Biochemical Society Transactions (2020)
https://doi.org/10.1042/BST20170168

PORTLAND PRESS

Review Article

Turning the Mre11/Rad50 DNA repair complex on its head: lessons from SMC protein hinges, dynamic coiled-coil movements and DNA loop-extrusion?

Ekaterina Zabolotnaya[1], Ioanna Mela[1,*], Robert M. Henderson[1] and Nicholas P. Robinson[2]

[1]Department of Pharmacology, University of Cambridge, Cambridge CB2 1PD, U.K.; [2]Division of Biomedical and Life Sciences, Faculty of Health and Medicine, Lancaster University, Lancaster LA1 4YG, U.K.

Correspondence: Nicholas P. Robinson (n.robinson2@lancaster.ac.uk)

The bacterial SbcC/SbcD DNA repair proteins were identified over a quarter of a century ago. Following the subsequent identification of the homologous Mre11/Rad50 complex in the eukaryotes and archaea, it has become clear that this conserved chromosomal processing machinery is central to DNA repair pathways and the maintenance of genomic stability in all forms of life. A number of experimental studies have explored this intriguing genome surveillance machinery, yielding significant insights and providing conceptual advances towards our understanding of how this complex operates to mediate DNA repair. However, the inherent complexity and dynamic nature of this chromosome-manipulating machinery continue to obfuscate experimental interrogations, and details regarding the precise mechanisms that underpin the critical repair events remain unanswered. This review will summarize our current understanding of the dramatic structural changes that occur in Mre11/Rad50 complex to mediate chromosomal tethering and accomplish the associated DNA processing events. In addition, undetermined mechanistic aspects of the DNA enzymatic pathways driven by this vital yet enigmatic chromosomal surveillance and repair apparatus will be discussed. In particular, novel and putative models of DNA damage recognition will be considered and comparisons will be made between the modes of action of the Rad50 protein and other related ATPases of the overarching SMC superfamily.

The Mre11/Rad50 (M/R) DNA repair complex: a machinery conserved throughout evolution for the resolution of DNA double-strand breaks

The maintenance of chromosomal integrity and genomic stability is central to cellular viability in all organisms. DNA double-strand breaks (DSBs), where both strands of the DNA duplex are concurrently broken, represent some of the most cytotoxic forms of genetic damage and require immediate, efficient and accurate repair in order to prevent genomic rearrangements and mutation [1,2]. Indeed, the failure to precisely repair DSBs in higher organisms is frequently associated with chromosomal instability and the development of cancer [3]. Cells can employ two broad DNA repair strategies at DSBs to restore the intact chromosome. The first method, known as non-homologous end-joining (NHEJ), reseals the fractured chromosome directly by blunt-ended ligation with minimal processing at the break [4,5]. Non-canonical 'alternative' NHEJ mechanisms have also been identified during which the ends of the DSB undergo limited processing prior to ligation and frequently the repair is thereby

*Present address: Department of Chemical Engineering and Biotechnology, University of Cambridge, Philippa Fawcett Drive, Cambridge, CB3 0AS, U.K.

Received: 24 March 2018
Revised: 19 October 2020
Accepted: 22 October 2020

Version of Record published: 10 December 2020

Biochemical Society Transactions (2020)
https://doi.org/10.1042/BST20170168

mediated via micro-homologies; such repair modes are therefore referred to as micro-homology mediated end-joining (MMEJ) [6]. In contrast, a second higher fidelity mechanism, homology-directed repair (HDR), requires a homologous chromosome as a template to restore the genetic content of the chromosome [1,7]. During this process, helicase and nuclease components are recruited to DSBs and perform long-range chromosome resection to generate the single-stranded tails required for the stand-invasion steps of homologous recombination (HR) [8–12] (Figure 1). These tails become coated by recombinase proteins of the Rad51/RadA/RecA superfamily, which are conserved across the divisions of life. The resultant proteo-filament is then able to invade the DNA duplex of the homologous chromosome and mediate HR driven DNA repair [13–15] (Figure 1). This HR pathway is, therefore, an ancient, conserved and essential DNA repair mechanism employed by all three divisions of life; the bacteria, the archaea and the eukaryotes [8]. In non-polyploid organisms, this high-fidelity mechanism is restricted to the S/G2 phases of the cell cycle when a copy of the genome is available as a homologous template, while by contrast the NHEJ pathways are possible at all cell-cycle stages [1].

It is well established that the Mre11–Rad50 (M/R) complex operates during the earliest stages of DSB repair, playing critical roles in the recognition and processing of these sites of damage and influencing which of the repair mechanisms is utilized by the cell [16–22]. The complex is also central to the DNA damage signalling pathways in eukaryotic organisms [23]. Interestingly, the M/R complex also plays roles in the MMEJ and NHEJ pathways [24]. The tight association of the enzymatically distinct Mre11 nuclease and the Rad50 ATPase at the core of this critical DNA repair complex is an arrangement conserved in all organisms [11,18], and M/R homologues are even encoded by dsDNA bacteriophages [25]. These two core protein components undergo dramatic ATP hydrolysis-mediated conformational changes that intrinsically link the nucleolytic activities of the Mre11 nuclease with the DNA tethering and chromosomal bridging functions of the Rad50 protein [18,19]. Nucleotide hydrolysis occurs within the catalytic domains of the Rad50 component, which is a divergent member of the structural maintenance of chromosomes (SMC) superfamily of ATPases [26–28]. In eukaryotic systems, a third accessory protein (Nbs1 in metazoans, or the Xrs2 homologue in yeasts) associates with M/R core (Figure 1), enabling the complex to fulfill critical functions in cell signalling and coordinating the enzymatic activities with cell-cycle checkpoints [29–31]. However, this tripartite arrangement seems to be a eukaryotic innovation, as homologues, or indeed even functional analogues of the Nbs1/Xrs2 protein, have not been identified to date in either bacterial or archaeal species.

Boiling down the mechanisms of M/R action: insight gleaned from thermophilic model systems

Much of our current understanding of the DNA-repair mechanisms of the M/R complex has arisen from structural observations garnered from studies of thermophilic archaeal and bacterial M/R complexes, in particular from the species *Pyrococcus furiosus*, *Methanocaldicoccus janaschii* and *Thermotoga maritima* [32–35]. It is often advantageous to investigate the ancestral, biochemically tractable and structurally rigid thermophilic homologues of eukaryotic proteins and in the case of the M/R complex these analyzes have been particularly illuminating [36]. Such studies have revealed that the catalytic core of the complex forms a tetrameric arrangement consisting of a dimer of Mre11 in conjunction with two Rad50 subunits [32–35]. The Mre11 dimer organizes via four-helix bundle interactions between the core nuclease domains, while juxtaposed 'nuclease-capping' domains, which regulate the accessibility of DNA substrates to the Mre11 active sites, extend from the catalytic domains, and these connect to C-terminal helix–loop–helix domains, which mediate binding to the Rad50 partners within the tetrameric core [18,19] (Figure 2A–E). The Rad50 ATPase, like the other members of the SMC superfamily, is a dumbbell-shaped protein consisting of two compact globular domains, which harbour the Walker A phosphate binding-loop and Walker B ATPase motifs respectively, linked by a long coiled-coil region typically 600–900 amino acids in length [27,28,37]. The V-shaped Rad50 molecule is angled around a cysteine-containing, zinc-coordinating motif (CXXC) that is located at the centre of the coiled-coil region [38]; this permits the two globular domains to approach each other and unite to form a functional ATPase catalytic site [18,19] (Figure 2A). The zinc-binding element, distal to the catalytic domains, is commonly referred to as the 'zinc-hook' and is analogous to the centrally located 'hinge' region of other SMC proteins [38–40]. M/R tetramers can remain associated via these zinc hook regions when the globular domains have separated (Figure 2B) [41–43]. It has also been suggested, based on atomic force microscopy (AFM) and electron microscopy studies that these zinc-hook regions play critical roles in mediating chromosomal bridging via intermolecular associations, with the hooks associating between reciprocal Rad50 molecules

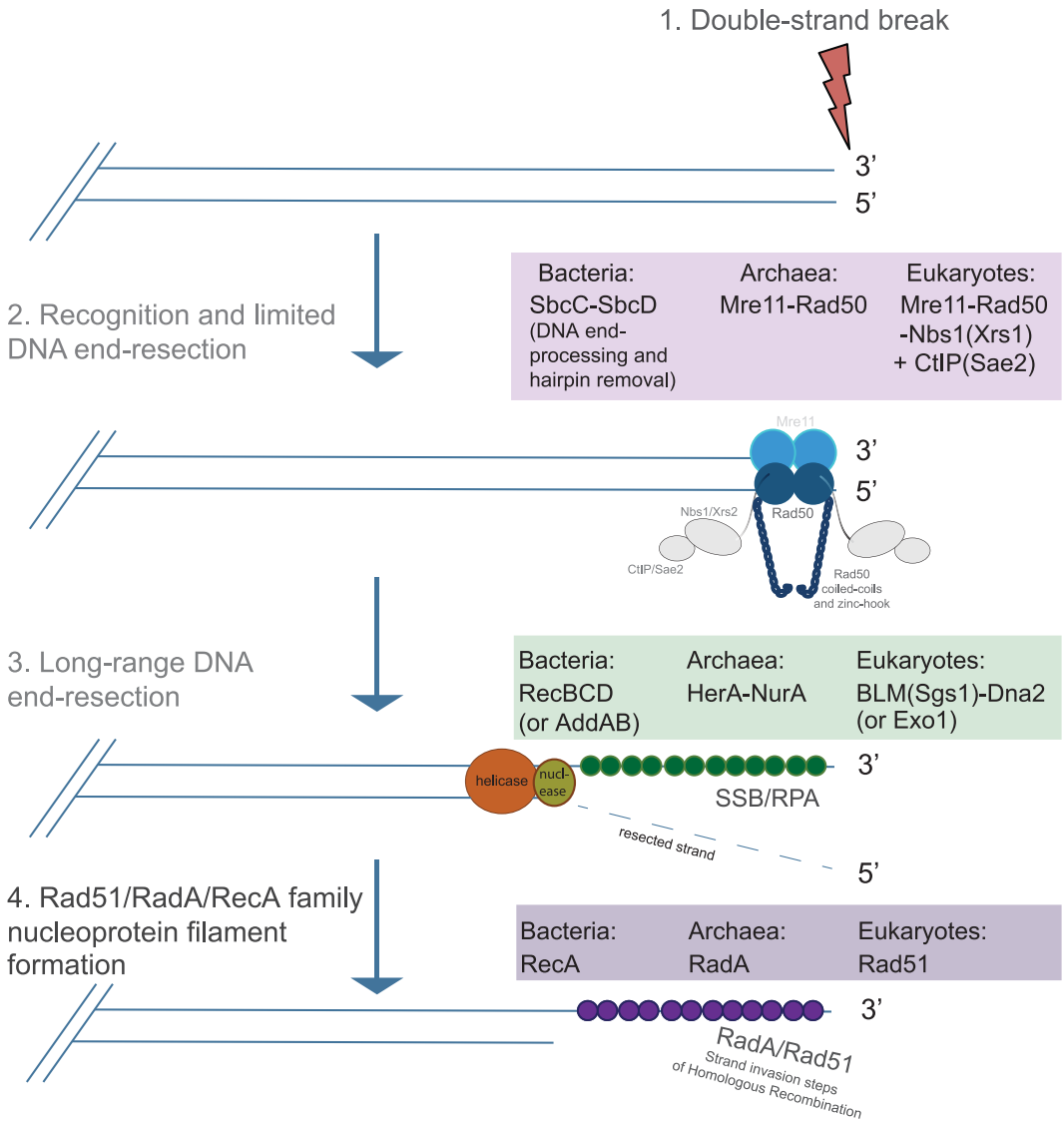

Figure 1. DNA Double-Strand Break (DSB) recognition and end-resection in the bacteria, archaea and eukaryotes.
Following generation of a DSB (step 1) the Mre11–Rad50 (M/R) complex is recruited at the site of damage (step 2). While the ancestral SbcC/SbcD Mre11/Rad50 complex of the archaea and bacteria is bipartite, the eukaryotic complex utilizes a third component Nbs1(Xrs2) that is not conserved in archaeal species. Initial processing at the break frequently utilizes the nucleolytic activity of Mre11, which in eukaryotes is potentiated by CtIP(Sae2). During generation of the 3′ single-stranded tails required for the strand invasion step during homologous recombination, the M/R complex facilitates the recruitment of the long-range end-resection machinery at the processed DNA-end. Bacteria use the RecBCD or AddAB, or AdnAB complexes to perform this resection, while in the archaea, this is performed by the HerA–NurA helicase/nuclease complex, while the RecQ family helicases such as BLM(Sgs1) or WRN and the Dna2 or Exo1 nucleases operate in eukaryotic cells (step 3). Unwound strands of the DNA duplex are coated by the single-stranded binding protein (SSB/RPA), which is displaced by the Rad51/RadA/RecA recombinase protein on the resultant 3′ single-stranded proteo-filament (step 4), which then invades the DNA duplex on the homologous chromosome to mediate the homologous recombination-driven repair event. The M/R complex also likely plays important architectural roles during the repair event, including tethering of the homologous chromosomes.

