on the face opposite to the DNA binding groove (23). As the Nbs1 N terminus interacts with several phosphorylated proteins, its flexible connection to its C-terminal interface across the Mre11 dimer extends the reach and network of the MRN complex for orchestrating DDRs (16, 29). For example, comparison of Nbs1-bound versus free structures unveils a long-range conformational change transmitted from the FHA domain bound to the

phospho-CtIP-peptide through the BRCT domains, which may impact their binding to their phospho-serine protein targets, to drive an ~30° domain rotation. These changes, which pull the linker to the Mre11 interface by ~30 Å (19), are postulated to alter the Mre11 dimer symmetry thought to distinguish nuclease binding and activity for two-ended dsDNA breaks (symmetric dimer) and one-ended DNA forks (asymmetric dimer) (19). Overall, NBS1 (Xrs2 in *S. cerevisiae*) acts a chaperone and adaptor coordinating interactions of the MR complex with other proteins (63, 64) and as a regulated activator of Mre11 nuclease (65).

Consistent with the implications from structures and biochemistry, CtIP binds to the NBS1 FHA domains and evidently promotes resection in HR (50, 66–70) as one mode of conformational control for MRN. Recently, structure analyses of the N terminus for human CtIP and *S. pombe* Ctp1 reveal its assembly (48, 49). The full length and N terminus of the CtIP predominantly form a tetramer in solution. Furthermore, an extended Zn-binding motif in the coiled-coil domain adjacent to the N terminus extends the dimeric interface in both directions (**Figure 2*c***). This CtIP interface resembles the MRN architecture and may tune the MRN complex in the interlinked complex that joins two sets of MRN–DNA complexes.

Using MRN's structural biology, we propose a testable model for MRN endo- and exonuclease activity during HR (**Figure 2*d***). Other nucleases, proteins, and spatial constraints likely impact this mechanism. Following initial recruitment to the damaged region by NBS1 N-terminal interactions (in eukaryotes) (71), ATP-bound MRN complexes load onto both sides of the DSB with RAD50 coiled-coil domains as intralinked complexes. Owing to their proximity, the Zn-hook and coiled-coil domains may switch into an interlinked complex, as seen in EM images (32). The ring-like conformation is suitable to permit MRN to move back from the break to allow access to other proteins, as the coiled-coil domain spans ~1,200 Å , or ~300 base pairs (bp) (32). Alternatively, a recent single-molecule imaging study (34) shows that the MRN complex can diffuse along the DNA, even in the presence of nucleosomes, to find the DNA end and carry out resection in association with EXO1. This study shows that Rad50 is critical for promoting facilitated diffusion along the nucleosomes-coated DNA, whereas Mre11 recognizes the DNA ends. This separation of function suggests one possible reason for the coevolution of Mre11 and Rad50 in all domains of life. Nbs1 is dispensable for both activities, allowing recruitment of the MR complex to the break in lower organisms for which Nbs1 is absent.

Through its interactions with both MRN and DNA (72, 73), the tetrameric CtIP configuration suggests a mechanical role in geometrically activating an MRE11 endonuclease cut in the 5′ strand of the duplex with possible associated or sequential ATP hydrolysis. Upon ATP hydrolysis, RAD50 opens to allow MRE11 to carry out 3′–5′ resection toward the break, and the arms may be freed from the interlinked complex. At the nick, other 5′–3′ enzymes, such as EXO1/DNA2, resect away from the break to form extended ssDNA as the committed step and 3′ single-strand substrate for HR. Upon reaching the DSB end, the CtIP dissociation upon a posttranslational modification of MRN may regulate MRN off rate and avoid release of unprotected DNA ends. To gain sequence-level information to test this model and other possible MRN mechanisms, it will likely be necessary to employ cryo–electron microscopy (cryo-EM), as enabled by advances in sample preparation for large complexes, plus EM detection technology and data analysis

(74, 75). Integrated models of NHEJ from component structures have proved useful to guide both biochemical and cellular experiments (76), so we hope this may be the case for MRN.

MRN AND DOUBLE-STRAND BREAK REPAIR

MRN's role in DDR is extensively studied for DSB repair. Unrepaired DSBs are toxic and can lead to chromosomal rearrangements (cancer hallmarks) and cell death through apoptosis (77). DSBs are caused by endogenous events (such as RF stalling; see Section 4) and severe damage from oxidation or ionizing radiation (IR) (78). MRN functions in both DSB sensing and repair: It is recruited to DSBs through interaction between NBS1 and cell cycle checkpoint protein RAD17 (71). RAD17 is phosphorylated by ATM and recruited to DSBs independent of the MRN complex (ATM-activation mechanisms are thoroughly reviewed in 79). Once at DSBs, the MRN complex recruits and activates ATM through its NBS1 interaction (Figure 3), with the likely participation of other unidentified interfaces. Activated ATM phosphorylates mediator of DNA damage checkpoint 1 (MDC1), which in turn recruits more MRN to DSBs and thereby amplifies MRN/ATM signaling (80).

MRN/ATM signaling is regulated in part by factors that influence the stability and loading of the complex to DSBs. Interestingly, ATM and NBS1 are protected by the molecular chaperone heat shock protein 90 (Hsp90) alpha from polyubiquitination-mediated proteasomal degradation (81). Similarly, PIH1D1, a subunit of Hsp90 cochaperone R2TP complex, stabilizes MRE11 through its C-terminal interactions (82). Ubiquitination of NBS1 by the ring finger protein RNF8 promotes optimal binding to DSBs (83), whereas MRE11 phosphorylation by Polo-like kinase 1 (Plk1) inhibits recruitment of the MRN complex to DSBs (84). In addition, ATM-interacting protein (ATMIN) competes with NBS1 for ATM binding, therefore decreasing DSB-related ATM signaling (85, 86). In contrast, the recently discovered MRN-interacting protein (MRNIP) promotes MRN loading onto DSBs, thus contributing positively to MRN/ATM signaling (87). Consequently, activated ATM phosphorylates many downstream substrates to regulate checkpoint signaling and allow cells time to repair damage (88).

There are two major pathways through which cells repair DSBs (89) (Figure 3): the error-prone canonical nonhomologous end joining (C-NHEJ) and the error-free HR. NHEJ mends DSBs by end joining with minimal end processing. This process is initiated by Ku heterodimer (Ku70/Ku80) binding at DSB ends and recruitment of DNA-PKcs to phosphorylate various substrates and aid end processing and ligation by the DNA ligase IV complex (90). Two added pathways that repair DSBs with minimum end processing are resection-dependent NHEJ (rd-NHEJ) and microhomology-mediated end joining (MMEJ) (91, 92). In rd-NHEJ, an initial resection step involves MRE11 exonuclease, EXD2, Plk3-phosphorylated CtIP/BRCA1, EXO1, and Artemis endonuclease before repair through C-NHEJ occurs. MRE11 endonuclease activity may be dispensable in rd-NHEJ (92), whereas in MMEJ, initial resection involves both endo- and exonuclease activity of the MRN complex plus other enzymes, such as CtIP, PARP1, FEN1, and DNA ligases I and III (93, 94). The minimum homology for ligation is ~5 bp (94). However, Werner syndrome helicase (WRN) and Bloom syndrome helicase (BLM) regulate these alternative pathways by

blocking MRE11 and CtIP recruitment to DSB ends to promote repair through C-NHEJ (95, 96).

In HR, DSB ends are extensively resected to generate 3 ssDNA overhangs that inhibit NHEJ repair (Figure 3). As noted above, the evolving general model suggests that the MRN complex can initiate and license resection through an MRE11 endonuclease cut (nicking several hundred base pairs back from the DSB). Subsequent MRE11 exonuclease (3′–5′ direction toward the DSB) acts in association with phosphorylated CtIP (Sae2 in yeast). At the nick, 5′–3′ nucleases, such as EXO1 or DNA2 in conjunction with BLM or WRN, promote extended resection away from the DSB to generate 3′ ssDNA overhangs more than 1,000 bp in length that constitute the committed step for HR (29). EXO1 catalyzes long-range resection of MRN-processed DNA ends in human cells (97, 98). However, EXO1 is strongly inhibited by replication protein A (RPA) binding to the resulting ssDNA (99, 100). Single-molecule imaging identified an additional, noncatalytic role for MRN in long-range resection. Here, MRN physically interacts with EXO1, traveling with the resection machinery as a processivity factor that prevents RPA inhibition (34).

Via interaction with the MRN complex or CtIP, extended DNA resection is stimulated by additional enzymes, including RECQL4 helicase and possibly by EXD2 exonuclease (101, 102), which has alternatively been localized to the cytoplasm and regulates translation in mitochondria (103). Regardless, the biochemistry for these regulating partners suggests changes in protein–DNA interaction stability and off rates. As described in the proposed model (**Figure 2*d***), Sae2 (CtIP in humans) may dislodge the yeast MRX complex (Mre11–Rad50–Xrs2, MRN in humans) from ssDNA ends after resection (104, 105). In the absence of Sae2, DSB repair is impaired due to prolonged MRX complex binding. This defect is rescued by depleting the yeast 53BP1-ortholog RAD9, as 53BP1 promotes NHEJ by inhibiting dsDNA resection for HR (106, 107). In humans, the tumor suppressor BRCA1 inhibits 53BP1 at breaks in association with MRN and CtIP to promote HR over NHEJ (107–110). Extended RPA-coated ssDNA can activate ATR signaling (discussed in Section 4). For HR, RPA is displaced from ssDNA by RAD51 that forms nucleofilaments after loading by BRCA2 (111). RAD51 filaments enable a homology search, subsequent strand invasion, and template-dependent HR repair, as reviewed previously (111–113). Besides contributing to HR pathway choice, the BRCA1–BARD1 complex also plays an indispensable role in stimulating RAD51 recombinase activity (114). On the basis of the in vitro EM imaging data, the MRN complex may support HR by clamping the invading strand and HR template independent of its nuclease properties (32). Also, Mre11 nuclease-deficient or null TK46 cells show delays in Rad51 foci resolution after IR-induced breaks compared with wild-type cells. This delay suggests that the MRN complex may function downstream (as well as upstream) of RAD51-filament formation for proper resolution of HR intermediates (115).

Key factors regulating pathway choice for DSB repair are the cell cycle (116) and the nature of DNA ends (69). Whereas NHEJ-based repair pathways are active throughout the cell cycle, HR requires a DNA template for the repair and is active in only the S and G2 phases, during which the homologous sister chromosome is present. The nature of the DSB ends also regulates the repair pathway choice (69). If the DSB ends are free of protein adducts

and damaged nucleotides, they are likely channeled through NHEJ for repair in human cells. Otherwise, they require processing through resection and the phosphatase activity of polynucleotide phosphatase/kinase (PNKP) (117). MRN biochemical endo- and exonuclease activities act on both 5′ and 3′ strands of DNA with protein adducts, and its endonuclease activity is stimulated by CtIP (72). In contrast, NBS1 inhibits the MR 3′–5′ exonuclease activity on clean DSB ends (72). The MRN complex can thus remove covalently linked topoisomerase complexes from genomic DNA in a mechanism requiring CtIP and BRCA1 (115, 118, 119). The MRN complex in association with CtIP also protects genomic loci at common fragile sites and palindromic repeats (120). Indeed, DSBs occurring at these sites often lead to the formation of secondary structures that can attract structure-specific nucleases and result in chromosomal rearrangements (120). At such sites, however, MRE11 nuclease activity promotes HR for error-free repair.

MRN AND REPLICATION FORK DYNAMICS

Accurate replication of genetic information is a prime genetic selection, and stress during replication can slow, stall, or collapse the RF (121). Sources for replication-induced stress include damaged DNA, chromatin compaction, non-B-DNA (i.e., DNA structures that do not conform to the canonical Watson-Crick double helix) forming sequences (G-quadruplex, small inverted repeats, trinucleotides repeats), covalent protein-DNA adducts, and DNA/RNA hybrids (121). Cells can overcome these barriers through RF stabilization, protection, remodeling, and restart (121–124) along with dormant origin firing in higher organisms (125–128). HR is implicated in fork transactions during fork recovery; however, HR protein responses at forks can be independent of dsDNA break repair (124). Although the HR response restores the replication robustness under stress, it can also contribute to genomic instability as a result of its intrinsic reliance on a homologous sequence (reviewed in 124). Given its HR-initiating roles, the MRN complex helps orchestrate RF stress responses (Figure 4). Its role at forks can be both beneficial and detrimental and thus requires regulation.

At forks, protein-coated ssDNA is a platform for binding by MRN and other DNA repair proteins that initiate damage signaling, fork protection, and repair (129). An encounter between the replication machine (or replisome) and a replication barrier can uncouple the replicative helicase and polymerase. This uncoupling results in long stretches of ssDNA, leading to an ATR checkpoint response that helps to stabilize the fork and allows time to resolve the replication barrier by arresting cell cycle progression (Figure 4) (121). Stretches of ssDNA are protected by ssDNA-binding proteins such as RPA or human single-stranded DNA binding protein 1 (hSSB1) (130, 131). RPA binding to ssDNA inhibits annealing of short homologous sequences (as in short repeats) through microhomology-mediated repair and reduces secondary structures recognized by nucleases (132). The resulting protein-coated ssDNA not only is protected but also promotes binding of MRN and other DNA repair proteins (133). ATR, along with ATRIP, is recruited to ssDNA-RPA regions through ATRIP and RPA interactions (134). At the damaged forks, RAD9–RAD1–HUS1 (also known as the 9–1–1 clamp), is loaded onto the DNA end adjacent to RPA-bound ssDNA by the clamp loader RAD17–RFC complex (134). The 9–1–1 clamp recruits the ATR-activator protein topoisomerase-binding protein 1 (TopBP1) that assists ATR in phosphorylating its

downstream substrates, including CHK1 and RPA (Figure 3) (134). Notably, TopBP1 was recruited independently of the 9–1–1 complex to the ATR-activating sites at ssDNA-dsDNA junctions at the RFs through the MRN complex in *Xenopus* egg extracts (135). This result is consistent with the observation that the MRN-depleted *Xenopus* extracts show a decrease but not complete loss of CHK1 phosphorylation (136).

In fact, ATR activation is a critical function for MRN in damage signaling. MRE11 (in addition to ATM) is required for ATR activation upon IR-induced damage (137, 138). Furthermore, NBS1 activates ATR in vitro independently of MRE11 (139). Notably, for replication-associated DSBs, a substantial amount of RPA phosphorylation by ATR is dependent on the NBS1–RPA interaction and is independent of RAD17 (140). These functions are likely conserved in yeast, in which RPA recruits the MRX complex to stalled forks and DSBs (141). Upon recruitment, MRX-mediated sister-chromatid tethering is essential for survival of genomic stress at both forks and breaks.