[33,38,41,42,44,45]. In the absence of a bound DNA substrate, it has been observed that the coiled-coils of a Rad50 homodimer associate intramolecularly, flexing to form a ring-like conformation, reminiscent of the arrangements seen in canonical SMC family proteins [41,44–47] (Figure 2D). These intramolecular linkages can be broken upon DNA binding to the globular domains and the seemingly flexible coiled-coils convert into

Biochemical Society Transactions (2020)
https://doi.org/10.1042/BST20170168

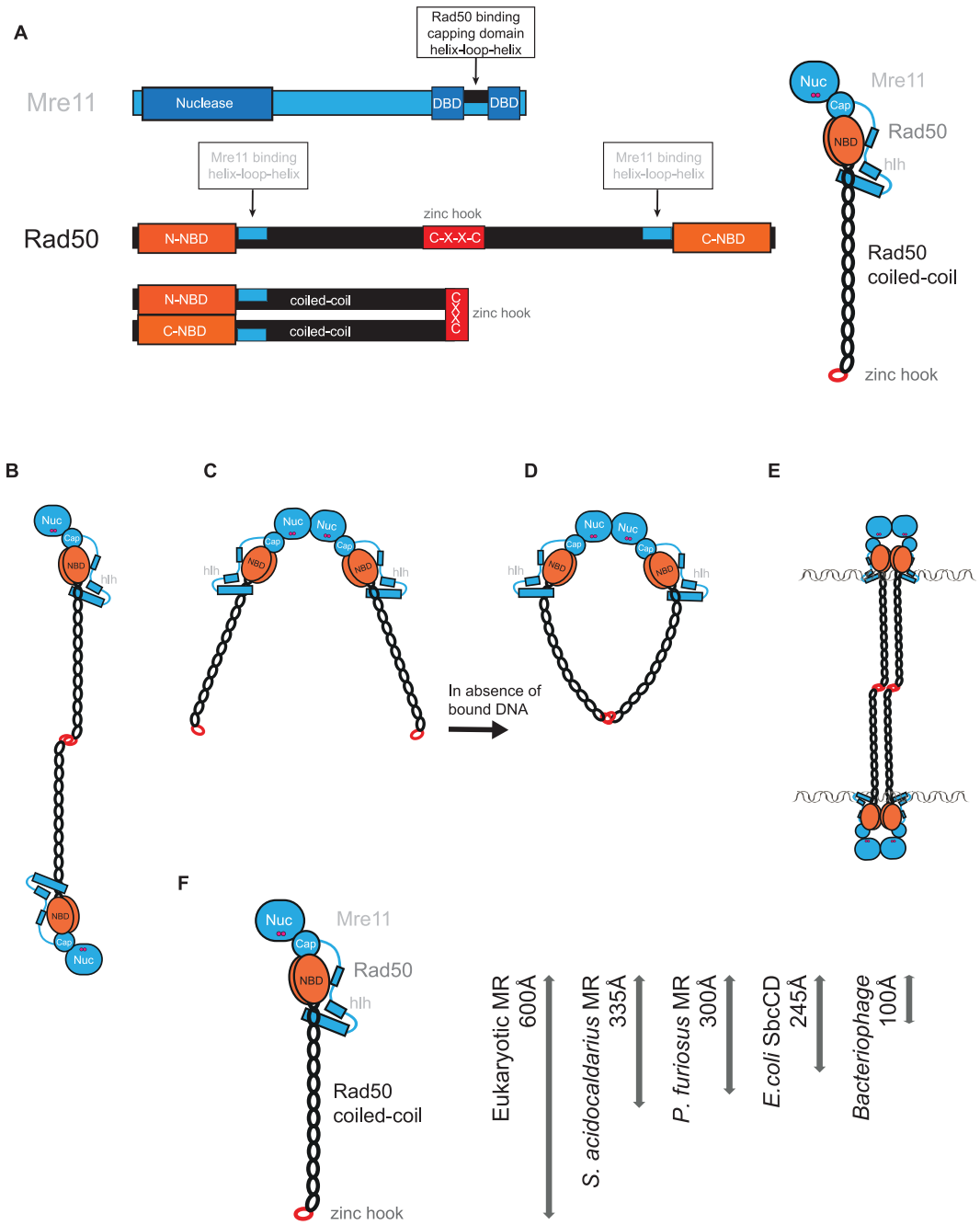

Figure 2. Schematic representation of the organization of the Mre11 and Rad50 domains in an M/R complex. Part 1 of 2 (**A**) Domain architecture of the Mre11 and Rad50 proteins. The nuclease domain of Mre11 is located at the N-terminus of the protein, while the capping domain and proximal helix–loop–helix Rad50 binding regions are located in the C-terminus of the protein adjacent to the DNA binding domains (DBD). N-terminal nucleotide-binding domain (N-NBD, ATPase-N) and C-terminal (C-NBD, ATPase-C) ATPase domains (in orange) are separated by a long antiparallel coiled coil region with a central zinc-hook domain (red). The Mre11 helix–loop–helix binding regions, proximal to the ATPase domains are indicated in blue. When folded along the zinc hook domain, the two domains compose a single catalytic ABC ATPase head from which a rod-like coiled-coil protrudes [21]. (Right) Schematic showing organization of the M/R complex: the two globular Rad50 NBD ATPase domains harbouring the Walker A and Walker B motifs, respectively (orange) are connected via the long coiled-coil region (black) which bends at the central 'zinc-hook' motif (red). The Mre11 nuclease (blue) interacts with the Rad50 catalytic domains via the 'capping' domain (Cap) and helix–loop–helix regions (hlh), which extend from the core nucleolytic domain (Nuc). (**B**–**E**) The M/R complex typically arranges as a tetramer (although higher order assemblies are possible [42,43], either via interactions at the

Figure 2. Schematic representation of the organization of the Mre11 and Rad50 domains in an M/R complex. Part 2 of 2
zinc-hook (**B**) or by association of the catalytic globular domains (**C**,**D**). The ring-shaped arrangement can form when the intramolecular associations are simultaneously formed at both the Rad50 zinc-hooks and the Mre11 globular domains (**D**). (**E**) Upon ATP binding the Rad50 globular domains are brought together resulting in the Rad50 coiled-coils associating to form an extended, rigid conformation that is thought to promote chromosomal bridging via association of the zinc-hooks between two complexes on homologous chromosomes. (**F**) A distance of 1200 Å (two-times 600 Å) could plausibly be bridged by the human M/R complex, or 600–670 Å by the archaeal complex (*Pyrococcus furiosus* or *Sulfolobus acidocaldarius*, respectively) or 490 Å (two-times 245 Å) by the *E. coli* homologous complex SbcCD, while the distance for bacteriophage coils would be shorter still (two-times 100 Å).

inflexible linear rods. Such changes permit the zinc hooks at the apices to associate intermolecularly with other M/R complexes in a manner that has been proposed to support DNA bridging during the repair events [41] (Figure 2E). Interestingly, the coiled-coil regions in bacterial species are somewhat shorter than their counterparts in the archaea, with reported lengths, when extended, of ∼250 Å compared with ∼300 Å, respectively [42,48]. Notably, bacteriophage homologues possess even shorter coiled-coils of ∼100 Å [49]. By comparison, the region in eukaryotic homologues is substantially longer, extending almost 600 Å [39,42] (Figure 2E). These variations in coiled-coil lengths are presumably reflective of the inherent inter-chromosomal distances and differences in chromosomal organization and complexity across the three divisions of life [39,48].

Early structural investigations of the M/R complex revealed the nature of the tetrameric assembly and also identified how the complex might bind to DNA ends [33,34]. However, it initially seemed unclear how the separable enzymatic functions of the Rad50 ATPase and the Mre11 nuclease might be coordinated during the recognition and subsequent repair of sites of DNA damage. Considerable insight was subsequently provided by a series of crystal structures and small-angle X-ray scattering (SAXS) analyzes performed in the presence or absence of ATP, or non-hydrolysable analogues of this nucleotide [23,32,37,50,51]. These studies unexpectedly revealed that the globular head domains of the Mre11/Rad50 complex experience extensive conformational shifts (Figure 3). Upon ATP binding a compact arrangement is formed, wherein the two ATPase domain are united, drawing all four of the globular domains of the M/R tetramer together in a closed conformation. However, the Rad50 ATPase domains separate dramatically upon nucleotide hydrolysis and the conformation opens into a distinctive 'W'-shaped structure, joined centrally by the Mre11 dimer, with the two Rad50 ATPase domains poised at opposite ends of the arrangement [18,19] (Figure 3). In this configuration the two Rad50 subunits attach to each of the Mre11 subunits via the helix–loop–helix region (Figure 3A). When arranged this way the Mre11 nuclease domains are accessible for DNA processing, but by contrast, these nucleolytic sites become partially masked in the more compact arrangement. When ATP binds to the Rad50 subunits the resulting conformational changes are transmitted to the Mre11 dimer through rotation of the helix–loop–helix region and this results in the M/R tetramer forming a clamp-like arrangement with increased affinity for DNA substrates [50]. Interestingly, in eukaryotic cells, it has been revealed that the Mre11-dependent nucleolytic activity of M/R complex plays a key role in preparing a DSB for HR-dependent repair [32,50,52,53]. In eukaryotes, DSBs are initially recognized and bound by the Ku DNA repair proteins. It has been revealed that before the G1/S phase transition of the cell cycle the endonucleolytic activity of the M/R complex, stimulated by the activity of the CtIP(Sae2) cofactor [54] (Figure 1), cleaves DNA proximal to the break, thereby removing Ku protein [52–56]. This permits the recruitment of the long-range DNA end-resection machinery, such as Dna2 and Exo1, effectively resulting in the selection of the HR pathway rather than the NHEJ mechanisms that are mediated by the end-binding Ku proteins in conjunction with DNA-PK and ligase [9,57].