ATR activation is also regulated by RAD50 (142). Once activated, ATR phosphorylates RAD50 at S635, which is required for cohesin complex (that holds together newly replicated chromatids) loading at the forks. Aside from phosphorylation, the neighboring Zn-hook sequence influences cohesin loading at replication sites to promote fork restart (143). These collective data suggest that multiple MRN components can orchestrate ATR signaling events that may themselves regulate the temporal sequence for MRN biochemical activities at RFs.

At forks, MRN-mediated resection plays a major functional role. MRN-mediated resection of nascent DNA likely frees the replisome from the stalled forks by promoting strand removal, fork inversion, and restart (123). MRE11 can generate 3′ overhangs to initiate HR in association with DNA2/EXO1. For example, in a recent study in a Werner syndrome–derived fibroblast cell line, the phosphorylation status of the S1133 of the WRN helicase influences the resection at breaks generated at the RF (144). Inhibition of S1133 phosphorylation in WRN results in impaired interaction with MRE11, defective MRN foci formation, and resection at the forks. Consequently, breaks at the fork can be repaired through DNA fusion by NHEJ with associated genome instability.

Although the MRN nuclease function can help resolve stalled RFs, unregulated resection can be catastrophic and lead to fork degradation through MRE11 exonuclease activity (145, 146). Thus, other proteins help regulate MRN-mediated nascent DNA degradation (147–150). The Fanconi anemia and breast cancer protein BRCA2 blocks MRE11-mediated resection of nascent ssDNA by promoting formation of stable nucleofilaments of RAD51 on nascent DNA (147, 151). However, cyclin-dependent kinase 2 (CDK2)–dependent phosphorylation of the BRCA2 C terminus results in disassembly of RAD51 filaments (152). This CDK2 activity is controlled by a complex formation with large tumor suppressor homolog 1 (LATS1) as part of the regulation of BRCA2 phosphorylation. Similarly, WRN helicase interacting protein 1 (WRNIP1) can stabilize RAD51 and thereby inhibit MRN-mediated degradation, a function independent of WRNIP1 ATPase activity (153). Like MRE11, WRN has an exonuclease domain with an associated coiled-coil region allowing assembly and partner interactions (154–156). Interestingly, the WRN exonuclease domain appears essential to inhibit MRE11/EXO1-dependent fork degradation under replication

stress induced with low doses of the TOP1 inhibitor camptothecin (157). MRN regulation at forks is thus enforced by protein interactions that block productive MRE11 nuclease binding.

By inhibiting Mre11 using endonuclease- and exonuclease-specific inhibitors, BRCA2 deficiency is rescued in tumor cells lacking functional BRCA2. Although BRCA-defective cancer cells can be killed by PARP inhibitors (PARPi), resistance to PARPi arises in cancer cells from the inactivation of proteins (such as PTIP, CHD4, and PARP1) that recruit MRE11 protein for nuclease processing at stalled forks (158). This unraveled resistance mechanism underscores the critical role for MRE11 in the regulated and error-free fork restart and how loss of MRE11 or its regulation at forks can rebalance damage responses in cancer cells. In fact, this may be the basis for genome instability from p53 mutations (159).

MRN AND DYSFUNCTIONAL TELOMERES

MRN responds to the disfunction of telomeres, which protect linear eukaryotic chromosome ends from DSB-related DDR and suppress replication instability (160). Telomeres consist of long (thousands of base pairs in length) duplex-DNA repeats (-TTAGGG-in humans) with shorter (up to several hundred base pairs) single-stranded 3′ overhangs and associated telomere protection–related proteins collectively termed the shelterin complex (161). Owing to the strict polarity restrictions of DNA polymerase, the 3′-DNA end sequence from the lagging strand is not duplicated at telomere ends, leading to shortening of the net telomere length with each replication cycle (162). Conversely, duplication of the leading strand always results in a blunt-ended dsDNA owing to correct polarity (Figure 5). Both leading and lagging strand telomeres are processed by nucleases Apollo and EXO1 to generate functional 3′ overhangs (163).

In addition to the shelterin complex, the reverse transcriptase telomerase maintains telomere length in budding yeast, stem cells, and many cancer cells (164). Yet interestingly, in most somatic cells, telomerase is inactive, leading to a limited number of replicative cycles and thereby regulating mutational accumulation and tumorigenesis (165–167). In budding yeast, the MRX complex may act in recruiting telomerase to telomeres (168). Also, telomere elongation may depend on X-ray repair cross-complementing protein 3 (XRCC3) and NBS1 in stem cells (169). The MRX complex may also assist Rad6 and Bre1 ubiquitin-conjugating enzymes in successful telomere replication (170).

In humans, telomeres function in tumor suppression. Cells either enter a replicative senescence state or undergo apoptosis once telomere length becomes critical, to avoid losing essential genes from linear chromosome ends (166). This regulatory mechanism relies on MRN and other proteins that assist the shelterin complex to avoid DSB repair at functional telomeres (reviewed in 171, 172).

At functional telomeres, DNA ends are protected. Since telomeres are G-rich sequences, they can form non-B-DNA structures such as G-quadruplexes that protect against strand degradation (173). As a result, replication in telomeric regions may have more frequent fork stalling (174) that may require resolution by MRN, but this role is uncertain.

In addition, the 3′ overhang can fold back on to the dsDNA to form a T-loop similar to strand invasion in HR (161, 175) (Figure 5). The six-protein shelterin complex contains telomeric repeat-binding factors 1 and 2 (TRF1 and TRF2), repressor and activator protein (RAP1), TRF1-interacting nuclear protein 2 (TIN2), protection of telomeres 1 (POT1), and TIN2- and POT1-interacting protein (TPP1) (176). TRF2 binds to dsDNA, protects the T-loop from helicases and resolvases (176), and inhibits ATM signaling, whereas POT1 preferentially binds ssDNA and inhibits ATR signaling (177) (Figure 5). Shelterin defects and telomerase abnormalities lead to dysfunctional telomeres, promoting MRN damage signaling and repair within a few cycles of replication (178).

The MRN complex may thus play dual roles at telomeres, where it may cause or inhibit genomic instability at dysfunctional telomeres by either promoting or inhibiting the NHEJ pathway, respectively (171). NBS1 co-crystallizes with TRF2 (179). NBS1 phosphorylation at S432 residue inhibits this interaction and promotes C-NHEJ at telomeres lacking TRF2 (179). Yet, NBS1 phosphorylation at the same site promotes A-NHEJ at telomeres lacking POT1–TPP1 (179). In addition to defective shelterin components, critically short or deleted ends lead to dysfunctional telomeres. Although most cancer cells use telomerase to replenish dysfunctional telomere lengths, some utilize MRE11-dependent and recombination-based alternative lengthening of telomere (ALT) for telomere maintenance (180, 181). Loss of both telomerase and the ALT pathway causes telomere dysfunction. In yeast cells with these characteristics, Mre11 opposes proliferation of cells lacking telomeres in the absence of Exo1 and checkpoint signaling (182).

In budding yeast lacking telomerase, the MRX complex promotes replicative senescence in association with Tel1 (ATM in humans) and RAP1-interacting factor 2 (Rif2) (183). Tel1 promotes MRX recruitment, whereas Rif2 acts as a negative regulator by enhancing ATP hydrolysis that opens Rad50, facilitating MRX release from DNA (184). In line with insights from yeast experiments, changes in expression and posttranslational modification of MRN and telomeric proteins are associated with various diseases in humans (185–187). In mouse embryonic fibro-blasts, the MRN complex is required for the response to telomere dysfunction, consistent with the idea that initiating this response shares common elements with the response to interstitial DNA damage (188). Interestingly, in patients with rheumatoid arthritis for which T cell aging occurs prematurely, low MRE11 levels lead to telomeric damage, juxtacentromeric heterochromatin unravelling, and upregulation of senescence marker. MRE11 inhibition in healthy T cells induced the aging phenotype, whereas MRE11 overexpression in rheumatoid arthritis T cells reverses it. The observation that the premature T cell aging is linked to MRE11 and telomere deprotection suggests MRE11 as a therapeutic target for immune aging and suppressing dysregulated inflammation (189). We expect that further knowledge of MRN biochemical mechanisms will be key to an improved understanding and intervention in multiple diseases including immune system aging.

MRN AND THE INNATE IMMUNE RESPONSE

MRN functions that sense, signal, and defend against viral infection (190) underscore the importance of deciphering the biochemical basis for its multifunctionality. MRN and other

damage recognition receptors detect viral DNA and initiate a cascade of innate immune and DNA damage signaling (191) (**Figure 6*a***). In the genome, activation of ATM by MRN is amplified over a mega-base region leading to formation of DNA damage foci, cell cycle arrest, and DNA repair (192). Upon viral infection, local but not global ATM activation and signaling are initiated by the MRN complex owing to the small size of viral DNA (193). This local ATM signaling avoids cell cycle arrest and global damage responses when a virus infects the cell (**Figure 6*a***) (193). Thus, MRN biochemical responses allow cells to differentiate genome damage signaling (global ATM signaling) for DNA repair compared with foreign-genome signaling (local ATM signaling) to block viral replication in the nucleolus (193). As part of its repair-related activities, the MRN complex in association with NHEJ repair proteins can further limit viral DNA replication in the nucleus by forming long viral DNA concatemers (190, 194, 195), which are too large to be packaged. Concatemers also interfere with replication: Replication origins generally present at the viral DNA termini are buried inside heterogeneously fused viral DNA and mutated through error-prone NHEJ (190, 196, 197) (**Figure 6*a***).

DNA in the cytoplasm is recognized as a potential viral infection and induces an immune response. MRN binding to viral DNA in the cytoplasm leads to production of type I interferons (IFNs) through activation of nuclear factor κβ (NF-κβ), a stimulator of interferon genes (STING), and interferon regulatory factor 3 (IRF3) (**Figure 6*a***) (198). Both MRE11 and RAD50, but not NBS1, are essential for producing IFNs. A cell line derived from an ATLD patient containing an MRE11 mutation that abrogates its DNA binding properties has defects in IFN production (198). Interestingly, RAD51 absence can also induce an innate response (199). RAD51 protects DNA inside the nucleus from MRE11-mediated nucleolytic degradation. Thus, in the absence of RAD51, MRE11 nuclease can degrade DNA, and the resulting DNA fragments are released into the cytoplasm where they activate the innate immune response. For interleukin-33 (IL-33), a member of the IL-1 superfamily of cytokines whose structure has been defined (200), stimulation upon adenoviral transduction depends on MRE11 status (201). Similarly, upon encountering cytosolic DNA, RAD50 forms a complex with innate immune system adaptor protein CARD9 in dendritic cells (202), which are the antigen-presenting cells that link the innate and adaptive immune systems. The cytosolic dsDNA-induced signaling complex (dsDNA–RAD50–CARD9) stimulates production of proinflammatory cytokine IL-1β through NF-κβ pathway activation (**Figure 6*a***) (202). However, viruses encode specific proteins that bind MRN and other host DNA repair proteins rendering them inactive through protein relocalization and degradation (**Figure 6*b***) by sumoylation and ubiquitination (190, 203). Yet, viruses can also rely on MRN and other host DNA repair proteins for successful lytic replication.

As a result, manipulation of MRN biochemistry by interactions with virus proteins is under active investigation, and several of these have been uncovered in human adenovirus (Ad) infection. Ad early region 4 (E4) encodes two proteins from open reading frame 3 (E4-ORF3) and open reading frame 6 (E4-ORF6). These proteins (with viral protein E1b-55K) inhibit concatemer formation by the MRN complex via relocalization and degradation (190, 204–207), avoiding MRN-mediated defects in late viral replication (205, 207). Upon

recognition, E4-ORF3 protein localizes the MRN complex to specific nuclear locations in the host cells known as promyelocytic leukemia protein domains (or PML bodies), rendering the complex inactive (**Figure 6*b***) (208). X-ray crystallography and mutational analyses show that E4-ORF3 assemble into both linear and branched polymeric structures by C-terminal tail swapping (209). This polymeric network spans the entire nuclear volume, forming multi-site interactions with PML bodies and MRN-binding interface in the C-terminal tail. Upon relocalization to PML, E4-ORF3 induces sumoylation of MRN complex components (210, 211). The E4-ORF6 in association with E1b-55K recruits other cellular proteins, such as cullin 5, Rbx1, and elongin B and C to form an active ubiquitin ligase complex (212, 213). This activated ligase complex ubiquitinates the MRN complex for proteasome-dependent degradation (190).

Building upon the Ad results, MRN roles are increasingly being elucidated in virus infection associated with human diseases—for example, high-risk human papillomavirus (HPV) that is implicated in cervical cancer and as well as in genital, head, and neck cancers (214). HPV-31 increases MRN complex half-life for productive viral replication (215). HPV-16 encodes transcription and replication factor E2 that interacts with RAD50-interacting protein 1 (Rint1) (216). Overexpression of exogenous Rint1 enhances HPV-16 replication, and a truncated mutant that lacks a RAD50-interacting domain reduces HPV-16 replication. Thus, HPV-16 may target MRN complex inactivation through Rint1 for productive replication. Similarly, the MRN complex inhibits replication of Kaposi sarcoma herpesvirus (KHSV) (217), which is implicated in Kaposi sarcoma, the most common cancer in HIV-infected individuals (218, 219). KHSV encodes a cytoplasmic isoform of latency-associated nuclear antigen (LANA). This isoform inhibits the host innate immune response by neutralizing MRN and cyclic GMP-AMP synthase DNA sensors (217).

The relationship linking viral infection, DNA repair, and innate immunity has biomedical importance, as oncolytic virus-based therapies are implemented in several cancers. For example, a modified oncolytic adenovirus mutant that lacks an antiapoptotic gene (*E1B19K*) sensitizes pancreatic cells in association with DNA-damage drugs (220), and the proposed mechanism is MRE11 inactivation. For oncolytic adenovirus type 5 being used in ovarian cancer cells (221), virus cytotoxicity is proposed to enable its ability to redistribute MRN (and other HR components). Overall, our evolving knowledge of interactions between viral infection, DNA repair, and the immune response requires defining multiple roles for MRN biochemistry.

SUMMARY AND PERSPECTIVES

A key aspect of the DNA double-helical structure, initially absent in the Watson and Crick model, is that it contains information not only for replication but also for repair of genomic damage (222) that is recognized by biologically conserved complexes such as MRN. Thus, MRN-DNA biochemistry is uncovering primary origins of normal and pathological cellular activities for DNA stress responses. Yet, this requires a path for a comprehensive characterization of proteins and nucleic acids, including their biologically relevant complexes and conformations (223). These observations have motivated our efforts to develop high-throughput and objective X-ray methods for comprehensive measurements of

complexes and conformations and flexibility under near-physiological conditions (e.g., see 224–229). Thus, X-ray scattering and diffraction methods coupled to cryo-EM and single-molecule data are helping to define the MRN structural biochemistry described here.

At the cellular level, MRN defects can cause chromosome instability. At the systems level, we see the importance of maintaining the B-DNA double helix structure, as potential non-B-DNA structures (PONDS) occur at high frequency in cancer as translocations and deletion break points (230). Furthermore, at RFs where the double helix is opened for processing, precise control of DNA ends is needed to avoid not only mutations but also template switching and large repeat expansions (231, 232). These and other data provide compelling support for the notion that DNA structures at forks, ends, and breaks are intrinsic risk factors that complexes such as MRN are under strong evolutionary pressure to tightly control.

Combined genetic and biochemical data suggest that MRN has evolved to coordinate cellular network responses to DNA damage linked to mutation, cancer, and cell death. Its modular components are integrated into complexes and pathway networks in different ways, but MRN core structures and activities are maintained and conserved across all domains of life. Collective data show that multiple DNA stress responses are dynamically interrelated by MRN in ways that depend on its interactions and conformations. These results have broad implications for damage responses, as we are learning that even direct damage reversal enzymes, such as ALKBH3 (233), surprisingly function within large complexes for DNA and RNA integrity (234).

The evolutionarily conserved MRN complex is thus decoding a biological language of dynamic shapes controlling chemistry, connections, and complex functionalities from self-assembled, sequence-encoded building blocks whose fidelity is maintained by complexes such as MRN. For example, mutants modulating RAD50 dynamic conformations can switch biological outcomes from DNA excision to end joining (52). The data reviewed here demonstrate how MRN interactions and resulting activities feed into cellular decision making in DNA stress responses including viral defenses (typically considered to be only broadly related to the DDR). MRN subunit biochemical activities depend upon their three-dimensional folds, but MRN biological functions are governed by its complexes and conformationally regulated interaction networks. Thus, HR and MMEJ are activated in irradiated human cells owing to phosphorylation-dependent formation of either NBS1 or XRCC1 repair complexes with MRE11–RAD50 (93). In fact, the use of the same MRN subunits in multiple response networks provides a physical means to coordinate sensing, signaling, chromatin remodeling, and repair processing. This helps ensure appropriate responses to DSBs and stalled forks that avoid destructive interference and thus chromosome breakage and aberrations. As its cellular selectivity and function are determined partly by its assembly with partners and damaged DNA, we have focused on what we know and would like to know about dynamic MRN complexes and their control of biological outcomes. From the results to date, we can expect that defining the biochemical activities of MRN components and their assemblies will provide a critical foundation for understanding multiple DNA stress responses.

As a consequence of its dynamic conformations and assemblies, MRN responses are DNA structure specific rather than sequence specific. For example, MRN complexes confer specificity to distinct nuclease processing at DNA breaks and forks. These processes include an endonuclease nick and the initiation of exonuclease processing that is not adjacent to the DSB. Moreover, the resulting combined MRE11 endo- and exonuclease activities can act as go/no-go controls in the selection of HR versus NHEJ pathways (27). MRN can thus orchestrate DDRs as a sensor, regulator, and effector within distinct DSB repair, RF, and telomere rescue complexes as well as in viral response networks.

We propose that MRN conducts, coordinates, and connects responses to DNA stress largely by orchestrating three processes: assembly (localization and protection at damage), conformation (creation of a productive geometry for interactions and active site chemistry), and disassembly (controlling access and handoffs of DNA intermediates). In chemistry, transition state theory instructs us on the control of the reaction barrier as the expected rate-limiting step. In the cellular biochemistry of DNA replication and repair, however, the rate-limiting step is often not chemistry, which has been evolutionarily optimized for high efficiency. Instead, we see that protein and DNA binding energy is converted into conformational change to regulate network responses on the basis of the cell cycle state and the nature of the DNA damage. At the nanoscale of MRN, charge, chemical bonding, molecular mechanics, and thermal energy share similar scales: This allows their efficient interconservation to regulate specific activities within metastable MRN–DNA and MRN–protein intermediates. We propose that this ability to transfer binding energy into functionally important conformational states is an evolutionarily optimized and emergent MRN property. In practical terms, targeting conformational switching, as proposed here for RAD50 on the basis of the exceptional patient response in an otherwise failed clinical trial (60), suggests a novel biochemical strategy for precision medicine.

More broadly, current and emerging MRN results are aiding in addressing grand challenges of the postgenomic era for biochemistry and cancer biology: (*a*) an accurate prediction of pathology for damage response sequence variants, (*b*) an actionable mechanistic understanding of oncogenic replication and repair stress for biology and medicine, and (*c*) an advanced assessment of functional biochemical complexes and networks controlling outcomes from molecules to cells (235). Existing insights are already changing our concepts of damage detection, signaling, and repair with implications for strategies of controlling MRN activities for cancer, T cell aging, immune responses, and viral infections. Going forward, we will increasingly need to link structures of intact dynamic assemblies from cryo-EM and X-ray scattering studies to outcomes in cells and furthermore test this multiscale understanding with separation-of-function mutations and activity-specific chemical inhibitors, as was done for MRE11 (27). Emerging robust methods for the coexpression of large, multiprotein complexes provide enabling technologies for examining functional assemblies(75). The resulting foundational knowledge defining how these molecular machineries act may allow control of DNA stress responses for biology and medicine. We suggest that targeting MRN biochemical activities in DNA damage and the innate immune response together may overcome the mutational phenotype that characterizes cancer cells (236), analogously to how antibiotic knockdown of pathogens allows their clearance by our immune system.

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LITERATURE CITED

1. Ciccia A , Elledge SJ . 2010 The DNA damage response: making it safe to play with knives. Mol. Cell40:179–20420965415
2. D’Amours D , Jackson SP . 2002 The MRE11 complex: at the crossroads of DNA repair and checkpoint signalling. Nat. Rev. Mol. Cell Biol 3:317–2711988766
3. Connelly JC , Leach DRF . 2002 Tethering on the brink: the evolutionarily conserved Mre11–Rad50 complex. Trends Biochem. Sci 27:410–1812151226
4. Stracker TH , Petrini JHJ . 2011 The MRE11 complex: starting from the ends. Nat. Rev. Mol. Cell Biol12:90–10321252998
5. Uchisaka N , Takahashi N , Sato M , Kikuchi A , Mochizuki S , et al. 2009 Two brothers with ataxia-telangiectasia-like disorder with lung adenocarcinoma. J. Pediatr 155:435–3819732584
6. Matsumoto Y , Miyamoto T , Sakamoto H , Izumi H , Nakazawa Y , et al. 2011 Two unrelated patients with MRE11A mutations and Nijmegen breakage syndrome-like severe microcephaly. DNA Repair 10:314–2121227757
7. Miyamoto R , Morino H , Yoshizawa A , Miyazaki Y , Maruyama H , et al. 2014 Exome sequencing reveals a novel MRE11 mutation in a patient with progressive myoclonic ataxia. J. Neurol. Sci 337:219–2324332946
8. Stewart GS , Maser RS , Stankovic T , Bressan DA , Kaplan MI , et al. 1999 The DNA double-strand break repair gene *hMRE11* is mutated in individuals with an ataxia-telangiectasia-like disorder. Cell 99:577–8710612394
9. Delia D 2004 MRE11 mutations and impaired ATM-dependent responses in an Italian family with ataxia-telangiectasia-like disorder. Hum. Mol. Genet 13:2155–6315269180
10. Fernet M , Gribaa M , Salih MAM , Seidahmed MZ , Hall J , Koenig M . 2005 Identification and functional consequences of a novel MRE11 mutation affecting 10 Saudi Arabian patients with the ataxia telangiectasia-like disorder. Hum. Mol. Genet 14:307–1815574463
11. Brandt S , Samartzis EP , Zimmermann A-K , Fink D , Moch H , et al. 2017 Lack of MRE11-RAD50-NBS1 (MRN) complex detection occurs frequently in low-grade epithelial ovarian cancer. BMC Cancer 17:4428073364
12. Gravells P , Grant E , Smith KM , James DI , Bryant HE . 2017 Specific killing of DNA damage-response deficient cells with inhibitors of poly(ADP-ribose) glycohydrolase. DNA Repair 52:81–9128254358
13. Kim I-K , Kiefer JR , Ho CMW , Stegeman RA , Classen S , et al. 2012 Structure of mammalian poly(ADP-ribose) glycohydrolase reveals a flexible tyrosine clasp as a substrate-binding element. Nat. Struct. Mol. Biol 19:653–5622609859

14. Wang Y , Gudikote J , Giri U , Yan J , Deng W , et al. 2018 RAD50 expression is associated with poor clinical outcomes after radiotherapy for resected non–small cell lung cancer. Clin. Cancer Res 24:341–5029030353
15. Lamarche BJ , Orazio NI , Weitzman MD . 2010 The MRN complex in double-strand break repair and telomere maintenance. FEBS Lett 584:3682–9520655309
16. Williams GJ , Lees-Miller SP , Tainer JA . 2010 Mre11–Rad50–Nbs1 conformations and the control of sensing, signaling, and effector responses at DNA double-strand breaks. DNA Repair 9:1299–30621035407
17. Lavin M , Kozlov S , Gatei M , Kijas A . 2015 ATM-dependent phosphorylation of all three members of the MRN complex: from sensor to adaptor. Biomolecules 5:2877–90226512707
18. Matsuoka S , Ballif BA , Smogorzewska A , McDonald ER , Hurov KE , et al. 2007 ATM and ATR substrate analysis reveals extensive protein networks responsive to DNA damage. Science 316:1160–6617525332
19. Williams RS , Dodson GE , Limbo O , Yamada Y , Williams JS , et al. 2009 Nbs1 flexibly tethers Ctp1 and Mre11-Rad50 to coordinate DNA double-strand break processing and repair. Cell 139:87–9919804755
20. Lloyd J , Chapman JR , Clapperton JA , Haire LF , Hartsuiker E , et al. 2009 A supramodular FHA/BRCT-repeat architecture mediates Nbs1 adaptor function in response to DNA damage. Cell 139:100–1119804756
21. Liu Y , Sung S , Kim Y , Li F , Gwon G , et al. 2016 ATP-dependent DNA binding, unwinding, and resection by the Mre11/Rad50 complex. EMBO J 35:743–5826717941
22. Sung S , Li F , Park YB , Kim JS , Kim AK , et al. 2014 DNA end recognition by the Mre11 nuclease dimer: insights into resection and repair of damaged DNA. EMBO J 33:2422–3525107472
23. Schiller CB , Lammens K , Guerini I , Coordes B , Feldmann H , et al. 2012 Structure of Mre11–Nbs1 complex yields insights into ataxia-telangiectasia–like disease mutations and DNA damage signaling. Nat. Struct. Mol. Biol 19:693–70022705791
24. Hammel M , Yu Y , Radhakrishnan SK , Chokshi C , Tsai M-S , et al. 2016 An intrinsically disordered APLF links Ku, DNA-PKcs, and XRCC4-DNA ligase IV in an extended flexible non-homologous end joining complex. J. Biol. Chem 291:26987–700627875301
25. Lavin MF . 2007 ATM and the Mre11 complex combine to recognize and signal DNA double-strand breaks. Oncogene 26:7749–5818066087
26. Marechal A , Zou L . 2013 DNA damage sensing by the ATM and ATR kinases. Cold Spring Harb. Perspect. Biol 5:a01271624003211
27. Shibata A , Moiani D , Arvai AS , Perry J , Harding SM , et al. 2014 DNA double-strand break repair pathway choice is directed by distinct MRE11 nuclease activities. Mol. Cell 53:7–1824316220
28. Trenz K , Smith E , Smith S , Costanzo V . 2006 ATM and ATR promote Mre11 dependent restart of collapsed replication forks and prevent accumulation of DNA breaks. EMBO J 25:1764–7416601701
29. Lafrance-Vanasse J , Williams GJ , Tainer JA . 2015 Envisioning the dynamics and flexibility of Mre11-Rad50-Nbs1 complex to decipher its roles in DNA replication and repair. Prog. Biophys. Mol. Biol 117:182–9325576492
30. Hopfner K-P , Karcher A , Shin DS , Craig L , Arthur LM , et al. 2000 Structural biology of Rad50 ATPase. Cell 101:789–80010892749
31. Cahill D , Carney JP . 2007 Dimerization of the Rad50 protein is independent of the conserved hook domain. Mutagenesis 22:269–7417426050
32. Hopfner K-P , Craig L , Moncalian G , Zinkel RA , Usui T , et al. 2002 The Rad50 zinc-hook is a structure joining Mre11 complexes in DNA recombination and repair. Nature 418:562–6612152085
33. Park YB , Hohl M , Padjasek M , Jeong E , Jin KS , et al. 2017 Eukaryotic Rad50 functions as a rod-shaped dimer. Nat. Struct. Mol. Biol 24:248–5728134932