Subsequent experimental interrogation of the distinct arrangements of the M/R complex revealed that the ATP-dependent actuation of the compact state promotes DNA-end recognition and also stimulates the chromosomal tethering activities of the M/R complex at DSBs [11,18,58]. In contrast, following ATP hydrolysis and the release of the complex into the more accessible state, the Mre11 catalytic sites are exposed potentiating the nucleolytic capability of the complex, facilitating processing of the DSB and initiating the end-resection process [11,18,58]. Thus, it has been proposed that the Mre11/Rad50 complex operates as an ATP dependent 'molecular-switch' providing a method of regulation that controls the separation of function between the disparate DNA break-recognition and end-resection roles [11,19,58]. Indeed, the seemingly low reported rate of *in vitro* ATP-hydrolysis by the M/R complex appears consistent with the energetic demands required by a

Biochemical Society Transactions (2020)
https://doi.org/10.1042/BST20170168

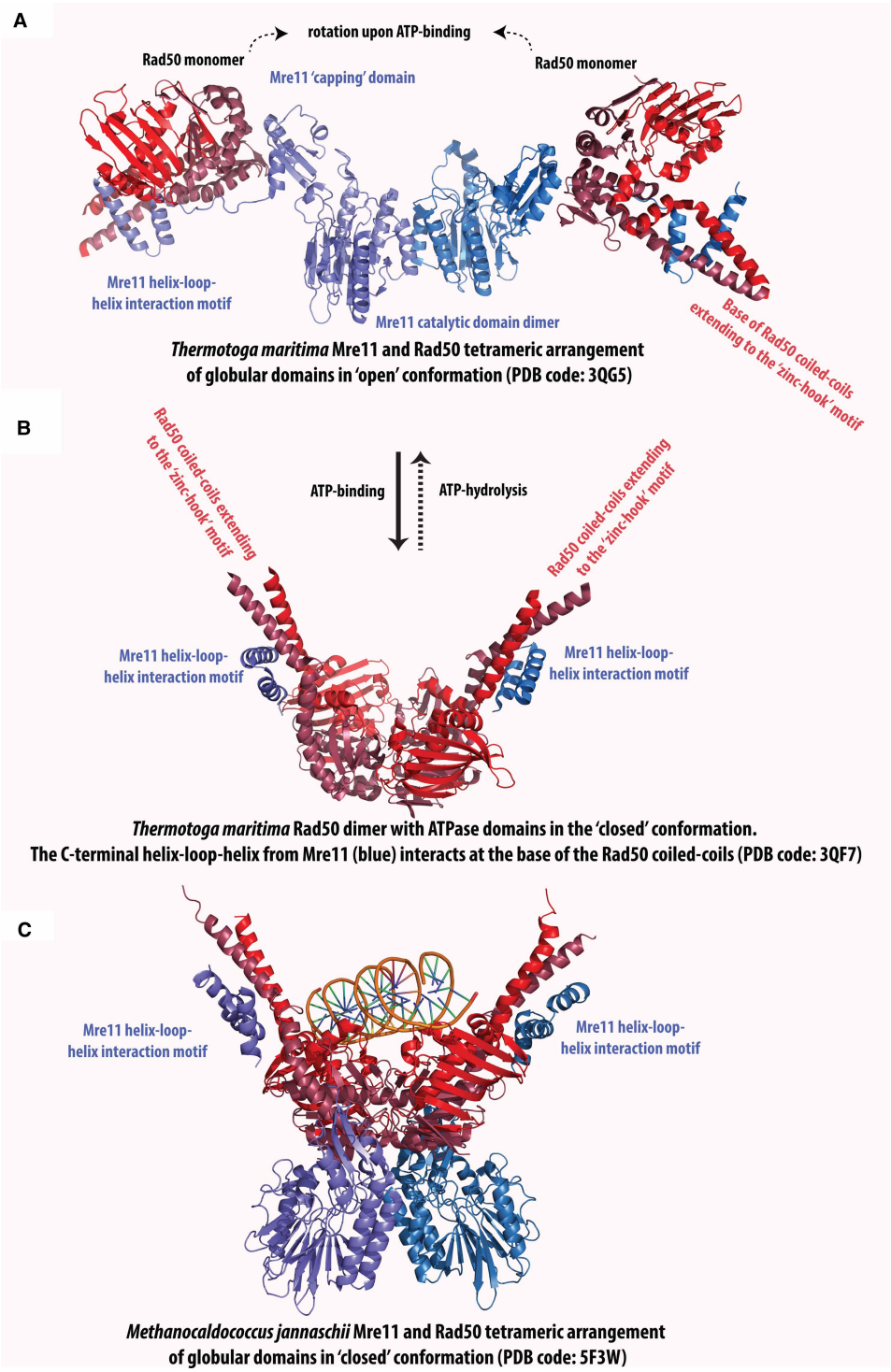

Figure 3. Conformational changes in the catalytic globular domain arrangement of the tetrameric M/R complex as a consequence of ATP binding, hydrolysis and release as revealed by crystallographic studies of thermophilic archaeal and bacterial homologues. Part 1 of 2

Mre11 components are coloured blue, and Rad50 in red. (**A**) an 'M'-shaped extended conformation is observed prior to ATP-binding; the tetramer is joined by dimerization at the Mre11 catalytic domains (ribbon representation of PDB: 3QG5; species: *Thermotoga maritima*). The two Rad50 subunits attach to each of the Mre11 subunits via the helix–loop–helix region. (**B**) the two Rad50 molecules associate upon ATP-binding to form the more compact tetrameric complex (ribbon representation of PDB: 3QF7; species:

Figure 3. Conformational changes in the catalytic globular domain arrangement of the tetrameric M/R complex as a consequence of ATP binding, hydrolysis and release as revealed by crystallographic studies of thermophilic archaeal and bacterial homologues. Part 2 of 2

Thermotoga maritima). Note that only the helix–loop–helix regions of Mre11 are shown in this structure. (**C**) ribbon representation of the closed conformation showing all four subunits of the tetramer and a dsDNA substrate bound to the Rad50 globular domains (PDB: 5F3W; species: *Methanocaldococcus jannaschii*). Note that the two halves of each Rad50 subunit (encompassing the two Walker boxes) are coloured in red and salmon, respectively, and only the base of the coiled-coil regions are depicted (these extend outwards and join at the zinc-hook hinged region). Figures were created in Pymol.

molecular switch. However, it has been observed that this rate of nucleotide turnover can be dramatically stimulated upon binding to double-stranded DNA, at least in prokaryotic or viral examples of the M/R complex [32,59]. It therefore currently remains undetermined if the energy derived from ATP hydrolysis by the M/R complex might also be utilized to drive the active manipulation of chromosomal substrates in addition to the well-characterized actions as a functional switch. Interestingly, recent insights into the mechanism of action of the canonical SMC family proteins, of which Rad50 is a member, may provide intriguing clues as to how the energy-dependent movement of the long coiled-coil regions could plausibly operate to organize and manipulate chromosomes [46].

Rad50 dynamism during DNA repair: insights from canonical SMC superfamily proteins?

The SMC superfamily is conserved across the bacterial, archaeal and eukaryotic divisions of life and includes the condensins, cohesins, SMC-5/6 DNA repair complexes, and prokaryotic SMCs [46]. Like the Rad50 members, these proteins arrange as dimers and adopt a distinctive ring-shaped arrangement mediated by the long and flexible coiled-coils, which encircle their DNA substrates. While Rad50 proteins form homodimers, the other SMC family members are heterodimeric formed of two distinct but closely related SMC subunits. One end of the ring is formed by the dimerization of the two SMC subunits at the hinge region, which is analogous to the zinc-hook region of Rad50 homologues. At the opposing end, the coiled-coil toroid is sealed by the association of the globular ATPase domains, stabilized by an additional kleisin subunit, which is conserved in the majority of prokaryotic and eukaroytic SMC complexes. In the Rad50 homologues, however, this ATPase domain stabilization is mediated by the unrelated Mre11 dimer [27,60] (Figure 4A). Although it was initially unclear how these proteins acted to manipulate their DNA substrates, it is now evident that SMC complexes operate as unconventional molecular machines, utilizing the energy liberated by ATP hydrolysis to drive critical chromosomal maintenance events [47,61,62]. This allows the complexes to actively sculpt the chromosomal landscape during their essential roles in gene expression, DNA repair and chromosome cohesion, compaction and organization. A growing body of experimental evidence has revealed that these complexes slide along DNA substrates in a process dependent on ATP hydrolysis. This substrate translocation is frequently linked to a dramatic extrusion of extensive DNA loops, which are critical for the organization of chromosomal architecture [47,63–72]. SMC complexes had been implicated in the formation of these critical looped chromosomal structures for some time, but there was initially some confusion as to how these unusual DNA fastening complexes could drive such dramatic events. It was initially hard to consolidate the chromosomal sculpting events with the inherently low ATP hydrolysis rates observed in SMC proteins, which ranges from less than 0.1 to 2 molecules of ATP per SMC dimer per second [63]. In contrast, most conventional DNA translocases display a considerably higher rate of ATP turnover frequently turning over thousands of ATP molecules per second [73]. Due to this disparity, it was originally suspected that additional molecular motors such as the powerful RNA polymerase of the transcriptional apparatus might provide the energy necessary to drive the formation of loops, with the SMC proteins simply topologically capturing the conformational changes. However, recent advancements in single molecule experimentation have now visualized the progressive extrusion of DNA loops mediated by SMC proteins alone [68,69,74–76]. These studies are now starting to reveal the mechanisms by which the SMC superfamily orchestrate chromosomal organization by DNA loop formation. Indeed, unique and unconventional translocation mechanisms have been observed, dependent on nucleotide hydrolysis in the ATPase domains of the SMC subunits [47,63–70,77].

Biochemical Society Transactions (2020)
https://doi.org/10.1042/BST20170168

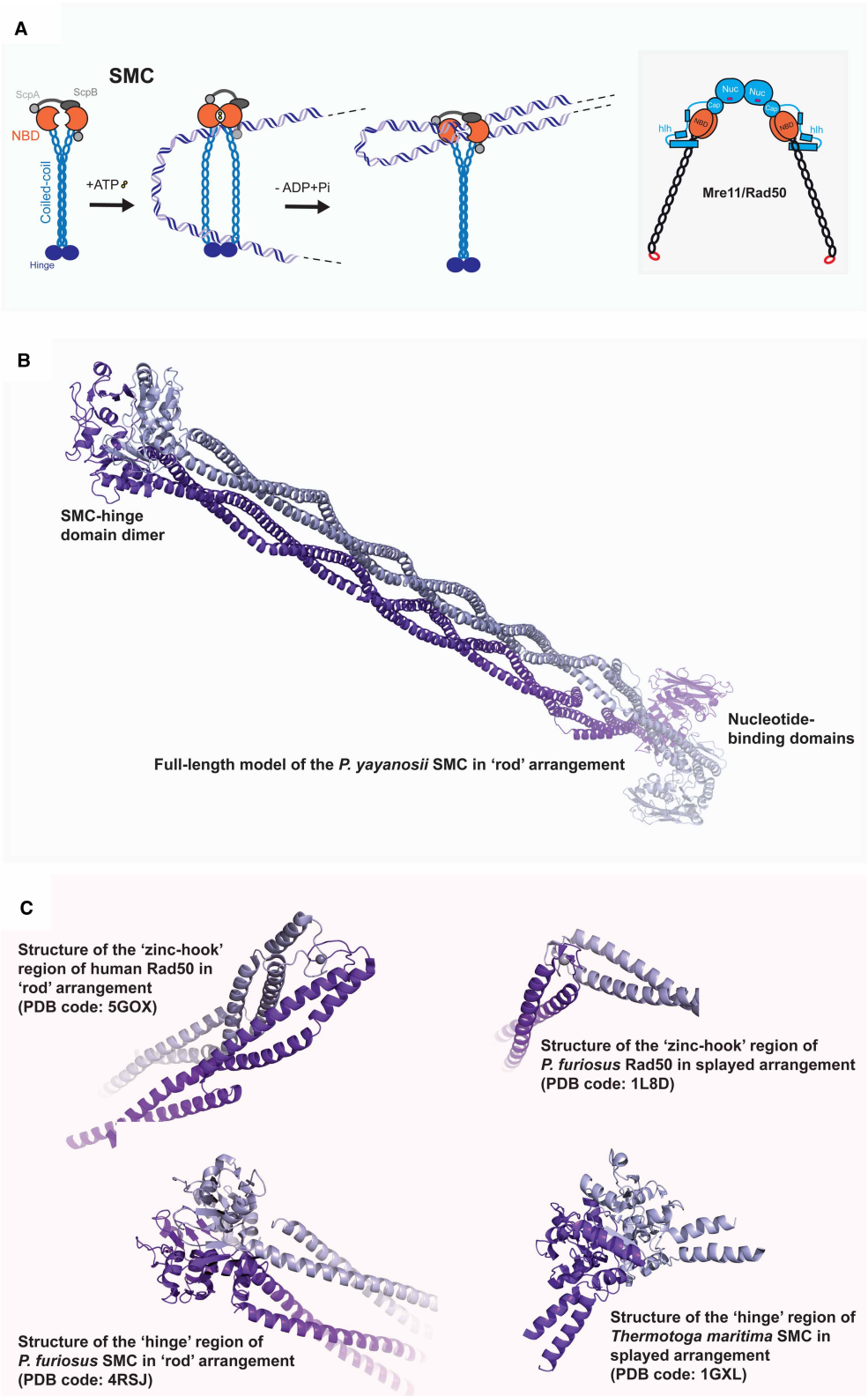

Figure 4. SMC family rod formation and ATP-dependent DNA transactions. Part 1 of 2

(**A**) Putative model for a DNA loop extrusion mechanism by an archaeal/bacterial SMC protein (as proposed in [79]). With the SMC in a ring-like conformation, a dsDNA substrate can associate with both the hinge and catalytic head domain regions.

Figure 4. SMC family rod formation and ATP-dependent DNA transactions. Part 2 of 2

ATP-hydrolysis results in the transition to the closed rod-like conformation resulting in the extrusion and capture of a DNA loop. The SMC nucleotide binding domains (NBD) are coloured orange, with the coiled-coils in blue and hinge region in purple. The archaeal/bacterial non-SMC subunits (Scps: **s**egregation and **c**ondensation proteins) are depicted in grey. (Right inset) A schematic of the Mre11/Rad50 complex is shown for comparison with SMC complex. (**B**) Full-length dimeric model of an archaeal SMC protein (species: *Pyrococcus yayanosii*) in 'rod' arrangement. The PDB coordinates used to produce this figure were taken from the Supplementary data of [79] [molcel6278mmc4_V1.pse]. The two subunits of the dimer are depicted in light and dark purple, respectively. (**C**) (top left) Arrangement of the 'zinc-hook' region of human Rad50 in closed 'rod' conformation (PDB: 5GOX); (top right) The 'zinc-hook' region of *Pyrococcus furiosus* Rad50 in an arrangement suitable for chromosomal bridging events (PDB: 1L8D); (bottom left) the analogous 'hinge' region of the *Pyrococcus furiosus* SMC protein in a 'rod' arrangement (PDB: 4RSJ); (bottom right) structure of the 'hinge' region of *Thermotoga maritima* SMC in splayed arrangement (PDB code: 1GXL). The structural figures shown in (**B**) and (**C**) were generated in Pymol.

Further efforts are required to elucidate the mechanisms by which the SMC complexes actively modulate chromosomal structures, although several models have been posited, including an 'inchworm' translocation mechanism, a 'hand-over-head' myosin-like stepping system and dynamic 'pumping' and 'scrunching' models [47,63,67]. These models have been formulated by taking into account the various experimental observations in the single molecule studies [68,69,74–76]. Currently, the 'pumping' and 'scrunching' models appear the most plausible and it remains possible that SMC action could work via a combination of these modes [47,63,67]. Furthermore, it has not yet been determined if the different members of the SMC superfamily operate via a common mechanism, or whether substantially different modes of action are employed by different SMC complexes.

It is widely accepted that the ATP-dependent engagement and release of the globular head domains is the driving force behind the dynamic and energy-dependent transactions that SMC superfamily proteins direct towards their chromosomal substrates [46] (Figure 4A). Indeed, early experimental observations of the prokaryotic SMCs have proposed models of action that suggest that architectural rearrangements transmitted between the ATPase head and hinge domains via the long coiled-coil arms appear critical for their respective roles in chromosomal organization [47,63,78–83]. Considering the clear evolutionary relationship between Rad50 and the other SMC family members, observable at both the level of amino-acid homology, and also in the overall architecture of these distinctive ATPases [28], it is tempting to speculate that commonalties might be retained in their respective modes of action. Whilst the globular catalytic domains of all the SMC family members are evolutionarily related to the overarching superfamily of ABC (ATP binding cassette) membrane transporters [27], the broad SMC class is distinguishable by the arrangement of the two globular ATPase domains at the N- and C-termini of the molecule, separated by the long coiled-coil regions. Indeed, the entire SMC class of proteins, which include the Rad50 homologues in addition to canonical SMCs, all form the characteristic V-shaped arrangement, which closes to form the toroid that can encircle the DNA substrates [46,47,84]. All of these SMC superfamily proteins share the common mechanistic feature of the engagement and disengagement of the disparate globular head domains as a result of ATP binding, hydrolysis and release, which results in conformational changes of the long coiled-coil regions [46,78,81,82,85] (Figure 4A). Indeed, studies of the archaeal Rad50 proteins have revealed that nucleotide hydrolysis results in a 30° rotation of the Rad50 globular lobes that effect the positioning of the coiled-coils, the accessibility of Mre11 nucleolytic sites, and may even culminate in localized unwinding of the DNA duplex at the DSB [32,37,86].

Whilst the canonical SMC superfamily proteins were originally envisaged as static DNA-clasping structures it has become evident that dynamic architectural rearrangements of the long coiled-coil regions occur during association with DNA substrates. Conformational changes of the coiled-coil arms are also coincident with the binding and hydrolysis of ATP at the head domains [47,63–67,78,81,85]. These allosterically regulated architectural arrangements appear critical for the established roles of the SMC family proteins in a variety of biological processes, which include sister-chromosome cohesion, chromosome condensation and DNA repair [28,47,60,79,81,85] (Figure 4A). When comparing the Rad50 homologues to other SMC family members it is evident that the hinged apex of the coiled coils is the most divergent region. This feature is considerably shorter in Rad50 proteins at around thirty amino acids in Rad50 [38] compared with around one-hundred and fifty amino acids in the SMC hinges [39] (Figure 4B,C). The Rad50 region also bears the unique 'zinc-hook'

Biochemical Society Transactions (2020)
https://doi.org/10.1042/BST20170168

motif (Figure 4C) that mediates zinc-dependent coiled-coil dimerization and promotes the intermolecular transactions that are believed to support chromosomal bridging [38]. Atomic force microscopy (AFM) and electron microscopy (EM) observations of the Rad50 complexes from both archaeal and eukaryotic species have revealed that the Rad50 coiled-coil regions undergo dramatic mesoscale conformational changes as a result of both DNA binding and ATP hydrolysis. [41,42,44,45,87] These events switch the conformation of the coiled-coils from seemingly flexible arrangements that encourage ring-shaped intramolecular associations via the zinc hooks, to rigid rod arrangements where the intramolecular interactions of the zinc hooks are broken [41,42,44,45] (Figure 2). Interestingly, the canonical SMC family proteins also adopt conformations that alternate between rod and ring shapes, [47,65] although the more extensive interaction interface observed at the canonical SMC hinge appears to promote and maintain the intramolecular associations at the hinge interface in both of these arrangements [78,79] (Figure 4A). Nevertheless, mounting experimental evidence appears suggestive that the mechanisms of chromosome organization and manipulation by both the classical SMC representatives and also the Rad50 proteins involve transitions between rod and ring shapes of the long coiled-coil regions.

Comparisons of the analogous Rad50 zinc hook and SMC hinge regions: an open and shut case?

When considering canonical SMC family proteins, it has been proposed that DNA substrates can enter or exit the SMC ring structure at one of two principal sites. These binding events are mediated by either the opening of the globular ATPase domains at the head, or alternatively occur following separation of heterodimer at the hinge region at the opposite end of the coiled-coils [88–90]. Certainly, in both prokaryotic and eukaryotic SMC proteins, the hinge region has been suggested to play essential roles in DNA-binding and act as a DNA entry gate, in addition to the established functions in hetero-dimerization [40,46,85,89–92]. In contrast, the analogous zinc hook region in the Rad50 has thus far only been implicated in either the intra-molecular coiled-coil associations, forming the closed ring-like or rod-like conformations [93,94], or alternatively in the inter-molecular interactions between disparate Rad50 molecules, which appear more prevalent following DNA binding [38,41]. However, little consideration has been given to putative roles of the zinc-hook region in direct DNA binding, in a manner evocative of the canonical SMC hinge. Indeed, the majority of experimental studies describe modes of M/R-mediated nucleic acid interaction that exclusively involve the globular ATPase domains and the localized coiled-coil regions that abut these globular catalytic termini [95,96]. Despite this commonly held perception, it perhaps should be noted that there is experimental evidence to suggest that the absolutely conserved Rad50 zinc-hook region might plausibly play an important role in direct DNA binding. Might the zinc-hook region of the M/R complex function as a counterpart of the SMC hinge, not only in terms of a coiled-coil interface, but also as an important site for mediating interactions with DNA substrates? In support of this view, it is particularly notable that mutations in the zinc hook region from *Saccharomyces cerevisiae* lead to phenotypes that are comparable with Rad50 null mutants, highlighting the essential nature of these regions [97]. Furthermore, it has been observed in mammalian cells that these zinc-hooks are essential for guiding the M/R complex to chromosomal DSBs following exogenously induced chromosomal damage [98]. Interestingly, it has also been revealed that the zinc-hook region from the archaeon *P. furiosus* can be substituted for the SMC hinge region of the prokaryotic condensin homologue in *Bacillus subtilis*, seemingly without affecting cellular viability under nutrient-rich conditions [80]. Similarly, it has been shown that substitution of the zinc-hook of the yeast M/R complex for the hinge region of the MukB bacterial condensin has minimal physiological effects on the resulting mutant yeast cells and thus the zinc-hook and hinge dimerization domains appear interchangeable [100].