34. Myler LR , Gallardo IF , Soniat MM , Deshpande RA , Gonzalez XB , et al. 2017 Single-molecule imaging reveals how Mre11-Rad50-Nbs1 initiates DNA break repair. Mol. Cell 67:891–98.e428867292
35. Williams GJ , Williams RS , Williams JS , Moncalian G , Arvai AS , et al. 2011 ABC ATPase signature helices in Rad50 link nucleotide state to Mre11 interface for DNA repair. Nat. Struct. Mol. Biol 18:423–4121441914
36. Williams RS , Moncalian G , Williams JS , Yamada Y , Limbo O , et al. 2008 Mre11 dimers coordinate DNA end bridging and nuclease processing in double-strand-break repair. Cell 135:97–10918854158
37. Das D , Moiani D , Axelrod HL , Miller MD , McMullan D , et al. 2010 Crystal structure of the first eubacterial Mre11 nuclease reveals novel features that may discriminate substrates during DNA repair. J. Mol. Biol 397:647–6320122942
38. Seifert FU , Lammens K , Stoehr G , Kessler B , Hopfner KP . 2016 Structural mechanism of ATP-dependent DNA binding and DNA end bridging by eukaryotic Rad50. EMBO J 35:759–7226896444
39. Seifert FU , Lammens K , Hopfner K-P . 2015 Structure of the catalytic domain of Mre11 from *Chaetomium thermophilum.* Acta Crystallogr. Sect. F 71:752–57
40. 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 105:473–8511371344
41. Lee JH . 2004 Direct activation of the ATM protein kinase by the Mre11/Rad50/Nbs1 complex. Science 304:93–9615064416
42. Falck J , Coates J , Jackson SP . 2005 Conserved modes of recruitment of ATM, ATR and DNA-PKcs to sites of DNA damage. Nature 434:605–1115758953
43. 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 25:5363–7915964794
44. Sibanda BL , Chirgadze DY , Ascher DB , Blundell TL . 2017 DNA-PKcs structure suggests an allosteric mechanism modulating DNA double-strand break repair. Science 355:520–2428154079
45. Stracker TH , Morales M , Couto SS , Hussein H , Petrini JHJ . 2007 The carboxy terminus of NBS1 is required for induction of apoptosis by the MRE11 complex. Nature 447:218–2117429352
46. Difilippantonio S , Celeste A , Kruhlak MJ , Lee Y , Difilippantonio MJ , et al. 2007 Distinct domains in Nbs1 regulate irradiation-induced checkpoints and apoptosis. J. Exp. Med 204:1003–1117485521
47. Moiani D , Ronato DA , Brosey CA , Arvai AS , Syed A , et al. 2017 Targeting allostery with avatars to design inhibitors assessed by cell activity: dissecting MRE11 endo- and exonuclease activities. Methods Enzymol 601:205–41
48. Davies OR , Forment JV , Sun M , Belotserkovskaya R , Coates J , et al. 2015 CtIP tetramer assembly is required for DNA-end resection and repair. Nat. Struct. Mol. Biol 22:150–5725558984
49. Andres SN , Appel CD , Westmoreland JW , Williams JS , Nguyen Y , et al. 2015 Tetrameric Ctp1 coordinates DNA binding and DNA bridging in DNA double-strand-break repair. Nat. Struct. Mol. Biol 22:158–6625580577
50. Andres SN , Williams RS . 2017 CtIP/Ctp1/Sae2, molecular form fit for function. DNA Repair 56:109–1728623092
51. Lammens K , Bemeleit DJ , Mockel C , Clausing E , Schele A , et al. 2011 The Mre11:Rad50 structure shows an ATP-dependent molecular clamp in DNA double-strand break repair. Cell 145:54–6621458667
52. Deshpande RA , Williams GJ , Limbo O , Williams RS , Kuhnlein J , et al. 2014 ATP-driven Rad50 conformations regulate DNA tethering, end resection, and ATM checkpoint signaling. EMBO J 33:482– 50024493214
53. Rojowska A , Lammens K , Seifert FU , Direnberger C , Feldmann H , Hopfner KP . 2014 Structure of the Rad50 DNA double-strand break repair protein in complex with DNA. EMBO J 33:2847–5925349191

54. He J , Shi LZ , Truong LN , Lu C-S , Razavian N , et al. 2012 Rad50 zinc hook is important for the Mre11 complex to bind chromosomal DNA double-stranded breaks and initiate various DNA damage responses. J. Biol. Chem 287:31747–5622833675
55. Barfoot T , Herdendorf TJ , Behning BR , Stohr BA , Gao Y , et al. 2015 Functional analysis of the bacteriophage T4 Rad50 homolog (gp46) coiled-coil domain. J. Biol. Chem 290:23905–1526242734
56. Roset R , Inagaki A , Hohl M , Brenet F , Lafrance-Vanasse J , et al. 2014 The Rad50 hook domain regulates DNA damage signaling and tumorigenesis. Genes Dev 28:451–6224532689
57. Hohl M , Kwon Y , Galvan SM , Xue X , Tous C , et al. 2011 The Rad50 coiled-coil domain is indispensable for Mre11 complex functions. Nat. Struct. Mol. Biol 18:1124–3121892167
58. Reindl S , Ghosh A , Williams GJ , Lassak K , Neiner T , et al. 2013 Insights into FlaI functions in archaeal motor assembly and motility from structures, conformations, and genetics. Mol. Cell 49:1069–8223416110
59. Acharya SN , Many AM , Schroeder AP , Kennedy FM , Savytskyy OP , et al. 2008 *Coprinus cinereus* Rad50 mutants reveal an essential structural role for Rad50 in axial element and synaptonemal complex formation, homolog pairing and meiotic recombination. Genetics 180:1889–90718940790
60. Al-Ahmadie H , Iyer G , Hohl M , Asthana S , Inagaki A , et al. 2014 Synthetic lethality in ATM-deficient RAD50-mutant tumors underlies outlier response to cancer therapy. Cancer Discov 4:1014–2124934408
61. Gao Y , Nelson SW . 2014 Autoinhibition of bacteriophage T4 Mre11 by its C-terminal domain. J. Biol. Chem 289:26505–1325077970
62. Tsutakawa SE , Lafrance-Vanasse J , Tainer JA . 2014 The cutting edges in DNA repair, licensing, and fidelity: DNA and RNA repair nucleases sculpt DNA to measure twice, cut once. DNA Repair 19:95–10724754999
63. Kim JH , Grosbart M , Anand R , Wyman C , Cejka P , Petrini JHJ . 2017 The Mre11-Nbs1 interface is essential for viability and tumor suppression. Cell Rep 18:496–50728076792
64. Oh J , Al-Zain A , Cannavo E , Cejka P , Symington LS . 2016 Xrs2 dependent and independent functions of the Mre11-Rad50 complex. Mol. Cell 64:405–1527746018
65. Crown KN , Savytskyy OP , Malik SB , Logsdon J , Williams RS , et al. 2013 A mutation in the FHA domain of *Coprinus cinereus* Nbs1 leads to Spo11-independent meiotic recombination and chromosome segregation. G3 3:1927–4324062528
66. Makharashvili N , Paull TT . 2015 CtIP: a DNA damage response protein at the intersection of DNA metabolism. DNA Repair 32:75–8125957490
67. Sartori AA , Lukas C , Coates J , Mistrik M , Fu S , et al. 2007 Human CtIP promotes DNA end resection. Nature 450:509–1417965729
68. Anand R , Ranjha L , Cannavo E , Cejka P . 2016 Phosphorylated CtIP functions as a co-factor of the MRE11-RAD50-NBS1 endonuclease in DNA end resection. Mol. Cell 64:940–5027889449
69. Liao S , Tammaro M , Yan H . 2016 The structure of ends determines the pathway choice and Mre11 nuclease dependency of DNA double-strand break repair. Nucleic Acids Res 44:5689–70127084932
70. Jensen KL , Russell P . 2016 Ctp1-dependent clipping and resection of DNA double-strand breaks by Mre11 endonuclease complex are not genetically separable. Nucleic Acids Res 44:8241–4927325741
71. Wang Q , Goldstein M , Alexander P , Wakeman TP , Sun T , et al. 2014 Rad17 recruits the MRE11-RAD50-NBS1 complex to regulate the cellular response to DNA double-strand breaks. EMBO J 33:862–7724534091
72. Deshpande RA , Lee J-H , Arora S , Paull TT . 2016 Nbs1 converts the human Mre11/Rad50 nuclease complex into an endo/exonuclease machine specific for protein-DNA adducts. Mol. Cell 64:593–60627814491
73. Cannavo E , Cejka P . 2014 Sae2 promotes dsDNA endonuclease activity within Mre11–Rad50–Xrs2 to resect DNA breaks. Nature 514:122–2525231868
74. Eisenstein M 2015 The field that came in from the cold. Nat. Methods 13:19–22

75. Gradia SD , Ishida JP , Tsai M-S , Jeans C , Tainer JA , Fuss JO . 2017 MacroBac: new technologies for robust and efficient large-scale production of recombinant multiprotein complexes. Methods Enzymol 592:1–2628668116
76. Williams GJ , Hammel M , Radhakrishnan SK , Ramsden D , Lees-Miller SP , Tainer JA . 2014 Structural insights into NHEJ: building up an integrated picture of the dynamic DSB repair super complex, one component and interaction at a time. DNA Repair 17:110–2024656613
77. Jackson SP , Bartek J . 2009 The DNA-damage response in human biology and disease. Nature 461:1071–7819847258
78. Lomax ME , Folkes LK , O'Neill P . 2013 Biological consequences of radiation-induced DNA damage: relevance to radiotherapy. Clin. Oncol 25:578–85
79. Paull TT . 2015 Mechanisms of ATM activation. Annu. Rev. Biochem 84:711–3825580527
80. Wu L , Luo K , Lou Z , Chen J . 2008 MDC1 regulates intra-S-phase checkpoint by targeting NBS1 to DNA double-strand breaks. PNAS 105:11200–518678890
81. Pennisi R , Antoccia A , Leone S , Ascenzi P , di Masi A . 2017 Hsp90αregulates ATM and NBN functions in sensing and repair of DNA double-strand breaks. FEBS J 284:2378–9528631426
82. von Morgen P , Burdova K , Flower TG , O'Reilly NJ , Boulton SJ , et al. 2017 MRE11 stability is regulated by CK2-dependent interaction with R2TP complex. Oncogene 36:4943–5028436950
83. Lu C-S , Truong LN , Aslanian A , Shi LZ , Li Y , et al. 2012 The RING finger protein RNF8 ubiquiti-nates Nbs1 to promote DNA double-strand break repair by homologous recombination. J. Biol. Chem 287:43984–9423115235
84. Li Z , Li J , Kong Y , Yan S , Ahmad N , Liu X . 2017 Plk1 phosphorylation of Mre11 antagonizes the DNA damage response. Cancer Res 77:3169–8028512243
85. Zhang T , Penicud K , Bruhn C , Loizou JI , Kanu N , et al. 2012 Competition between NBS1 and ATMIN controls ATM signaling pathway choice. Cell Rep 2:1498–50423219553
86. Zhang T , Cronshaw J , Kanu N , Snijders AP , Behrens A . 2014 UBR5-mediated ubiquitination of ATMIN is required for ionizing radiation-induced ATM signaling and function. PNAS 111:12091–9625092319
87. Staples CJ , Barone G , Myers KN , Ganesh A , Gibbs-Seymour I , et al. 2016 MRNIP/C5orf45 interacts with the MRN complex and contributes to the DNA damage response. Cell Rep 16:2565–7527568553
88. Abraham RT . 2001 Cell cycle checkpoint signaling through the ATM and ATR kinases. Genes Dev 15:2177–9611544175
89. Shin DS , Chahwan C , Huffman JL , Tainer JA . 2004 Structure and function of the double-strand break repair machinery. DNA Repair 3:863–7315279771
90. Mahaney BL , Meek K , Lees-Miller SP . 2009 Repair of ionizing radiation-induced DNA double-strand breaks by non-homologous end-joining. Biochem. J 417:639–5019133841
91. Sfeir A , Symington LS . 2015 Microhomology-mediated end joining: A back-up survival mechanism or dedicated pathway? Trends Biochem. Sci 40:701–1426439531
92. Biehs R , Steinlage M , Barton O , Juhász S , Künzel J , et al. 2017 DNA double-strand break resection occurs during non-homologous end joining in G1 but is distinct from resection during homologous recombination. Mol. Cell 65:671–84.e528132842
93. Dutta A , Eckelmann B , Adhikari S , Ahmed KM , Sengupta S , et al. 2016 Microhomology-mediated end joining is activated in irradiated human cells due to phosphorylation-dependent formation of the XRCC1 repair complex. Nucleic Acids Res 45:2585–99
94. Sharma S , Javadekar SM , Pandey M , Srivastava M , Kumari R , Raghavan SC . 2015 Homology and enzymatic requirements of microhomology-dependent alternative end joining. Cell Death Dis 6:e169725789972
95. Shamanna RA , Lu H , de Freitas JK , Tian J , Croteau DL , Bohr VA . 2016 WRN regulates pathway choice between classical and alternative non-homologous end joining. Nat. Commun 7:1378527922005
96. Grabarz A , Guirouilh-Barbat J , Barascu A , Pennarun G , Genet D , et al. 2013 A role for BLM in double-strand break repair pathway choice: prevention of CtIP/Mre11-mediated alternative nonhomologous end-joining. Cell Rep 5:21–2824095737

97. Tomimatsu N , Mukherjee B , Deland K , Kurimasa A , Bolderson E , et al. 2012 Exo1 plays a major role in DNA end resection in humans and influences double-strand break repair and damage signaling decisions. DNA Repair 11:441–4822326273
98. Tomimatsu N , Mukherjee B , Hardebeck MC , Ilcheva M , Camacho CV , et al. 2014 Phosphorylation of EXO1 by CDKs 1 and 2 regulates DNA end resection and repair pathway choice. Nat. Commun 5:356124705021
99. Myler LR , Gallardo IF , Zhou Y , Gong F , Yang S-H , et al. 2016 Single-molecule imaging reveals the mechanism of Exo1 regulation by single-stranded DNA binding proteins. PNAS 113:E1170–7926884156
100. Genschel J , Modrich P . 2003 Mechanism of 5′-directed excision in human mismatch repair. Mol. Cell 12:1077–8614636568
101. Lu H , Shamanna RA , Keijzers G , Anand R , Rasmussen LJ , et al. 2016 RECQL4 promotes DNA end resection in repair of DNA double-strand breaks. Cell Rep 16:161–7327320928
102. Broderick R , Nieminuszczy J , Baddock HT , Deshpande RA , Gileadi O , et al. 2016 EXD2 promotes homologous recombination by facilitating DNA end resection. Nat. Cell Biol 18:271–8026807646
103. Silva J , Aivio S , Knobel PA , Bailey LJ , Casali A , et al. 2018 EXD2 governs germ stem cell homeostasis and lifespan by promoting mitoribosome integrity and translation. Nat. Cell Biol 20:162–7429335528
104. Puddu F , Oelschlaegel T , Guerini I , Geisler NJ , Niu H , et al. 2015 Synthetic viability genomic screening defines Sae2 function in DNA repair. EMBO J 34:1509–2225899817
105. Chen H , Donnianni RA , Handa N , Deng SK , Oh J , et al. 2015 Sae2 promotes DNA damage resistance by removing the Mre11–Rad50–Xrs2 complex from DNA and attenuating Rad53 signaling. PNAS 112:E1880–8725831494
106. Nitiss JL , Ferrari M , Dibitetto D , De Gregorio G , Eapen VV , et al. 2015 Functional interplay between the 53BP1-ortholog Rad9 and the Mre11 complex regulates resection, end-tethering and repair of a double-strand break. PLOS Genet 11:e100492825569305
107. Bunting SF , Callen E , Wong N , Chen H-T , Polato F , et al. 2010 53BP1 inhibits homologous recombination in Brca1-deficient cells by blocking resection of DNA breaks. Cell 141:243–5420362325
108. Bothmer A , Robbiani DF , Feldhahn N , Gazumyan A , Nussenzweig A , Nussenzweig MC . 2010 53BP1 regulates DNA resection and the choice between classical and alternative end joining during class switch recombination. J. Exp. Med 207:855–6520368578
109. Bouwman P , Aly A , Escandell JM , Pieterse M , Bartkova J , et al. 2010 53BP1 loss rescues BRCA1 deficiency and is associated with triple-negative and BRCA-mutated breast cancers. Nat. Struct. Mol. Biol 17:688–9520453858
110. Daley JM , Sung P . 2014 53BP1, BRCA1, and the choice between recombination and end joining at DNA double-strand breaks. Mol. Cell. Biol 34:1380–8824469398
111. Thompson LH . 2012 Recognition, signaling, and repair of DNA double-strand breaks produced by ionizing radiation in mammalian cells: the molecular choreography. Mutat. Res 751:158–24622743550
112. Prakash R , Zhang Y , Feng W , Jasin M . 2015 Homologous recombination and human health: the roles of BRCA1, BRCA2, and associated proteins. Cold Spring Harb. Perspect. Biol 7:a01660025833843
113. Symington LS . 2016 Mechanism and regulation of DNA end resection in eukaryotes. Crit. Rev. Biochem. Mol. Biol 51:195–21227098756
114. Zhao W , Steinfeld JB , Liang F , Chen X , Maranon DG , et al. 2017 BRCA1–BARD1 promotes RAD51-mediated homologous DNA pairing. Nature 550:360–6528976962
115. Takeda S , Hoa NN , Sasanuma H . 2016 The role of the Mre11–Rad50–Nbs1 complex in double-strand break repair—facts and myths. J. Radiat. Res 57:i25–3227311583
116. Branzei D , Foiani M . 2008 Regulation of DNA repair throughout the cell cycle. Nat. Rev. Mol. Cell Biol 9:297–30818285803