Might these experimental observations imply that Rad50 zinc-hook regions can interact directly with DNA substrates? To date, it remains undetermined precisely how the M/R complex initially locates and then associates with sites of DNA damage. Indeed, it is particularly unclear which functional domains of the complex might be involved in the detection of DSBs. However, a recent single-molecule study has revealed that the M/R complex is considerably more mobile on chromosomes than was previously anticipated [100] This study also demonstrated that the M/R complex is able to translocate along even histone-occupied chromatin, effectively scanning for broken DNA ends via facilitated diffusion along the substrate [100]. This unexpected DNA-tracking mechanism appears dependent on the Rad50 globular domains of the M/R complex. Presumably, the coiled-coils arms of the Rad50 dimer also encircle the chromosome during this scanning

process in a manner evocative of the previously described movements of other canonical SMC proteins upon chromosomal substrates [61,63,77,88,101]. Certainly, other studies are indicative of the coiled-coil regions associating with DNA substrates [60,102]. It therefore appears that the M/R complex tracks chromosomes via associations with the Rad50 globular domains utilizing an as yet undetermined interaction mode. It has also been revealed that upon arrival at the DSB the Mre11 component of the M/R complex then recognizes the broken DNA terminus, which it binds with high-affinity [94,100]. It should be noted that previous models for DSB recognition have almost exclusively depicted the M/R complex DNA loading events occurring directly at the break without prior chromosomal translocation [11,18]. This dynamic DNA end-surveillance system, dependent on chromosomal-tracking revolutionized our views of DSB recognition by the M/R complex. Furthermore, in support of this model, a recent study of the M/R complex from the thermophilic archaeon *Sulfolobus acidocaldarius*, using real-time AFM, has demonstrated that the zinc-hook region of Rad50 appears to associate transiently with DNA substrates [43]. This study also predicted, by classical molecular dynamics simulations of the archaeal Rad50 hinged region on double-stranded DNA, that the zinc-hook region and juxtaposed coiled coils are seemingly able to track the minor groove of a DNA duplex until an end is encountered [43]. It therefore seems plausible that the Rad50 zinc-hook and associated coiled-coils may aid the facilitated diffusion along DNA substrates in addition to established transactions with the Rad50 globular domains [32,50,95,96]. It remains to be determined if any DNA binding activity can also occur close to the zinc-hooks when the region is in the dimerized conformation.

Running rings around DNA substrates: is ATP-hydrolysis powered manipulation of chromosomes common to all the SMC superfamily members?

Following the recently described method of DNA translocation by the M/R complex, it seems logical to question whether this scanning for broken DNA ends might be limited to facilitated diffusion. Could, for instance, the active architectural rearrangements in the M/R complex brought about by ATP hydrolysis be harnessed to actively drive these translocation events along DNA duplexes? While the single-molecule study of the *S. cerevisiae* M/R proteins did not establish a link between the Rad50 mediated diffusion and ATP hydrolysis [100], it is noteworthy that the AFM study of the *S. acidocaldarius* M/R complex identified unexpected and unprecedented evidence of ATP-dependent DNA duplex strand separation [43]. This dramatic DNA unwinding, which extended over several hundred base-pairs, was observable as 'bubble' structures on double-stranded DNA substrates. These duplex strand-separations might conceivably arise as a consequence of supercoiling or writhe generated from the energy-dependent manipulation of the DNA substrate by the archaeal M/R complex [43]. Fast-scan AFM analyzes in fluid also revealed that archaeal M/R complex can manipulate DNA substrates in a manner consistent with translocation and loop formation [43]. While further experimental interrogation is required to verify how the M/R complexes are involved in the active manipulation of DNA substrates, the mounting data provided by studies of the canonical SMC proteins indicate that the ATP-dependent architectural contortions induced by these complexes are indeed linked to DNA translocation and chromosomal loop extrusion [43,68,69,74–77,80]. Furthermore, the growing body of evidence from studies of prokaryotic and eukaryotic SMC proteins indicates that the transitions between closed rod and ring conformations of the coiled-coil arms appear to mediate the DNA loop extrusion (Figure 4A); these events involve allosteric communication between the globular domains and the hinge regions via the coiled-coils of the SMC proteins [78–81]. Indeed, the ATPase globular domains appear to be able to exist in at least three arrangements: a so-called engaged 'J-state' rod-like arrangement, or fully disengaged in the absence of ATP, or the engaged ring-like 'E-state' in the presence of ATP [47]. Conformational changes in the vicinity of the SMC hinge, alternating between juxtaposed 'J-state' coils and the ring-like 'E-states', as a result of either DNA or ATP binding at the globular domains, appear critical for the actuation of these structural shifts [47]. These events seem to be key for powering the translocation of the SMC complexes along chromosomal substrates and loop extrusion. Indeed, after the pioneering single-molecule study of the *S. cerevisiae* condensin revealed that this class of SMC proteins are indeed able to translocate along immobilized DNA substrates *in vitro* [61], it has subsequently become clear that similar events appear to be associated with the formation of DNA loops in condensins, cohesins and bacterial SMCs alike [63,66,67]. While the mechanism of action is still currently being investigated and debated, 'scrunching' models, where the coiled coils bend at non-helical 'elbow' regions located in the middle of coiled-coils [65,70,103,104], seem a likely scenario [47,63,65,66]. It therefore appears that canonical

Biochemical Society Transactions (2020)
https://doi.org/10.1042/BST20170168

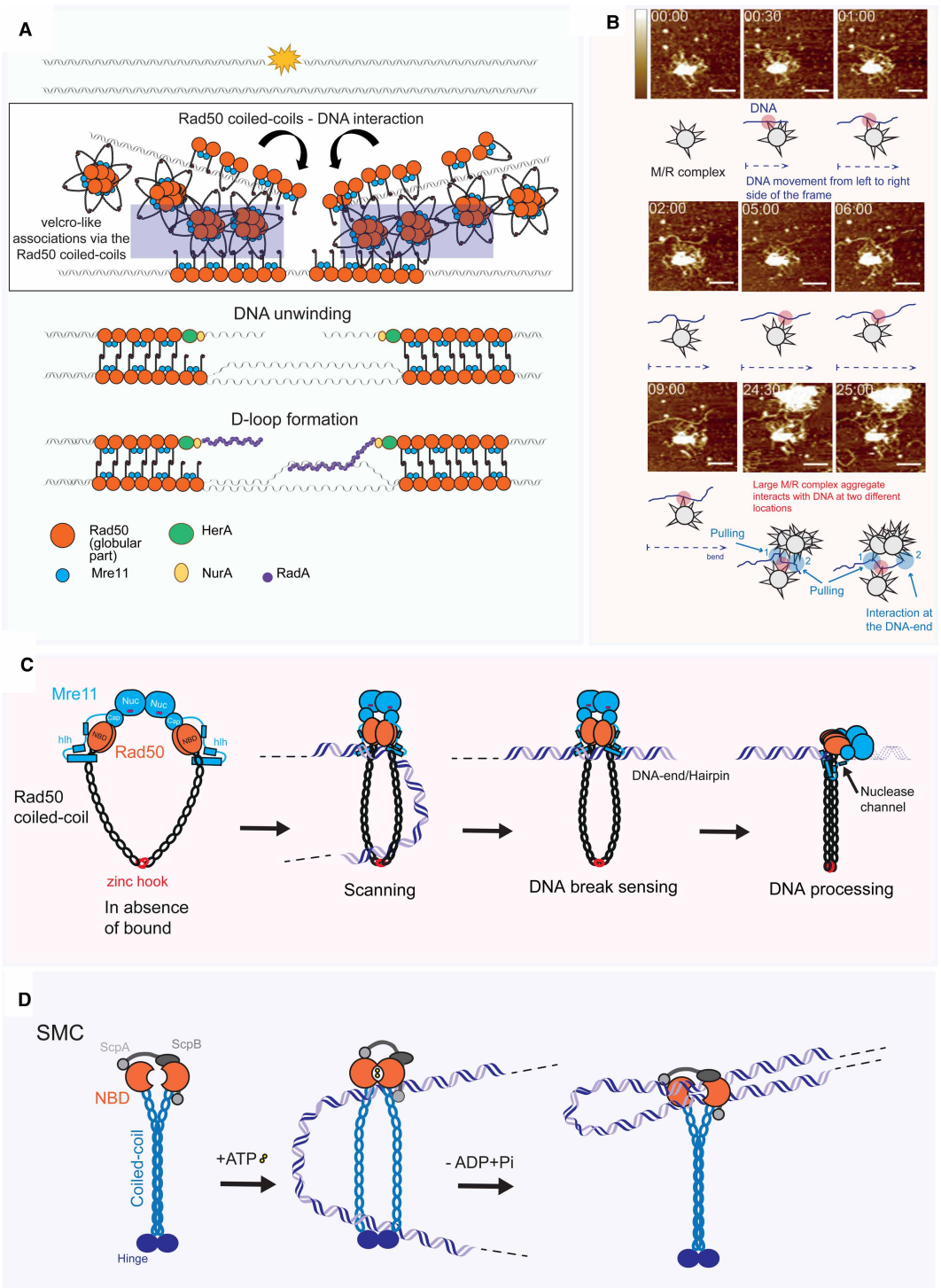

Figure 5. Final reflections on M/R and SMC complex activities. Part 1 of 2

(**A**) Proposed modes of action of the *S. acidocaldarius* Mre11/Rad50 (M/R) complex during DNA repair events. Associations with the homologous chromosome may form via *Velcro*®-like interchromosomal connections between the Rad50 zinc-hooks and coiled-coiled regions (blue shaded region). M/R oligomers may also link the broken DNA termini while the homologous chromosome remains held in proximity by Rad50 intermolecular associations. The M/R protein complexes scan the DNA double-helix and slide a towards a ds-break, facilitated by associations with the Rad50 coiled-coils. We speculate that M/R unwinding activity would facilitate the strand-invasion steps of HR. In *S. acidocaldarius*, the HerA–NurA DNA end-resection

Figure 5. Final reflections on M/R and SMC complex activities. Part 2 of 2

machinery generates long-range 3′ ss-DNA overhangs. The intrinsic unwinding activity of the M/R complex may open the DNA duplex on the homologous chromosome to facilitate D-loop formation and strand-invasion by the RadA proteo-filament, although this hypothesis requires experimental verification. (**B**) Real-time FS-AFM imaging of DNA binding and translocation following association with the Rad50 coiled-coil apices of the wild-type M/R complex. Selected images of the *S. acidocaldarius* M/R complex captured during Fast Scan-AFM imaging as described by Zabolotnaya et al. [43]. M/R can interact with DNA substrates via the Rad50 coiled coils apices (cartoons below images, red shaded circles). Examination of individual Rad50 apices reveals a pattern of alternate binding and disengagement from the DNA substrate resulting in the intermittent movement that displaces the DNA strand from left to right (cartoons, blue dotted arrows). A second M/R protein oligomer arrives at the observed area (24:30) and binds to another region of the DNA strand via the ends of Rad50 coiled coils making two different contact points (blue shaded circles, 1 & 2), as depicted in the cartoons. This interaction causes a transient pulling of the DNA substrate towards the newly arrived protein complex at the first contact point (blue shaded circle, 1) (24:30–25:00). These dramatic ATP-dependent movements seem consistent with an active DNA scanning mechanism. (**C**) Model proposed by Kashammer et al. [94] for the sensing and processing of DNA ends by *E. coli* MR. Upon binding at an internal DNA site the M/R complex enters into a scanning mode (with relaxed coiled-coils) until a blocked end or dsDNA break is encountered, upon which the coiled-coils undergo a ring-to-rod transition forming the high-affinity cutting state at or near DNA ends. (**D**) Illustration of SMC family ATP-dependent coiled-coil transitions from ring-to-rod conformational changes, illustrating a putative model for a DNA loop extrusion mechanism by an SMC protein (as proposed in [79] (see Figure 4).

SMC family of proteins do indeed utilize a previously uncharacterized ATP-dependent and processive mechano-chemical motor mechanism, which is essential for the extrusion of DNA loops through the SMC arms [47,61,63].

It is noteworthy that recently the structure of both the human and *E. coli* Rad50 zinc-hook regions have been determined and these can form rigid rod-like structures upon DNA binding [93,94] (Figure 4C), distinct from the previously described splayed zinc hook structure that was revealed using the hinged region from the thermophilic archaeon *Pyrococcus furiosus* Rad50 [38] (Figure 4C). The observation of these two different conformations at the Rad50 zinc-hook region, reminiscent of the two characterized analogous arrangements of the canonical SMC hinges adds weight to the intriguing possibility that the M/R complex may also be an unconventional chromosome manipulating motor. Indeed, models have suggested communication between the catalytic head and zinc-hook regions, via transmission through the long Rad50 coiled-coils [87,94].

Perspective: unifying concepts and methodologies to elucidate the modes of action of the M/R complex and the SMC family ATPases

Over the last three decades, considerable progress has been made towards elucidating the action of the M/R complex. Despite these experimental insights, many mechanistic details regarding how this intriguing DNA repair complex targets DSBs and regulates chromosomal stability remain unexplained. The recent discovery that the M/R complex is considerably more mobile on chromatin than was previously anticipated, coupled with the idea that the essential zinc-hook region may play crucial roles in chromosomal transactions, provides us with tantalizing clues that suggest that Rad50 may share unanticipated similarities to the other canonical SMC-like proteins during chromosomal processing events. Considering the recent advances in our understanding of the modes of action of the canonical SMCs, it remains an intriguing possibility that this dynamic and enigmatic complex may utilize the ATPase activity of Rad50 activity to drive an energy-dependent search for DSBs. Interestingly, it has also been observed that the M/R complex in yeast appears to co-migrate along a chromosomal substrate with the Exo1 exonuclease, which is required for DNA end-resection [8,105]. It will therefore be important to explore in the future how the M/R machinery is involved in the recruitment of the long-range DNA end resection machinery. Perhaps energy-dependent chromosomal manipulation by the M/R complex may even facilitate the critical downstream processing of the DSB to produce the single-stranded tails required for strand invasion [43] (see Figure 5 for proposed models).

Given the inherent complexities associated with studying the dynamic SMC superfamily of proteins it has become clear that to completely ascertain the intricacies of the action of these proteins it will likely be necessary

Biochemical Society Transactions (2020)
https://doi.org/10.1042/BST20170168

to employ the latest state-of-the-art single molecule approaches in combination with classical structural and biophysical approaches. Experimentally tractable thermophilic archaeal homologues, which exhibit the intrinsic catalytic activities, even in the absence of accessory factors such as CtIP, will no doubt play an important role in the future experimental pursuit of our understanding of these mechanisms. However, to ultimately comprehend the complicated chromosomal arrangements mediated by Rad50 and the other SMC family proteins it may be necessary to study these events in the context of the localized cellular environment. Indeed, it seems likely that the development of new technologies for analyzing chromosome organization within cells will doubtlessly be important for enhancing our understanding of the wide range of essential biological events mediated by the SMC superfamily of proteins.

Perspective

- The Mre11/Rad50 complex is an essential DNA repair machinery used by all forms of life with homologues identified in the bacteria, archaea, and eukarya. The disparate catalytic functions of the Mre11 nuclease and the Rad50 ATPase (a member of the Structural Maintenance of Chromosomes (SMC) superfamily of ATPases that include the condensins and cohesins) work together to drive the DNA repair events that maintain chromosomal integrity.
- Several decades of research have provided critical insights into how the Mre11/Rad50 complex orchestrates these essential DNA repair events, although many questions remain still unanswered concerning the modes of action that this chromosomal maintenance machinery utilizes ensure the fidelity of the genetic code. There has been considerable emphasis on the study of the catalytic 'head' domains of the Rad50 ATPase, while the coordinated movements of associated long coil-coiled regions of that connect the 'head' and 'zinc hooks' (analogous to SMC hinge regions) of Rad50 are less well understood.
- Recent pioneering studies have advanced our understanding of how canonical SMC complexes, such as the condensins, orchestrate dramatic ATP-driven conformational changes within their coiled-coil regions, acting as molecular machines that physically extrude DNA loops. Similar modes of activity may be involved during DNA repair events mediated by the Mre11/Rad50 complex, perhaps by 'turning the M/R complex on its head', using analagous movements within the coiled-coiled regions to those that have recently been described for the canonical SMC proteins.

Competing Interests

The authors declare that there are no competing interests associated with the manuscript.

Funding

The research performed in the N.P.R. and R.M.H. laboratories described in this review was funded by the Medical Research Council [Career Development Award G0701443] an Isaac Newton Trust Research Grant (Trinity College and Department of Biochemistry, Cambridge) and start-up funds from the Division of Biomedical and Life Sciences (Lancaster University) and a Biotechnology and Biological Sciences Research Council Grant BB/J018236/1, which provided funds for the purchase of the Bruker FastScan atomic force microscope. N.P.R. is currently funded by the Leverhulme Trust (RPG-2019-297).

Open Access

Open access for this article was enabled by the participation of Lancaster University in an all-inclusive *Read & Publish* pilot with Portland Press and the Biochemical Society under a transformative agreement with JISC.

Author Contributions

All authors were involved in the preparation of this review manuscript.

Abbreviations

ABC, ATP binding cassette; AFM, atomic force microscopy; DSBs, double-strand breaks; HR, homologous recombination; MMEJ, micro-homology mediated end-joining; NHEJ, non-homologous end-joining; SMC, structural maintenance of chromosomes.

References

1 Chapman, J.R., Taylor, M.R. and Boulton, S.J. (2012) Playing the end game: DNA double-strand break repair pathway choice. *Mol. Cell* **47**, 497–510 https://doi.org/10.1016/j.molcel.2012.07.029
2 Ranjha, L., Howard, S.M. and Cejka, P. (2018) Main steps in DNA double-strand break repair: an introduction to homologous recombination and related processes. *Chromosoma* **127**, 187–214 https://doi.org/10.1007/s00412-017-0658-1
3 Thompson, L.H. and Schild, D. (2002) Recombinational DNA repair and human disease. *Mutat. Res.* **509**, 49–78 https://doi.org/10.1016/S0027-5107(02)00224-5
4 Weterings, E. and van Gent, D.C. (2004) The mechanism of non-homologous end-joining: a synopsis of synapsis. *DNA Repair* **3**, 1425–1435 https://doi.org/10.1016/j.dnarep.2004.06.003
5. Lieber, M.R. (2010) The mechanism of double-strand DNA break repair by the nonhomologous DNA end-joining pathway. *Annu. Rev. Biochem.* **79**, 181–211 https://doi.org/10.1146/annurev.biochem.052308.093131
6 Seol, J.H., Shim, E.Y. and Lee, S.E. (2017) Microhomology-mediated end joining: good, bad and ugly. *Mutat. Res.* **809**, 81–87 https://doi.org/10.1016/j.mrfmmm.2017.07.002
7 Maher, R.L., Branagan, A.M. and Morrical, S.W. (2011) Coordination of DNA replication and recombination activities in the maintenance of genome stability. *J. Cell. Biochem.* **112**, 2672–2682 https://doi.org/10.1002/jcb.23211
8 Blackwood, J.K., Rzechorzek, N.J., Bray, S.M., Maman, J.D., Pellegrini, L. and Robinson, N.P. (2013) End-resection at DNA double-strand breaks in the three domains of life. *Biochem. Soc. Trans.* **41**, 314–320 https://doi.org/10.1042/BST20120307
9 Symington, L.S. (2016) Mechanism and regulation of DNA end resection in eukaryotes. *Crit. Rev. Biochem. Mol. Biol* **51**, 195–212 https://doi.org/10.3109/10409238.2016.1172552
10 Yeeles, J.T. and Dillingham, M.S. (2010) The processing of double-stranded DNA breaks for recombinational repair by helicase-nuclease complexes. *DNA Repair (Amst)* **9**, 276–285 https://doi.org/10.1016/j.dnarep.2009.12.016
11 Schiller, C.B., Seifert, F.U., Linke-Winnebeck, C. and Hopfner, K.P. (2014) Structural studies of DNA end detection and resection in homologous recombination. *Cold Spring Harb. Perspect. Biol.* **6**, a017962 https://doi.org/10.1101/cshperspect.a017962
12 Rzechorzek, N.J., Blackwood, J.K., Bray, S.M., Maman, J.D., Pellegrini, L. and Robinson, N.P. (2014) Structure of the hexameric HerA ATPase reveals a mechanism of translocation-coupled DNA-end processing in archaea. *Nat. Commun.* **5**, 5506 https://doi.org/10.1038/ncomms6506
13 Kowalczykowski, S.C. (2015) An overview of the molecular mechanisms of recombinational DNA repair. *Cold Spring Harb. Perspect. Biol.* **7**, a016410 https://doi.org/10.1101/cshperspect.a016410
14 Spies, M. (2017) A time for promiscuity in a eukaryotic recombinase. *J. Biol. Chem.* **292**, 11136–11137 https://doi.org/10.1074/jbc.H117.787614
15 Yu, X., Jacobs, S.A., West, S.C., Ogawa, T. and Egelman, E.H. (2001) Domain structure and dynamics in the helical filaments formed by RecA and Rad51 on DNA. *Proc. Natl Acad. Sci. U.S.A.* **98**, 8419–8424 https://doi.org/10.1073/pnas.111005398
16 Thompson, L.H. (2012) Recognition, signaling, and repair of DNA double-strand breaks produced by ionizing radiation in mammalian cells: the molecular choreography. *Mutat. Res.* **751**, 158–246 https://doi.org/10.1016/j.mrrev.2012.06.002
17 Gobbini, E., Cassani, C., Villa, M., Bonetti, D. and Longhese, M.P. (2016) Functions and regulation of the MRX complex at DNA double-strand breaks. *Microb. Cell* **3**, 329–337 https://doi.org/10.15698/mic2016.08.517
18 Lafrance-Vanasse, J., Williams, G.J. and Tainer, J.A. (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**, 182–193 https://doi.org/10.1016/j.pbiomolbio.2014.12.004
19 Hopfner, K.P. (2014) ATP puts the brake on DNA double-strand break repair: a new study shows that ATP switches the Mre11-Rad50-Nbs1 repair factor between signaling and processing of DNA ends. *Bioessays* **36**, 1170–1178 https://doi.org/10.1002/bies.201400102
20 Stracker, T.H. and Petrini, J.H. (2011) The MRE11 complex: starting from the ends. *Nat. Rev. Mol. Cell Biol.* **12**, 90–103 https://doi.org/10.1038/nrm3047
21 Lamarche, B.J., Orazio, N.I. and Weitzman, M.D. (2010) The MRN complex in double-strand break repair and telomere maintenance. *FEBS Lett.* **584**, 3682–3695 https://doi.org/10.1016/j.febslet.2010.07.029
22 Liu, Y., Sung, S., Kim, Y., Li, F., Gwon, G., Jo, A. et al. (2016) ATP-dependent DNA binding, unwinding, and resection by the Mre11/Rad50 complex. *EMBO J.* **35**, 743–758 https://doi.org/10.15252/embj.201592462
23 D'Amours, D. and Jackson, S.P. (2002) The Mre11 complex: at the crossroads of DNA repair and checkpoint signalling. *Nat. Rev. Mol. Cell Biol.* **3**, 317–327 https://doi.org/10.1038/nrm805
24 Truong, L.N., Li, Y., Shi, L.Z., Hwang, P.Y., He, J., Wang, H. et al. (2013) Microhomology-mediated end joining and homologous recombination share the initial end resection step to repair DNA double-strand breaks in mammalian cells. *Proc. Natl Acad. Sci. U.S.A.* **110**, 7720–7725 https://doi.org/10.1073/pnas.1213431110
25 Connelly, J.C. and Leach, D.R. (2002) Tethering on the brink: the evolutionarily conserved Mre11-Rad50 complex. *Trends Biochem. Sci.* **27**, 410–418 https://doi.org/10.1016/S0968-0004(02)02144-8
26 Connelly, J.C., Kirkham, L.A. and Leach, D.R. (1998) The SbcCD nuclease of *Escherichia coli* is a structural maintenance of chromosomes (SMC) family protein that cleaves hairpin DNA. *Proc. Natl Acad. Sci. U.S.A.* **95**, 7969–7974 https://doi.org/10.1073/pnas.95.14.7969
27 Hopfner, K.P. and Tainer, J.A. (2003) Rad50/SMC proteins and ABC transporters: unifying concepts from high-resolution structures. *Curr. Opin. Struct. Biol.* **13**, 249–255 https://doi.org/10.1016/S0959-440X(03)00037-X
28 Kinoshita, E., van der Linden, E., Sanchez, H. and Wyman, C. (2009) RAD50, an SMC family member with multiple roles in DNA break repair: how does ATP affect function? *Chromosome Res.* **17**, 277–288 https://doi.org/10.1007/s10577-008-9018-6
29 Rupnik, A., Lowndes, N.F. and Grenon, M. (2010) MRN and the race to the break. *Chromosoma* **119**, 115–135 https://doi.org/10.1007/s00412-009-0242-4