117. Aceytuno RD , Piett CG , Havali-Shahriari Z , Edwards RA , Rey M , et al. 2017 Structural and functional characterization of the PNKP–XRCC4–LigIV DNA repair complex. Nucleic Acids Res 45:6238–5128453785
118. Aparicio T , Baer R , Gottesman M , Gautier J . 2016 MRN, CtIP, and BRCA1 mediate repair of topo-isomerase II–DNA adducts. J. Cell Biol 212:399–40826880199
119. Lee KC , Padget K , Curtis H , Cowell IG , Moiani D , et al. 2012 MRE11 facilitates the removal of human topoisomerase II complexes from genomic DNA. Biol. Open 1:863–7323213480
120. Wang H , Li Y , Truong LN , Shi LZ , Hwang PY-H , et al. 2014 CtIP maintains stability at common fragile sites and inverted repeats by end resection-independent endonuclease activity. Mol. Cell 54:1012–2124837675
121. Zeman MK , Cimprich KA . 2014 Causes and consequences of replication stress. Nat. Cell Biol 16:2–924366029
122. Neelsen KJ , Lopes M . 2015 Replication fork reversal in eukaryotes: from dead end to dynamic response. Nat. Rev. Mol. Cell Biol 16:207–2025714681
123. Aze A , Zhou JC , Costa A , Costanzo V . 2013 DNA replication and homologous recombination factors: acting together to maintain genome stability. Chromosoma 122:401–1323584157
124. Carr AM , Lambert S . 2013 Replication stress-induced genome instability: the dark side of replication maintenance by homologous recombination. J. Mol. Biol 425:4733–4423643490
125. Kawabata T , Luebben SW , Yamaguchi S , Ilves I , Matise I , et al. 2011 Stalled fork rescue via dormant replication origins in unchallenged S phase promotes proper chromosome segregation and tumor suppression. Mol. Cell 41:543–5321362550
126. Woodward AM , Göhler T , Luciani MG , Oehlmann M , Ge X , et al. 2006 Excess Mcm2–7 license dormant origins of replication that can be used under conditions of replicative stress. J. Cell Biol 173:673–8316754955
127. Ge XQ , Jackson DA , Blow JJ . 2007 Dormant origins licensed by excess Mcm2–7 are required for human cells to survive replicative stress. Genes Dev 21:3331–4118079179
128. Blow JJ , Ge XQ , Jackson DA . 2011 How dormant origins promote complete genome replication. Trends Biochem. Sci 36:405–1421641805
129. Xu X , Vaithiyalingam S , Glick GG , Mordes DA , Chazin WJ , Cortez D . 2008 The basic cleft of RPA70N binds multiple checkpoint proteins, including RAD9, to regulate ATR signaling. Mol. Cell. Biol 28:7345–5318936170
130. Fanning E 2006 A dynamic model for replication protein A (RPA) function in DNA processing pathways. Nucleic Acids Res 34:4126–3716935876
131. Bolderson E , Petermann E , Croft L , Suraweera A , Pandita RK , et al. 2014 Human single-stranded DNA binding protein 1 (hSSB1/NABP2) is required for the stability and repair of stalled replication forks. Nucleic Acids Res 42:6326–3624753408
132. Deng SK , Chen H , Symington LS . 2015 Replication protein A prevents promiscuous annealing between short sequence homologies: implications for genome integrity. BioEssays 37:305–1325400143
133. Oakley GG , Tillison K , Opiyo SA , Glanzer JG , Horn JM , Patrick SM . 2009 Physical interaction between replication protein A (RPA) and MRN: involvement of RPA2 phosphorylation and the N-terminus of RPA1. Biochemistry 48:7473–8119586055
134. Cimprich KA , Cortez D . 2008 ATR: an essential regulator of genome integrity. Nat. Rev. Mol. Cell Biol 9:616–2718594563
135. Duursma AM , Driscoll R , Elias JE , Cimprich KA . 2013 A role for the MRN complex in ATR activation via TOPBP1 recruitment. Mol. Cell 50:116–2223582259
136. Lee J , Dunphy WG . 2013 The Mre11-Rad50-Nbs1 (MRN) complex has a specific role in the activation of Chk1 in response to stalled replication forks. Mol. Biol. Cell 24:1343–5323468519
137. Myers JS , Cortez D . 2006 Rapid activation of ATR by ionizing radiation requires ATM and Mre11. J. Biol. Chem 281:9346–5016431910
138. Jazayeri A , Falck J , Lukas C , Bartek J , Smith GCM , et al. 2005 ATM-and cell cycle-dependent regulation of ATR in response to DNA double-strand breaks. Nat. Cell Biol 8:37–4516327781

139. Kobayashi M , Hayashi N , Takata M , Yamamoto K-i. 2013 NBS1 directly activates ATR independently of MRE11 and TOPBP1. Genes Cells 18:238–4623368512
140. Shiotani B , Nguyen Hai D , Håkansson P , Marechal A , Tse A , et al. 2013 Two distinct modes of ATR activation orchestrated by Rad17 and Nbs1. Cell Rep. 3:1651–62
141. Seeber A , Hegnauer AM , Hustedt N , Deshpande I , Poli J , et al. 2016 RPA mediates recruitment of MRX to forks and double-strand breaks to hold sister chromatids together. Mol. Cell 64:951–6627889450
142. Gatei M , Kijas AW , Biard D , Dörk T , Lavin MF . 2014 RAD50 phosphorylation promotes ATR down-stream signaling and DNA restart following replication stress. Hum. Mol. Genet 23:4232–4824694934
143. Tittel-Elmer M , Lengronne A , Davidson MB , Bacal J , Francois P , et al. 2012 Cohesin association to replication sites depends on Rad50 and promotes fork restart. Mol. Cell 48:98–10822885006
144. Palermo V , Rinalducci S , Sanchez M , Grillini F , Sommers JA , et al. 2016 CDK1 phosphorylates WRN at collapsed replication forks. Nat. Commun 7:1288027634057
145. Hashimoto Y , Chaudhuri AR , Lopes M , Costanzo V . 2010 Rad51 protects nascent DNA from Mre11-dependent degradation and promotes continuous DNA synthesis. Nat. Struct. Mol. Biol 17:1305–1120935632
146. Vallerga MB , Mansilla SF , Federico MB , Bertolin AP , Gottifredi V . 2015 Rad51 recombinase prevents Mre11 nuclease-dependent degradation and excessive PrimPol-mediated elongation of nascent DNA after UV irradiation. PNAS 112:E6624–3326627254
147. Schlacher K , Christ N , Siaud N , Egashira A , Wu H , Jasin M . 2011 Double-strand break repair-independent role for BRCA2 in blocking stalled replication fork degradation by MRE11. Cell 145:529–4221565612
148. Schlacher K , Wu H , Jasin M . 2012 A distinct replication fork protection pathway connects Fanconi anemia tumor suppressors to RAD51-BRCA1/2. Cancer Cell 22:106–1622789542
149. Kim TM , Son MY , Dodds S , Hu L , Luo G , Hasty P . 2014 RECQL5 and BLM exhibit divergent functions in cells defective for the Fanconi anemia pathway. Nucleic Acids Res 43:893–90325520194
150. Huh MS , Ivanochko D , Hashem LE , Curtin M , Delorme M , et al. 2016 Stalled replication forks within heterochromatin require ATRX for protection. Cell Death Dis 7:e222027171262
151. Kolinjivadi AM , Sannino V , De Antoni A , Zadorozhny K , Kilkenny M , et al. 2017 Smarcal1-mediated fork reversal triggers Mre11-dependent degradation of nascent DNA in the absence of Brca2 and stable Rad51 nucleofilaments. Mol. Cell 67:867–8128757209
152. Pefani DE , O'Neill E . 2015 Safeguarding genome stability: RASSF1A tumor suppressor regulates BRCA2 at stalled forks. Cell Cycle 14:1624–3025927241
153. Leuzzi G , Marabitti V , Pichierri P , Franchitto A . 2016 WRNIP1 protects stalled forks from degradation and promotes fork restart after replication stress. EMBO J 35:1437–5127242363
154. Trego KS , Chernikova SB , Davalos AR , Perry JJP , Finger LD , et al. 2014 The DNA repair endonuclease XPG interacts directly and functionally with the WRN helicase defective in Werner syndrome. Cell Cycle 10:1998–2007
155. Perry JJP , Asaithamby A , Barnebey A , Kiamanesch F , Chen DJ , et al. 2010 Identification of a coiled coil in Werner syndrome protein that facilitates multimerization and promotes exonuclease processivity. J. Biol. Chem 285:25699–70720516064
156. Perry JJP , Yannone SM , Holden LG , Hitomi C , Asaithamby A , et al. 2006 WRN exonuclease structure and molecular mechanism imply an editing role in DNA end processing. Nat. Struct. Mol. Biol 13:414–2216622405
157. Iannascoli C , Palermo V , Murfuni I , Franchitto A , Pichierri P . 2015 The WRN exonuclease domain protects nascent strands from pathological MRE11/EXO1-dependent degradation. Nucleic Acids Res 43:9788–80326275776
158. Chaudhuri AR , Callen E , Ding X , Gogola E , Duarte AA , et al. 2016 Replication fork stability confers chemoresistance in BRCA-deficient cells. Nature 535:382–8727443740

159. Roy S , Tomaszowski K-H , Luzwick JW , Park S , Li J , et al. 2018 p53 orchestrates DNA replication restart homeostasis by suppressing mutagenic RAD52 and POLθ pathways. eLife 7:e3172329334356
160. Zhu X-D , Küster B , Mann M , Petrini JHJ , de Lange T 2000 Cell-cycle-regulated association of RAD50/MRE11/NBS1 with TRF2 and human telomeres. Nat. Genet 25:347–5210888888
161. O'Sullivan RJ , Karlseder J . 2010 Telomeres: protecting chromosomes against genome instability. Nat. Rev. Mol. Cell Biol 11:171–8120125188
162. Levy MZ , Allsopp RC , Futcher AB , Greider CW , Harley CB . 1992 Telomere end-replication problem and cell aging. J. Mol. Biol 225:951–601613801
163. Wu P , Takai H , de Lange T . 2012 Telomeric 3′ overhangs derive from resection by Exo1 and Apollo and fill-in by POT1b-associated CST. Cell 150:39–5222748632
164. Shippen-Lentz D , Blackburn EH . 1990 Functional evidence for an RNA template in telomerase. Science 247:546–521689074
165. Hastie ND , Dempster M , Dunlop MG , Thompson AM , Green DK , Allshire RC . 1990 Telomere reduction in human colorectal carcinoma and with ageing. Nature 346:866–682392154
166. Harley CB , Futcher AB , Greider CW . 1990 Telomeres shorten during ageing of human fibroblasts. Nature 345:458–602342578
167. Allsopp RC , Vaziri H , Patterson C , Goldstein S , Younglai EV , et al. 1992 Telomere length predicts replicative capacity of human fibroblasts. PNAS 89:10114–181438199
168. Takata H , Tanaka Y , Matsuura A . 2005 Late S phase-specific recruitment of Mre11 complex triggers hierarchical assembly of telomere replication proteins in *Saccharomyces cerevisiae.* Mol. Cell 17:573–8315721260
169. Rivera T , Haggblom C , Cosconati S , Karlseder J . 2016 A balance between elongation and trimming regulates telomere stability in stem cells. Nat. Struct. Mol. Biol 24:30–3927918544
170. Wu Z , Liu J , Zhang Q-D , Lv D-K , Wu N-F , Zhou J-Q . 2017 Rad6–Bre1-mediated H2B ubiquitination regulates telomere replication by promoting telomere-end resection. Nucleic Acids Res 45:3308–2228180293
171. Marcand S 2014 How do telomeres and NHEJ coexist? Mol. Cell. Oncol 1:e96343827308342
172. Marcomini I , Gasser SM . 2015 Nuclear organization in DNA end processing: telomeres versus double-strand breaks. DNA Repair 32:134–4026004856
173. Williamson JR , Raghuraman MK , Cech TR . 1989 Monovalent cation-induced structure of telomeric DNA: the G-quartet model. Cell 59:871–802590943
174. Higa M , Fujita M , Yoshida K . 2017 DNA replication origins and fork progression at mammalian telomeres. Genes 8:112
175. Denchi EL . 2009 Give me a break: How telomeres suppress the DNA damage response. DNA Repair 8:1118–2619482563
176. Palm W , de Lange T . 2008 How shelterin protects mammalian telomeres. Annu. Rev. Genet 42:301–3418680434
177. Denchi EL , de Lange T . 2007 Protection of telomeres through independent control of ATM and ATR by TRF2 and POT1. Nature 448:1068–7117687332
178. Déjardin J , Kingston RE 2009 Purification of proteins associated with specific genomic loci. Cell 136:175–8619135898
179. Rai R , Hu C , Broton C , Chen Y , Lei M , Chang S . 2017 NBS1 phosphorylation status dictates repair choice of dysfunctional telomeres. Mol. Cell 65:801–17.e428216226
180. Cesare AJ , Reddel RR . 2010 Alternative lengthening of telomeres: models, mechanisms and implications. Nat. Rev. Genet 11:319–3020351727
181. Shay JW , Reddel RR , Wright WE . 2012 Cancer and telomeres–an ALTernative to telomerase. Science 336:1388–9022700908
182. Xue Y , Marvin ME , Ivanova IG , Lydall D , Louis EJ , Maringele L . 2016 Rif1 and Exo1 regulate the genomic instability following telomere losses. Aging Cell 15:553–6227004475
183. Ballew BJ , Lundblad V . 2013 Multiple genetic pathways regulate replicative senescence in telomerase-deficient yeast. Aging Cell 12:719–2723672410