30 Lee, J.H. and Paull, T.T. (2007) Activation and regulation of ATM kinase activity in response to DNA double-strand breaks. *Oncogene* **26**, 7741–7748 https://doi.org/10.1038/sj.onc.1210872
31 Williams, R.S., Dodson, G.E., Limbo, O., Yamada, Y., Williams, J.S., Guenther, G. et al. (2009) Nbs1 flexibly tethers Ctp1 and Mre11-Rad50 to coordinate DNA double-strand break processing and repair. *Cell* **139**, 87–99 https://doi.org/10.1016/j.cell.2009.07.033
32 Lim, H.S., Kim, J.S., Park, Y.B., Gwon, G.H. and Cho, Y. (2011) Crystal structure of the Mre11-Rad50-ATPgammaS complex: understanding the interplay between Mre11 and Rad50. *Genes Dev.* **25**, 1091–1104 https://doi.org/10.1101/gad.2037811
33 Hopfner, K.P., Karcher, A., Craig, L., Woo, T.T., Carney, J.P. and Tainer, J.A. (2001) Structural biochemistry and interaction architecture of the DNA double-strand break repair Mre11 nuclease and Rad50-ATPase. *Cell* **105**, 473–485 https://doi.org/10.1016/S0092-8674(01)00335-X
34 Williams, R.S., Moncalian, G., Williams, J.S., Yamada, Y., Limbo, O., Shin, D.S. et al. (2008) Mre11 dimers coordinate DNA end bridging and nuclease processing in double-strand-break repair. *Cell* **135**, 97–109 https://doi.org/10.1016/j.cell.2008.08.017
35 Das, D., Moiani, D., Axelrod, H.L., Miller, M.D., McMullan, D., Jin, K.K. 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**, 647–663 https://doi.org/10.1016/j.jmb.2010.01.049
36 Shin, D.S., Pratt, A.J. and Tainer, J.A. (2014) Archaeal genome guardians give insights into eukaryotic DNA replication and damage response proteins. *Archaea* **2014**, 206735 https://doi.org/10.1155/2014/206735
37 Williams, G.J., Williams, R.S., Williams, J.S., Moncalian, G., Arvai, A.S., Limbo, O. et al. (2011) ABC ATPase signature helices in Rad50 link nucleotide state to Mre11 interface for DNA repair. *Nat. Struct. Mol. Biol.* **18**, 423–431 https://doi.org/10.1038/nsmb.2038
38 Hopfner, K.P., Craig, L., Moncalian, G., Zinkel, R.A., Usui, T., Owen, B.A. et al. (2002) The Rad50 zinc-hook is a structure joining Mre11 complexes in DNA recombination and repair. *Nature* **418**, 562–566 https://doi.org/10.1038/nature00922
39 Truebestein, L. and Leonard, T.A. (2016) Coiled-coils: the long and short of it. *Bioessays* **38**, 903–916 https://doi.org/10.1002/bies.201600062
40 Hirano, M. and Hirano, T. (2002) Hinge-mediated dimerization of SMC protein is essential for its dynamic interaction with DNA. *EMBO J.* **21**, 5733–5744 https://doi.org/10.1093/emboj/cdf575
41 Moreno-Herrero, F., de Jager, M., Dekker, N.H., Kanaar, R., Wyman, C. and Dekker, C. (2005) Mesoscale conformational changes in the DNA-repair complex Rad50/Mre11/Nbs1 upon binding DNA. *Nature* **437**, 440–443 https://doi.org/10.1038/nature03927
42 de Jager, M., Trujillo, K.M., Sung, P., Hopfner, K.P., Carney, J.P., Tainer, J.A. et al. (2004) Differential arrangements of conserved building blocks among homologs of the Rad50/Mre11 DNA repair protein complex. *J. Mol. Biol.* **339**, 937–949 https://doi.org/10.1016/j.jmb.2004.04.014
43 Zabolotnaya, E., Mela, I., Williamson, M.J., Bray, S.M., Yau, S.K., Papatziamou, D. et al. (2020) Modes of action of the archaeal Mre11/Rad50 DNA-repair complex revealed by fast-scan atomic force microscopy. *Proc. Natl Acad. Sci. U.S.A.* **117**, 14936–14947 https://doi.org/10.1073/pnas.1915598117
44 de Jager, M., van Noort, J., van Gent, D.C., Dekker, C., Kanaar, R. and Wyman, C. (2001) Human Rad50/Mre11 is a flexible complex that can tether DNA ends. *Mol. Cell* **8**, 1129–1135 https://doi.org/10.1016/S1097-2765(01)00381-1
45 van Noort, J. , van Der Heijden, T., de Jager, M., Wyman, C., Kanaar, R. and Dekker, C. (2003) The coiled-coil of the human Rad50 DNA repair protein contains specific segments of increased flexibility. *Proc. Natl Acad. Sci. U.S.A.* **100**, 7581–7586 https://doi.org/10.1073/pnas.1330706100
46 Uhlmann, F. (2016) SMC complexes: from DNA to chromosomes. *Nat. Rev. Mol. Cell Biol.* **17**, 399–412 https://doi.org/10.1038/nrm.2016.30
47 Yatskevich, S., Rhodes, J. and Nasmyth, K. (2019) Organization of chromosomal DNA by SMC complexes. *Annu. Rev. Genet.* **53**, 445–482 https://doi.org/10.1146/annurev-genet-112618-043633
48 Rose, A., Schraegle, S.J., Stahlberg, E.A. and Meier, I. (2005) Coiled-coil protein composition of 22 proteomes–differences and common themes in subcellular infrastructure and traffic control. *BMC Evol. Biol.* **5**, 66 https://doi.org/10.1186/1471-2148-5-66
49 Barfoot, T., Herdendorf, T.J., Behning, B.R., Stohr, B.A., Gao, Y., Kreuzer, K.N. and Nelson, S.W. (2015) Functional analysis of the bacteriophage T4 Rad50 homolog (gp46) coiled-coil domain. *J. Biol. Chem.* **290**, 23905–23915 https://doi.org/10.1074/jbc.M115.675132
50 Lammens, K., Bemeleit, D.J., Mockel, C., Clausing, E., Schele, A., Hartung, S. et al. (2011) The Mre11:Rad50 structure shows an ATP-dependent molecular clamp in DNA double-strand break repair. *Cell* **145**, 54–66 https://doi.org/10.1016/j.cell.2011.02.038
51 Mockel, C., Lammens, K., Schele, A. and Hopfner, K.P. (2012) ATP driven structural changes of the bacterial Mre11:Rad50 catalytic head complex. *Nucleic Acids Res.* **40**, 914–927 https://doi.org/10.1093/nar/gkr749
52 Wang, W., Daley, J.M., Kwon, Y., Krasner, D.S. and Sung, P. (2017) Plasticity of the Mre11-Rad50-Xrs2-Sae2 nuclease ensemble in the processing of DNA-bound obstacles. *Genes Dev.* **31**, 2331–2336 https://doi.org/10.1101/gad.307900.117
53 Anand, R., Ranjha, L., Cannavo, E. and Cejka, P. (2016) Phosphorylated CtIP functions as a co-factor of the MRE11-RAD50-NBS1 endonuclease in DNA end resection. *Mol. Cell* **64**, 940–950 https://doi.org/10.1016/j.molcel.2016.10.017
54 Andres, S.N. and Williams, R.S. (2017) CtIP/Ctp1/Sae2, molecular form fit for function. *DNA Repair* **56**, 109–117 https://doi.org/10.1016/j.dnarep.2017.06.013
55 Langerak, P., Mejia-Ramirez, E., Limbo, O. and Russell, P. (2011) Release of Ku and MRN from DNA ends by Mre11 nuclease activity and Ctp1 is required for homologous recombination repair of double-strand breaks. *PLoS Genet.* **7**, e1002271 https://doi.org/10.1371/journal.pgen.1002271
56 Reginato, G., Cannavo, E. and Cejka, P. (2017) Physiological protein blocks direct the Mre11-Rad50-Xrs2 and Sae2 nuclease complex to initiate DNA end resection. *Genes Dev.* **31**, 2325–2330 https://doi.org/10.1101/gad.308254.117
57 Gnugge, R. and Symington, L.S. (2017) Keeping it real: MRX-Sae2 clipping of natural substrates. *Genes Dev.* **31**, 2311–2312 https://doi.org/10.1101/gad.310771.117
58 Deshpande, R.A., Williams, G.J., Limbo, O., Williams, R.S., Kuhnlein, J., Lee, J.H. et al. (2014) ATP-driven Rad50 conformations regulate DNA tethering, end resection, and ATM checkpoint signaling. *EMBO J.* **33**, 482–500 https://doi.org/10.1002/embj.201386100
59 Herdendorf, T.J., Albrecht, D.W., Benkovic, S.J. and Nelson, S.W. (2011) Biochemical characterization of bacteriophage T4 Mre11-Rad50 complex. *J. Biol. Chem.* **286**, 2382–2392 https://doi.org/10.1074/jbc.M110.178871
60 Williams, G.J., Lees-Miller, S.P. and Tainer, J.A. (2010) Mre11-Rad50-Nbs1 conformations and the control of sensing, signaling, and effector responses at DNA double-strand breaks. *DNA Repair* **9**, 1299–1306 https://doi.org/10.1016/j.dnarep.2010.10.001
61 Terakawa, T., Bisht, S., Eeftens, J.M., Dekker, C., Haering, C.H. and Greene, E.C. (2017) The condensin complex is a mechanochemical motor that translocates along DNA. *Science* **358**, 672–676 https://doi.org/10.1126/science.aan6516

62 Kamada, K. and Barilla, D. (2018) Combing chromosomal DNA mediated by the SMC complex: structure and mechanisms. *Bioessays* **40** https://doi.org/10.1002/bies.201700166
63 Hassler, M., Shaltiel, I.A. and Haering, C.H. (2018) Towards a unified model of SMC complex function. *Curr. Biol.* **28**, R1266–R1281 https://doi.org/10.1016/j.cub.2018.08.034
64 Banigan, E.J., van den Berg, A.A., Brandao, H.B., Marko, J.F. and Mirny, L.A. (2020) Chromosome organization by one-sided and two-sided loop extrusion. *eLife* **9**, e53558 https://doi.org/10.7554/eLife.53558
65 Sedeno Cacciatore, A. and Rowland, B.D. (2019) Loop formation by SMC complexes: turning heads, bending elbows, and fixed anchors. *Curr. Opin. Genet. Dev.* **55**, 11–18 https://doi.org/10.1016/j.gde.2019.04.010
66 Banigan, E.J. and Mirny, L.A. (2020) Loop extrusion: theory meets single-molecule experiments. *Curr. Opin. Cell Biol.* **64**, 124–138 https://doi.org/10.1016/j.ceb.2020.04.011
67 Datta, S., Lecomte, L. and Haering, C.H. (2020) Structural insights into DNA loop extrusion by SMC protein complexes. *Curr. Opin. Struct. Biol.* **65**, 102–109 https://doi.org/10.1016/j.sbi.2020.06.009
68 Davidson, I.F. et al. (2019) DNA loop extrusion by human cohesin. *Science* **366**, 1338–1345 https://doi.org/10.1126/science.aaz3418
69 Ganji, M., Shaltiel, I.A., Bisht, S., Kim, E., Kalichava, A., Haering, C.H. and Dekker, C. (2018) Real-time imaging of DNA loop extrusion by condensin. *Science* **360**, 102–105 https://doi.org/10.1126/science.aar7831
70 Burmann, F., Lee, B.G., Than, T., Sinn, L., O'Reilly, F.J., Yatskevich, S. et al. (2019) A folded conformation of MukBEF and cohesin. *Nat. Struct. Mol. Biol.* **26**, 227–236 https://doi.org/10.1038/s41594-019-0196-z
71 Goloborodko, A., Imakaev, M.V., Marko, J.F. and Mirny, L. (2016) Compaction and segregation of sister chromatids via active loop extrusion. *eLife* **5**, e14864 https://doi.org/10.7554/eLife.14864
72 Mirny, L.A., Imakaev, M. and Abdennur, N. (2019) Two major mechanisms of chromosome organization. *Curr. Opin. Cell Biol.* **58**, 142–152 https://doi.org/10.1016/j.ceb.2019.05.001
73 Singleton, M.R., Dillingham, M.S. and Wigley, D.B. (2007) Structure and mechanism of helicases and nucleic acid translocases. *Annu. Rev. Biochem.* **76**, 23–50 https://doi.org/10.1146/annurev.biochem.76.052305.115300
74 Kong, M. et al. (2020) Human condensin I and II drive extensive ATP-dependent compaction of nucleosome-bound DNA. *Mol. Cell* **79**, 99–114 e119 https://doi.org/10.1016/j.molcel.2020.04.026
75 Golfier, S., Quail, T., Kimura, H. and Brugues, J. (2020) Cohesin and condensin extrude DNA loops in a cell cycle-dependent manner. *eLife* **9**, e53885 https://doi.org/10.7554/eLife.53885
76 Kim, Y., Shi, Z., Zhang, H., Finkelstein, I.J. and Yu, H. (2019) Human cohesin compacts DNA by loop extrusion. *Science* **366**, 1345–1349 https://doi.org/10.1126/science.aaz4475
77 Wang, X., Hughes, A.C., Brandao, H.B., Walker, B., Lierz, C., Cochran, J.C. et al. (2018) In vivo evidence for ATPase-dependent DNA translocation by the *Bacillus subtilis* SMC condensin complex. *Mol. Cell* **71**, 841–847 e845 https://doi.org/10.1016/j.molcel.2018.07.006
78 Soh, Y.M., Burmann, F., Shin, H.C., Oda, T., Jin, K.S., Toseland, C.P. et al. (2015) Molecular basis for SMC rod formation and its dissolution upon DNA binding. *Mol. Cell* **57**, 290–303 https://doi.org/10.1016/j.molcel.2014.11.023
79 Diebold-Durand, M.L., Lee, H., Ruiz Avila, L.B., Noh, H., Shin, H.C., Im, H. et al. (2017) Structure of full-length SMC and rearrangements required for chromosome organization. *Mol. Cell* **67**, 334–347 e335 https://doi.org/10.1016/j.molcel.2017.06.010
80 Burmann, F., Basfeld, A., Vazquez Nunez, R., Diebold-Durand, M.L., Wilhelm, L. and Gruber, S. (2017) Tuned SMC arms drive chromosomal loading of prokaryotic condensin. *Mol. Cell* **65**, 861–872 e869 https://doi.org/10.1016/j.molcel.2017.01.026
81 Minnen, A., Burmann, F., Wilhelm, L., Anchimiuk, A., Diebold-Durand, M.L. and Gruber, S. (2016) Control of Smc coiled coil architecture by the ATPase heads facilitates targeting to chromosomal parB/parS and release onto flanking DNA. *Cell Rep.* **14**, 2003–2016 https://doi.org/10.1016/j.celrep.2016.01.066
82 Hu, B., Itoh, T., Mishra, A., Katoh, Y., Chan, K.L., Upcher, W. (2011) ATP hydrolysis is required for relocating cohesin from sites occupied by its Scc2/4 loading complex. *Curr. Biol.* **21**, 12–24 https://doi.org/10.1016/j.cub.2010.12.004
83 Hinshaw, S.M., Makrantoni, V., Kerr, A., Marston, A.L. and Harrison, S.C. (2015) Structural evidence for Scc4-dependent localization of cohesin loading. *eLife* **4**, e06057 https://doi.org/10.7554/eLife.06057
84 Peterson, C.L. (1994) The SMC family: novel motor proteins for chromosome condensation? *Cell* **79**, 389–392 https://doi.org/10.1016/0092-8674(94)90247-X
85 Hirano, M. and Hirano, T. (2006) Opening closed arms: long-distance activation of SMC ATPase by hinge-DNA interactions. *Mol. Cell* **21**, 175–186 https://doi.org/10.1016/j.molcel.2005.11.026
86 Cannon, B., Kuhnlein, J., Yang, S.H., Cheng, A., Schindler, D., Stark, J.M. et al. (2013) Visualization of local DNA unwinding by Mre11/Rad50/Nbs1 using single-molecule FRET. *Proc. Natl Acad. Sci. U.S.A.* **110**, 18868–18873 https://doi.org/10.1073/pnas.1309816110
87 Hohl, M., Kochanczyk, T., Tous, C., Aguilera, A., Krezel, A. and Petrini, JH. (2015) Interdependence of the rad50 hook and globular domain functions. *Mol. Cell* **57**, 479–491 https://doi.org/10.1016/j.molcel.2014.12.018
88 Nolivos, S. and Sherratt, D. (2014) The bacterial chromosome: architecture and action of bacterial SMC and SMC-like complexes. *FEMS Microbiol. Rev.* **38**, 380–392 https://doi.org/10.1111/1574-6976.12045
89 Shintomi, K. and Hirano, T. (2007) How are cohesin rings opened and closed? *Trends Biochem. Sci.* **32**, 154–157 https://doi.org/10.1016/j.tibs.2007.02.002
90 Murayama, Y. and Uhlmann, F. (2015) DNA entry into and exit out of the cohesin ring by an interlocking gate mechanism. *Cell* **163**, 1628–1640 https://doi.org/10.1016/j.cell.2015.11.030
91 Griese, J.J., Witte, G. and Hopfner, K.P. (2010) Structure and DNA binding activity of the mouse condensin hinge domain highlight common and diverse features of SMC proteins. *Nucleic Acids Res.* **38**, 3454–3465 https://doi.org/10.1093/nar/gkq038
92 Kurze, A., Michie, K.A., Dixon, S.E., Mishra, A., Itoh, T., Khalid, S. et al. (2011) A positively charged channel within the Smc1/Smc3 hinge required for sister chromatid cohesion. *EMBO J.* **30**, 364–378 https://doi.org/10.1038/emboj.2010.315
93 Park, Y.B., Hohl, M., Padjasek, M., Jeong, E., Jin, K.S., Krezel, A. et al. (2017) Eukaryotic Rad50 functions as a rod-shaped dimer. *Nat. Struct. Mol. Biol.* **24**, 248–257 https://doi.org/10.1038/nsmb.3369

94 Kashammer, L., Saathoff, J.H., Lammens, K., Gut, F., Bartho, J., Alt, A. et al. (2019) Mechanism of DNA end sensing and processing by the Mre11-Rad50 complex. *Mol. Cell* **76**, 382–394 e386 https://doi.org/10.1016/j.molcel.2019.07.035

95 Rojowska, A., Lammens, K., Seifert, F.U., Direnberger, C., Feldmann, H. and Hopfner, K.P. (2014) Structure of the Rad50 DNA double-strand break repair protein in complex with DNA. *EMBO J.* **33**, 2847–2859 https://doi.org/10.15252/embj.201488889

96 Seifert, F.U., Lammens, K., Stoehr, G., Kessler, B. and Hopfner, K.P. (2016) Structural mechanism of ATP-dependent DNA binding and DNA end bridging by eukaryotic Rad50. *EMBO J.* **35**, 759–772 https://doi.org/10.15252/embj.201592934

97 Wiltzius, J.J., Hohl, M., Fleming, J.C. and Petrini, J.H. (2005) The Rad50 hook domain is a critical determinant of Mre11 complex functions. *Nat. Struct. Mol. Biol.* **12**, 403–407 https://doi.org/10.1038/nsmb928

98 He, J., Shi, L.Z., Truong, L.N., Lu, C.S., Razavian, N., Li, Y. 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**, 31747–31756 https://doi.org/10.1074/jbc.M112.384750

99 Tatebe, H., Lim, C.T., Konno, H., Shiozaki, K., Shinohara, A., Uchihashi, T. and Furukohri, A. (2020) Rad50 zinc hook functions as a constitutive dimerization module interchangeable with SMC hinge. *Nat. Commun.* **11**, 370 https://doi.org/10.1038/s41467-019-14025-0

100 Myler, L.R., Gallardo, I.F., Soniat, M.M., Deshpande, R.A., Gonzalez, X.B., Kim, Y. et al. (2017) Single-molecule imaging reveals How Mre11-Rad50-Nbs1 initiates DNA break repair. *Mol. Cell* **67**, 891–898 e894 https://doi.org/10.1016/j.molcel.2017.08.002

101 Gruber, S. (2018) SMC complexes sweeping through the chromosome: going with the flow and against the tide. *Current Opin. Microbiol.* **42**, 96–103 https://doi.org/10.1016/j.mib.2017.10.004

102. Lee, J.H., Mand, M.R., Deshpande, R.A., Kinoshita, E., Yang, S.H., Wyman, C. and Paull, T.T. (2013) Ataxia telangiectasia-mutated (ATM) kinase activity is regulated by ATP-driven conformational changes in the Mre11/Rad50/Nbs1 (MRN) complex. *J. Biol. Chem.* **288**, 12840–12851 https://doi.org/10.1074/jbc.M113.460378

103 Yoshimura, S.H., Hizume, K., Murakami, A., Sutani, T., Takeyasu, K. and Yanagida, M. (2002) Condensin architecture and interaction with DNA: regulatory non-SMC subunits bind to the head of SMC heterodimer. *Curr. Biol.* **12**, 508–513 https://doi.org/10.1016/S0960-9822(02)00719-4

104 Haering, C.H., Lowe, J., Hochwagen, A. and Nasmyth, K. (2002) Molecular architecture of SMC proteins and the yeast cohesin complex. *Mol. Cell* **9**, 773–788 https://doi.org/10.1016/S1097-2765(02)00515-4

105 Symington, L.S. (2014) End resection at double-strand breaks: mechanism and regulation. *Cold Spring Harb. Perspect. Biol.* **6**, a016436 https://doi.org/10.1101/cshperspect.a016436