184. Durocher D , Cassani C , Gobbini E , Wang W , Niu H , et al. 2016 Tel1 and Rif2 regulate MRX functions in end-tethering and repair of DNA double-strand breaks. PLOS Biol 14:e100238726901759

185. Saretzki G , Panero J , Stella F , Schutz N , Fantl DB , Slavutsky I . 2015 Differential expression of non-shelterin genes associated with high telomerase levels and telomere shortening in plasma cell disorders. PLOS ONE 10:e013797226366868

186. Hoxha M , Fabris S , Agnelli L , Bollati V , Cutrona G , et al. 2014 Relevance of telomere/telomerase system impairment in early stage chronic lymphocytic leukemia. Genes Chromosomes Cancer 53:612–2124706380

187. Heng J , Zhang F , Guo X , Tang L , Peng L , et al. 2017 Integrated analysis of promoter methylation and expression of telomere related genes in breast cancer. Oncotarget 8:25442–5428424414

188. Attwooll CL , Akpinar M , Petrini JH . 2009 The Mre11 complex and the response to dysfunctional telomeres. Mol. Cell. Biol 29:5540–5119667076

189. Li Y , Shen Y , Hohensinner P , Ju J , Wen Z , et al. 2016 Deficient activity of the nuclease MRE11A induces T cell aging and promotes arthritogenic effector functions in patients with rheumatoid arthritis. Immunity 45:903–1627742546

190. Stracker TH , Carson CT , Weitzman MD . 2002 Adenovirus oncoproteins inactivate the Mre11–Rad50– NBS1 DNA repair complex. Nature 418:348–5212124628

191. Lilley CE , Schwartz RA , Weitzman MD . 2007 Using or abusing: viruses and the cellular DNA damage response. Trends Microbiol 15:119–2617275307

192. Polo SE , Jackson SP . 2011 Dynamics of DNA damage response proteins at DNA breaks: a focus on protein modifications. Genes Dev 25:409–3321363960

193. Shah GA , O'Shea CC . 2015 Viral and cellular genomes activate distinct DNA damage responses. Cell 162:987–100226317467

194. Weiden MD , Ginsberg HS . 1994 Deletion of the E4 region of the genome produces adenovirus DNA concatemers. PNAS 91:153–578278357

195. Boyer J , Rohleder K , Ketner G . 1999 Adenovirus E4 34k and E4 11k inhibit double strand break repair and are physically associated with the cellular DNA-dependent protein kinase. Virology 263:307–1210544104

196. Rawlins DR , Rosenfeld PJ , Wides RJ , Challberg MD , Kelly TJ . 1984 Structure and function of the adenovirus origin of replication. Cell 37:309–196722875

197. Bernstein JA , Porter JM , Challberg MD . 1986 Template requirements for in vivo replication of adenovirus DNA. Mol. Cell. Biol 6:2115–243785188

198. Kondo T , Kobayashi J , Saitoh T , Maruyama K , Ishii KJ , et al. 2013 DNA damage sensor MRE11 rec-ognizes cytosolic double-stranded DNA and induces type I interferon by regulating STING trafficking. PNAS 110:2969–7423388631

199. Bhattacharya S , Srinivasan K , Abdisalaam S , Su F , Raj P , et al. 2017 RAD51 interconnects between DNA replication, DNA repair and immunity. Nucleic Acids Res 45:4590–60528334891

200. Liu X , Hammel M , He Y , Tainer JA , Jeng US , et al. 2013 Structural insights into the interaction of IL-33 with its receptors. PNAS 110:14918–2323980170

201. Stav-Noraas TE , Edelmann RJ , Poulsen LLC , Sundnes O , Phung D , et al. 2017 Endothelial IL-33 expression is augmented by adenoviral activation of the DNA damage machinery. J. Immunol 198:3318–2528258201

202. Roth S , Rottach A , Lotz-Havla AS , Laux V , Muschaweckh A , et al. 2014 Rad50-CARD9 interactions link cytosolic DNA sensing to IL-1β production. Nat. Immunol 15:538–4524777530

203. Sohn S-Y , Hearing P . 2012 Adenovirus regulates Sumoylation of Mre11-Rad50-Nbs1 components through a paralog-specific mechanism. J. Virol 86:9656–6522740413

204. Bridge E , Ketner G . 1989 Redundant control of adenovirus late gene expression by early region 4. J. Virol 63:631–382911117

205. Halbert DN , Cutt JR , Shenk T . 1985 Adenovirus early region 4 encodes functions required for efficient DNA replication, late gene expression, and host cell shutoff. J. Virol 56:250–574032537

206. Huang MM , Hearing P . 1989 Adenovirus early region 4 encodes two gene products with redundant effects in lytic infection. J. Virol 63:2605–152724411

207. Weinberg DH , Ketner G . 1986 Adenoviral early region 4 is required for efficient viral DNA replication and for late gene expression. J. Virol 57:833–383485200

208. Evans JD , Hearing P . 2005 Relocalization of the Mre11-Rad50-Nbs1 complex by the adenovirus E4 ORF3 protein is required for viral replication. J. Virol 79:6207–1515858005

209. Ou HD , Kwiatkowski W , Deerinck TJ , Noske A , Blain KY , et al. 2012 A structural basis for the assembly and functions of a viral polymer that inactivates multiple tumor suppressors. Cell 151:304–1923063122

210. Sohn SY , Hearing P . 2012 Adenovirus regulates sumoylation of Mre11-Rad50-Nbs1 components through a paralog-specific mechanism. J. Virol 86:9656–6522740413

211. Bridges RG , Sohn SY , Wright J , Leppard KN , Hearing P . 2016 The adenovirus E4-ORF3 protein stimulates SUMOylation of general transcription factor TFII-I to direct proteasomal degradation. mBio 7:e02184–1526814176

212. Harada JN , Shevchenko A , Shevchenko A , Pallas DC , Berk AJ . 2002 Analysis of the adenovirus E1B-55K-anchored proteome reveals its link to ubiquitination machinery. J. Virol 76:9194–20612186903

213. Querido E 2001 Degradation of p53 by adenovirus E4orf6 and E1B55K proteins occurs via a novel mechanism involving a Cullin-containing complex. Genes Dev 15:3104–1711731475

214. zur Hausen H 2009 Papillomaviruses in the causation of human cancers—a brief historical account. Virology 384:260–6519135222

215. Johnson BA , Aloor HL , Moody CA . 2017 The Rb binding domain of HPV31 E7 is required to maintain high levels of DNA repair factors in infected cells. Virology 500:22–3427770701

216. Campos-León K , Wijendra K , Siddiqa A , Pentland I , Feeney KM , et al. 2017 Association of human papil-lomavirus 16 E2 with Rad50-interacting protein 1 enhances viral DNA replication. J. Virol 91:e02305–1628031358

217. Robertson ES , Mariggio` G , Koch S , Zhang G , Weidner-Glunde M , et al. 2017 Kaposi Sarcoma Her-pesvirus (KSHV) latency-associated nuclear antigen (LANA) recruits components of the MRN (Mre11-Rad50-NBS1) repair complex to modulate an innate immune signaling pathway and viral latency. PLOS Pathog 13:e100633528430817

218. Chang Y , Cesarman E , Pessin MS , Lee F , Culpepper J , et al. 1994 Identification of herpesvirus-like DNA sequences in AIDS-associated Kaposi's sarcoma. Science 266:1865–697997879

219. Bouvard V , Baan R , Straif K , Grosse Y , Secretan B , et al. 2009 A review of human carcinogens–Part B: biological agents. Lancet Oncol 10:321–2219350698

220. Pantelidou C , Cherubini G , Lemoine NR , Halldén G 2016 The E1B19K-deleted oncolytic adenovirus mutant AdΔ19K sensitizes pancreatic cancer cells to drug-induced DNA-damage by down-regulating Claspin and Mre11. Oncotarget 7:15703–2426872382

221. Tookman LA , Browne AK , Connell CM , Bridge G , Ingemarsdotter CK , et al. 2015 RAD51 and BRCA2 enhance oncolytic adenovirus type 5 activity in ovarian cancer. Mol. Cancer Res 14:44–5526452665

222. Crick F 1974 The double helix: a personal view. Nature 248:766–694599081

223. Harrison SC . 2007 Comments on the NIGMS PSI. Structure 15:1344–4617997957

224. Hura GL , Menon AL , Hammel M , Rambo RP , Poole FL , et al. 2009 Robust, high-throughput solution structural analyses by small angle X-ray scattering (SAXS). Nat. Methods 6:606–1219620974

225. Rambo RP , Tainer JA . 2011 Characterizing flexible and intrinsically unstructured biological macro-molecules by SAS using the Porod-Debye law. Biopolymers 95:559–7121509745

226. Hura GL , Budworth H , Dyer KN , Rambo RP , Hammel M , et al. 2013 Comprehensive macromolecular conformations mapped by quantitative SAXS analyses. Nat. Methods 10:453–5423624664

227. Rambo RP , Tainer JA . 2013 Accurate assessment of mass, models and resolution by small-angle scattering. Nature 496:477–8123619693

228. Rambo RP , Tainer JA . 2013 Super-resolution in solution X-ray scattering and its applications to structural systems biology. Annu. Rev. Biophys 42:415–4123495971

229. Brosey CA , Ho C , Long WZ , Singh S , Burnett K , et al. 2016 Defining NADH-driven allostery regulating apoptosis-inducing factor. Structure 24:2067–7927818101

230. Bacolla A , Tainer JA , Vasquez KM , Cooper DN . 2016 Translocation and deletion breakpoints in cancer genomes are associated with potential non-B DNA-forming sequences. Nucleic Acids Res 44:5673–8827084947

231. Tsutakawa SE , Thompson MJ , Arvai AS , Neil AJ , Shaw SJ , et al. 2017 Phosphate steering by Flap Endonuclease 1 promotes 5′-flap specificity and incision to prevent genome instability. Nat. Commun 8:1585528653660

232. Rashid F , Harris PD , Zaher MS , Sobhy MA , Joudeh LI , et al. 2017 Single-molecule FRET unveils induced-fit mechanism for substrate selectivity in flap endonuclease 1. eLife 6:e2188428230529

233. Sundheim O , Vågbø CB , Bjørås M , Sousa MML , Talstad V , et al. 2006 Human ABH3 structure and key residues for oxidative demethylation to reverse DNA/RNA damage. EMBO J 25:3389–9716858410

234. Dango S , Mosammaparast N , Sowa ME , Xiong L-J , Wu F , et al. 2011 DNA unwinding by ASCC3 helicase is coupled to ALKBH3-dependent DNA alkylation repair and cancer cell proliferation. Mol. Cell 44:373–8422055184

235. Brosey CA , Ahmed Z , Lees-Miller SP , Tainer JA . 2017 What combined measurements from structures and imaging tell us about DNA damage responses. Methods Enzymol 592:417–5528668129

236. Loeb LA , Loeb KR , Anderson JP . 2003 Multiple mutations and cancer. PNAS 100:776–8112552134

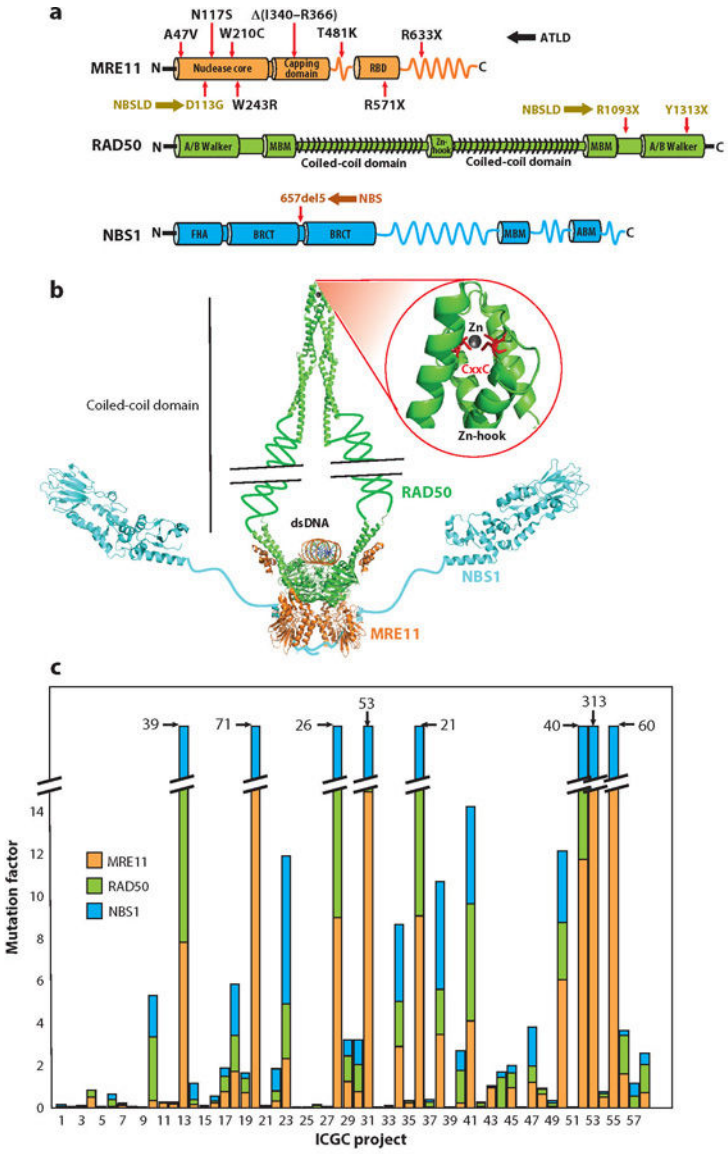

Figure 1.

MRN domains, complex assembly, and mutations. (*a*) Key domains of MRN subunits MRE11 (*orange*), RAD50 (*green*), and NBS1 (*cyan*). Mutated sites (*red arrows*) for human inherited diseases (color coded for ATLD, NBS, and NBSLD; mutations corresponding to each disease are color coded with respective abbreviations) are shown on each subunit with respective substitutions, where X represents the stop codon. (*b*) Modeled assembly of MRN complex and dsDNA based on experimentally derived partial crystal structures (PDB IDs: 5F3W, 5GOX, and 3HUE; color coding as in panel *a*) showing a dimer of a heterotrimer. The allosterically linked Zn-hook is highlighted in the inset along with CxxC motifs (*red*) on two RAD50 monomers. (*c*) The extent of mutations (reported as mutation factor) in subunits of the MRN complex in cancers documented in the International Cancer Genome Consortium (ICGC) projects. A mutation factor is defined as a number of observed mutations multiplied by the fraction of mutation-positive cases. ICGC projects with high

mutation factors (>15 in total) include the following: #13, Breast Cancer, ER+ and HER2−, European Union/United Kingdom; #20, Esophageal Adenocarcinoma, United Kingdom; #28 Liver Cancer, China; #31, Liver Cancer, RIKEN, Japan; #36, Malignant Lymphoma, Germany; #52, Skin Adenocarcinoma, Brazil; #53, Skin Cancer, Australia; and #55, Soft Tissue Cancer, Leiomyosarcoma, France. A complete list of the 58 projects is provided in **Supplemental Table 1**. Abbreviations: ABM, ATM binding motif; ATLD, ataxia-telangiectasia-like disorder; BRCT, BRCA1 C-terminal domain; dsDNA, double-stranded DNA; FHA, forkhead-associated domain; MBM, MRE11 binding motif; MRE11, meiotic recombination 11 homolog 1; NBS, Nijmegen breakage syndrome; NBSLD, NBS-like disorder; PDB, Protein Data Bank; RAD50, DNA repair protein RAD50; RBD, RAD50 binding domain.

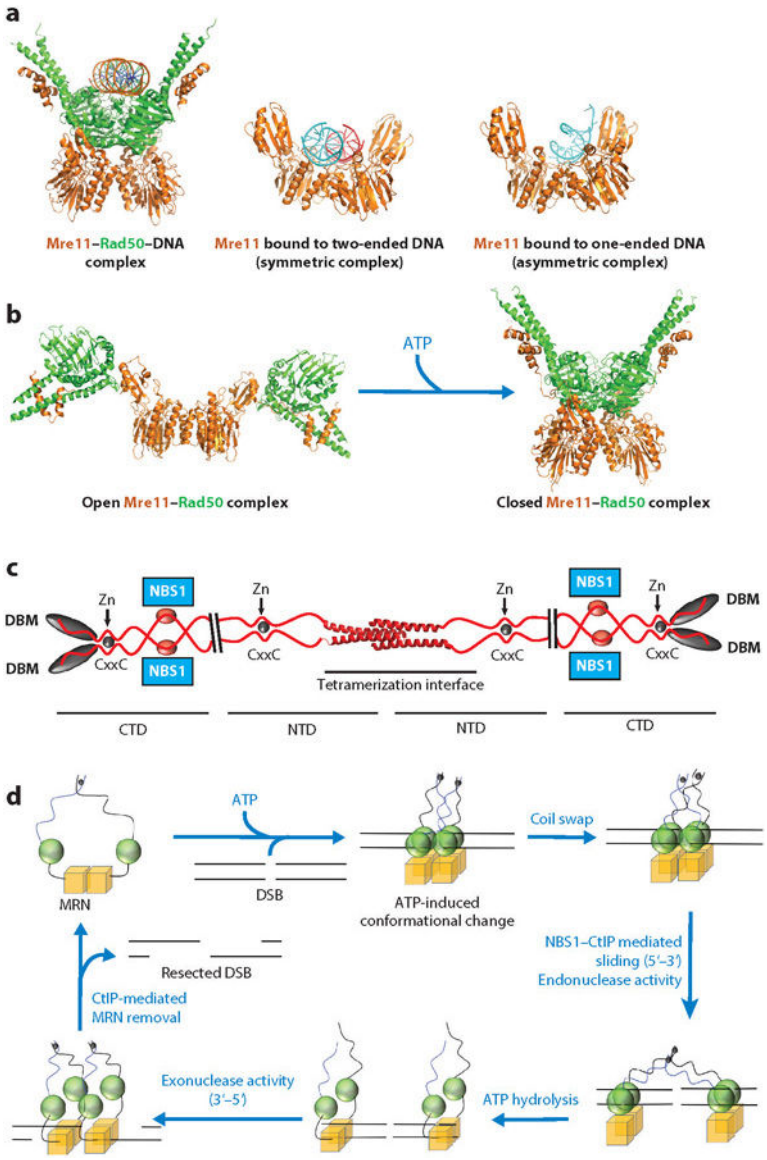

Figure 2.

The Mre11–Rad50 (MR) heterotetramer: DNA-binding interface, ATP-mediated conformational changes, and a unified model for MRN's functions in DSB resection (color coding as in Figure 1) are shown. (*a*) Crystal structures of ATPγS-bound *Methanococcus jannaschii* (*Mj*) Mre11—Rad50–DNA complex (PDB ID: 5F3W) and the symmetric (PDB ID: 3DSC) and asymmetric (PDB ID: 3DSD) *Pyrococcus furiosus* Mre11 DNA-bound states, highlighting the DNA-binding interface at the Rad50 and Mre11 dimerization interface. (*b*) Crystal structures of the MR complex in the nucleotide-free state (PDB ID: 3QG5, *Thermotoga maritima* MR) and in the ATPγS-bound state (PDB ID: 3AV0, *Mj*MR), highlighting the conformational switch in the MRN core complex. (*c*) Human CtIP model from the recent crystal structure of CtIP N-terminal tetramerization domain (PDB ID: 4D2H). Plausible NBS1 binding sites on four CtIP and C-terminal DBMs, along with CxxC motifs and Zn coordination, are shown. (*d*) A testable pathway for DSB resection by the

MRN complex. For clarity, only MRE11 (M) and RAD50 (R) subunits are shown. First, two molecules of MRN bind to DSB ends in an ATP-bound state, with MRE11 nuclease activity inhibited by the closed RAD50 conformation. Then, MRN forms an interlinked complex through a RAD50 coil swap. At this point, CtIP–NBS1 interaction allows MRN dimers to slide away from the break, and MRE11 makes the initial licensing cut through its endonuclease activity. Upon complete ATP hydrolysis, the RAD50 dimerization interface opens and dsDNA becomes accessible to the MRE11 exonuclease. DNA resection begins in the 3′–5′ direction and proceeds toward the break. Upon reaching the break, the MRN complex may be dislodged from DSB with the help of CtIP and NBS1 (NBS1 not shown for clarity). Because only M and R are shown in the model, NBS1 (N) is colored gray in MRN notation. Abbreviations: CTD, C-terminal domain; CtIP, C-terminal binding protein–interacting protein; DBMs, DNA binding motifs; DSBs, double-strand breaks; dsDNA, double-stranded DNA; MRE11, meiotic recombination 11 homolog 1; MRN, MRE11–RAD50–NBS1 complex; NBS1, Nijmegen breakage syndrome protein 1; NTD, N-terminal domain; PDB, Protein Data Bank; RAD50, DNA repair protein RAD50.

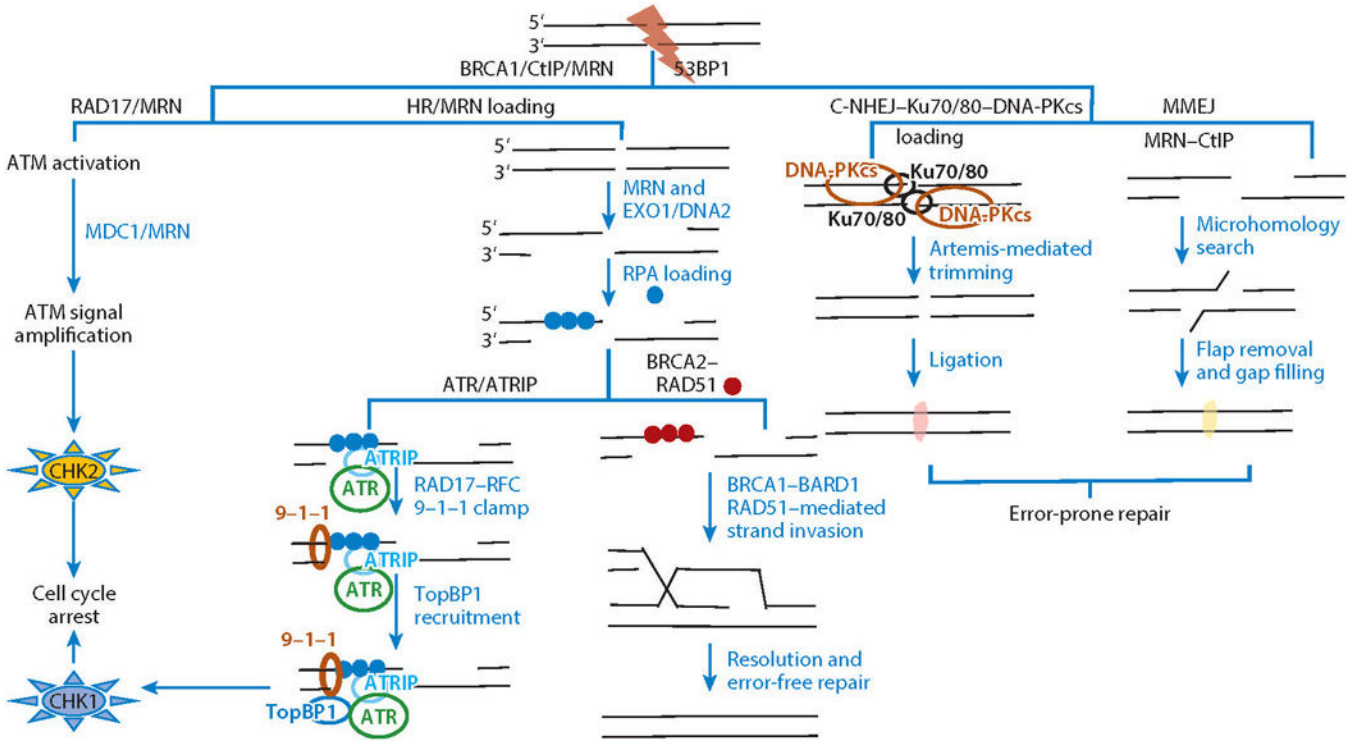

Figure 3.

The MRN complex activates possible (alternative) pathways at DSBs. The MRN complex can initially be recruited to a DSB by RAD17 and activates the ATM kinase. Once activated, ATM phosphorylates downstream substrates including MDC1, which in turn recruits MRN, and the ATM signal is further amplified at the DSB. ATM checkpoint signaling leads to cell cycle regulation through CHK2 activation (*far left*). On the basis of the cell cycle phase and nature of the DSB ends, DSBs are channeled through either HR or NHEJ for repair (*central paths*). In HR, the MRN complex initiates the 5′ resection followed by long-range resection by EXO1 or DNA2. The resulting 3′ ssDNA overhangs are loaded with ssDNA-binding protein RPA. The RPA-coated ssDNA can activate a second checkpoint pathway: ATR signaling. ATR is recruited to the RPA-coated ssDNA through ATRIP and RPA interaction. Then the RAD17–RFC clamp loading complex loads the 9–1–1 (RAD9–HUS1–RAD1) checkpoint clamp at the ssDNA and dsDNA junction on the RPA-coated ssDNA arms. The ATR activator TopBP1 is loaded by RAD9, leading to activation of CHK1 kinase and cell cycle regulation. For high-fidelity DSB repair, the BRCA2–RAD51 complex nucleates the formation of RAD51 nucleofilaments and displaces RPA from ssDNA. RAD51 nucleofilaments promote homology search and strand invasion in association with BRCA1–BARD1 for error-free HR repair through the resolution of repair intermediates. The two NHEJ pathways (*right*) depend upon DNA-PKcs and MRN–CtIP. In C-NHEJ, DSB ends are bluntly fused through Ku70/80–DNA-PKcs complex tethering, Artemis-mediated end processing, ligase IV, and XLF-XRCC4 scaffolding factor–mediated ligation. The C-NHEJ is independent of the MRN complex; however, MMEJ operates through MRN–CtIP-mediated resection followed by priming based on microhomology, flap removal, and gap filling. Abbreviations: ATM, ataxia-telangiectasia mutated; ATR, ataxia-telangiectasia and

RAD3-related; ATRIP, ATR-interacting protein; C-NHEJ, canonical nonhomologous end joining; DNA-PKcs, DNA-dependent protein kinase catalytic subunit; DSB, double-strand break; HR, homologous recombination; MMEJ, microhomology-mediated end joining; MRE11, meiotic recombination 11 homolog 1; MRN, MRE11–RAD50–NBS1 complex; NBS1, Nijmegen breakage syndrome protein 1; NHEJ, nonhomologous end joining; RAD50, DNA repair protein RAD50; RFC, replication factor C; RPA, replication protein A; ssDNA, single-stranded DNA.

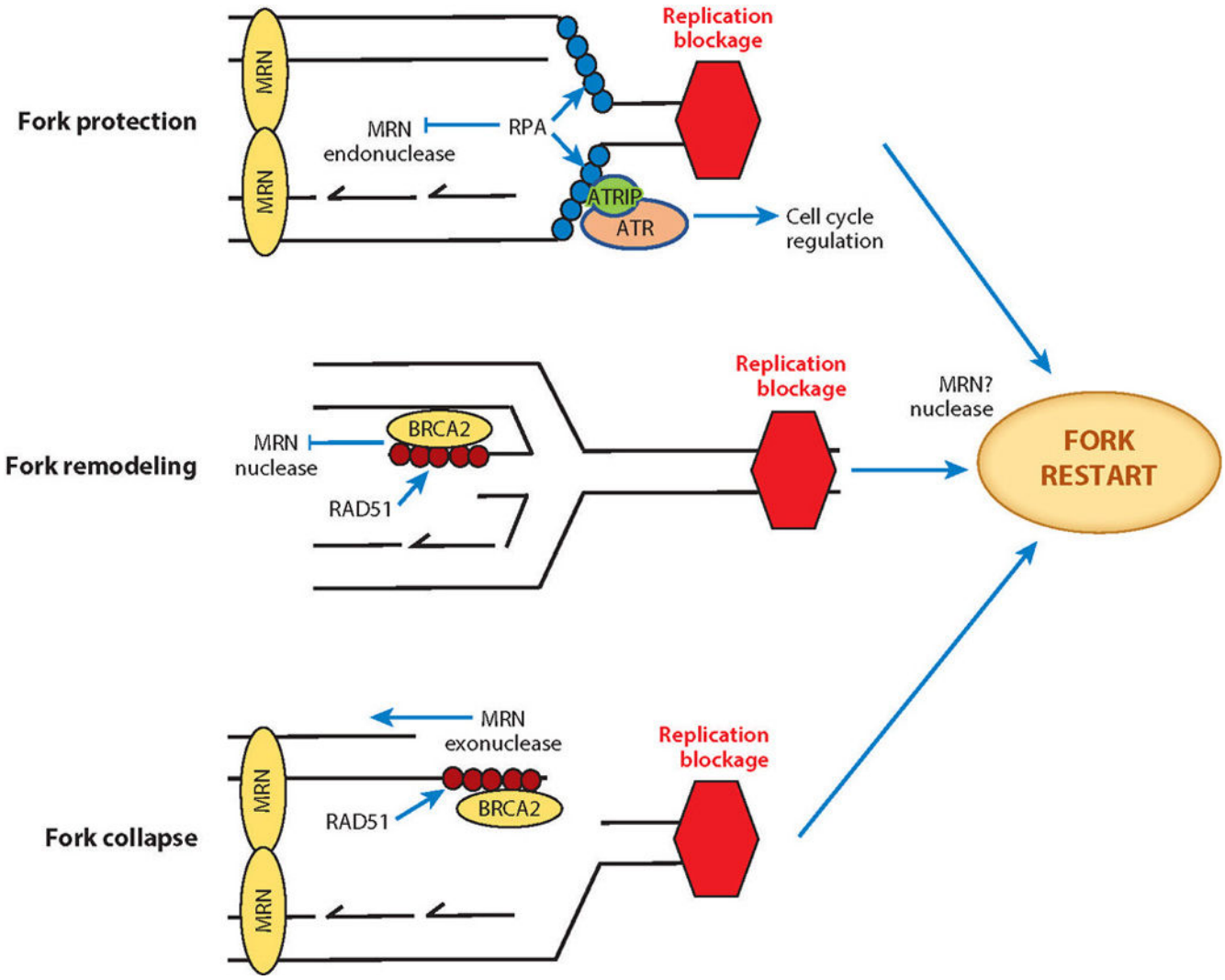

Figure 4.

The MRN complex with RPA and ATR orchestrates replication fork protection, remodeling, and restart as well as HR rescue of collapsed forks. At stalled forks, the MRN complex participates in fork protection through non-nuclease functions (e.g., DNA tethering), and its ssDNA endonuclease activity is inhibited by RPA. RPA-coated ssDNA initiates ATR checkpoint signaling that provides cells time for repair and restart. Replication barriers are removed by remodeling through fork reversal that must then be processed for fork restart. The nascent ssDNA at reversed forks is protected against MRN exonuclease activity by RAD51 loading in association with BRCA2. For restart, MRE11 excision of one strand may facilitate fork reversal and restart. Unrepaired collapsed forks can become DSBs where the MRN complex initiates homologous recombination by initiating resection. Abbreviations: ATR, ataxia-telangiectasia and RAD3-related; ATRIP, ATR-interacting protein; MRE11, meiotic recombination 11 homolog 1; MRN, MRE11–RAD50–NBS1 complex; NBS1, Nijmegen breakage syndrome protein 1; RAD50, DNA repair protein RAD50; RPA, replication protein A; ssDNA, single-stranded DNA.

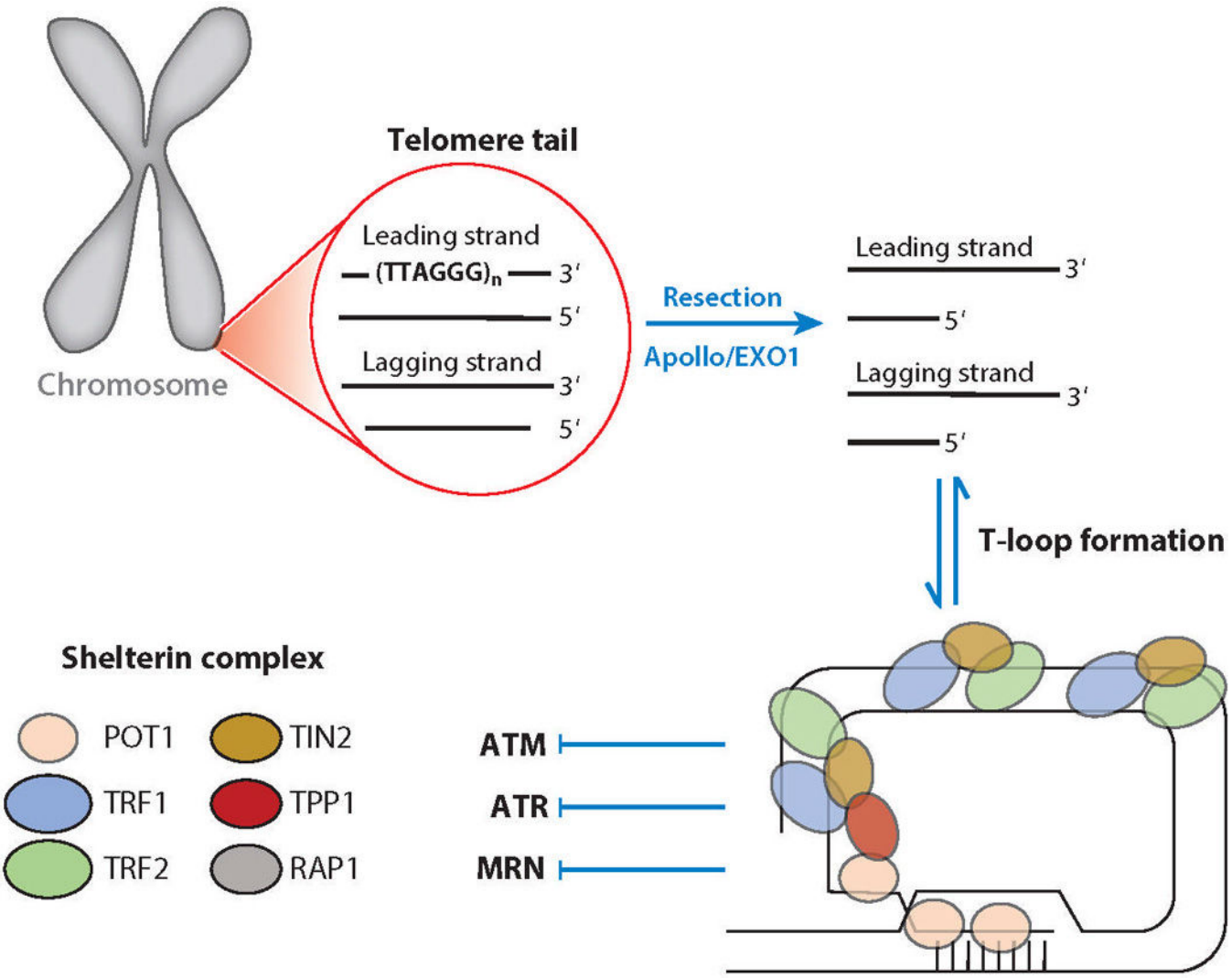

Figure 5.

Telomere structure and dynamics regulate MRN interactions and distinguish chromosome ends from breaks. Human telomeres consist of a long stretch of repetitive TTAGGG sequences and interacting proteins. Telomeres forming from the replication of a leading strand result in tails with blunt ends, whereas the replication of a lagging strand generates telomeres with 3′ overhangs. The 5′ resection of a blunt-ended telomere inhibits telomere fusion through NHEJ. The telomeres with 3′ overhangs form transient T-loop structures. The shelterin complex, which helps protect telomere ends, consists of telomeric repeat-binding factors 1 and 2 (TRF1 and TRF2), repressor and activator protein (RAP1), TRF1-interacting nuclear protein 2 (TIN2), protection of telomeres 1 (POT1), and TIN2- and POT1-interacting protein (TPP1). Abbreviations: ATM, ataxia-telangiectasia mutated; ATR, ataxia-telangiectasia and RAD3-related; CtIP, C-terminal binding protein–interacting protein; MRE11, meiotic recombination 11 homolog 1; MRN, MRE11–RAD50–NBS1 complex; NBS1, Nijmegen breakage syndrome protein 1; NHEJ, nonhomologous end joining; RAD50, DNA repair protein RAD50; T-loop, telomere loop.

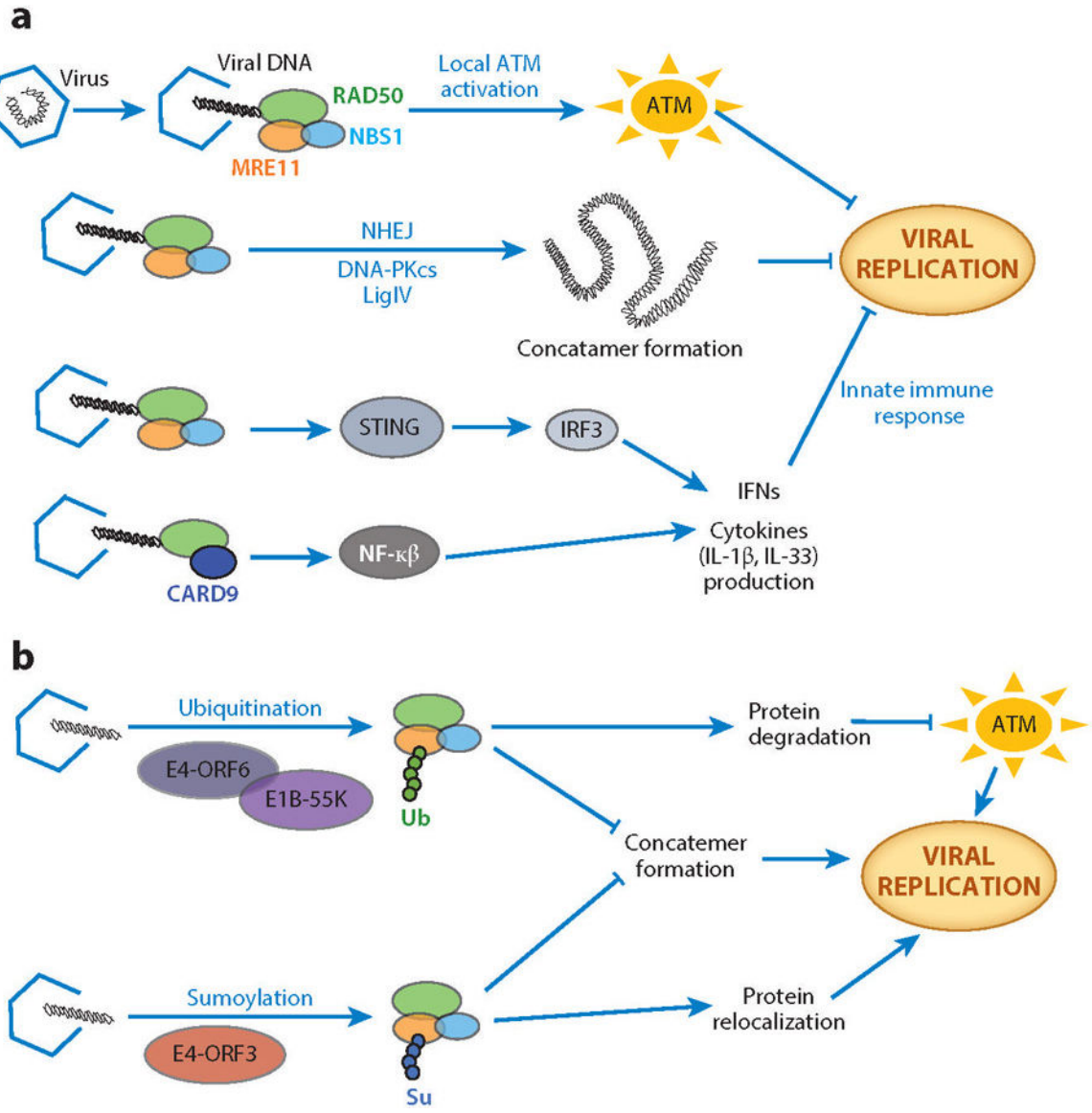

Figure 6.

MRN in host and virus responses. (*a*) The MRN complex inhibits viral replication via pathways linking DNA repair proteins and the innate immune response. The interaction between the MRN complex and viral DNA leads to local ATM activation that avoids cell cycle arrest and apoptosis, which is characteristic of global ATM signaling in DNA damage. The MRN complex promotes the formation of viral DNA concatemers (randomly fused long chains of viral DNA) in association with NHEJ proteins to help inhibit viral replication. MRN components furthermore promote the innate immune response by inducing interferon and cytokine production through pathways involving STING, IRF3, and NF-κβ. (*b*) Viruses neutralize the MRN complex by relocalization and/or degradation. The viral protein complex of E4-ORF6 and E1B-55K directs the MRN complex for degradation through ubiquitination (Ub). The viral protein E4-ORF3 relocalizes the MRN complex to the nucleus through sumoylation (Su) to promote viral replication by limiting MRN-promoted viral

DNA concatemer formation. Abbreviations: ATM, ataxia-telangiectasia mutated; CARD9, caspase recruitment domain-containing protein 9; DNA-PKcs, DNA-dependent protein kinase catalytic subunit; E1B-55K, a 55-kDa protein of adenovirus *E1B* gene; E4-ORF3, early region 4 of adenovirus open reading frame 3 protein; E4-ORF6, early region 4 of adenovirus open reading frame 6 protein; IFNs, type I interferons; IL-1β, interleukin 1 beta; IL-33, interleukin 33; IRF3, interferon regulatory factor 3; MRE11, meiotic recombination 11 homolog 1; MRN, MRE11–RAD50–NBS1 complex; NBS1, Nijmegen breakage syndrome protein 1; NF-κβ, nuclear factor κβ; NHEJ, nonhomologous end joining; RAD50, DNA repair protein RAD50; STING, stimulator of interferon genes.