ABC ATPase signature helices in Rad50 link nucleotide state to Mre11 interface for DNA repair

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The Mre11–Rad50 complex, or Mre11–Rad50–Nbs1 (MRN) in higher eukaryotes, coordinates detection, signaling and repair of cytotoxic and mutagenic DNA double-strand breaks (DSBs). Mre11 3′–5′ exonuclease and single-stranded DNA (ssDNA) endonuclease activities are regulated by Rad50 ATP binding and hydrolysis within the MRN complex.1,2 Combined structural, biochemical and cell biology results show MRN serves as a DNA damage sensor, an enzymatic effector and a transducer of cell-cycle checkpoint signals for DNA double-strand break repair (DSBR).3,4 MRN tumor-suppressor functions are crucial. NBS1 mutations cause the radiosensitive and chromosome-instability disorder Nijmegen breakage syndrome (NBS)5, MRE11 mutations cause ataxia telangiectasia-like disorder and RAD50 mutations result in an NBS-like syndrome.6 Other MRE11 variants (including L473F) are linked to colorectal cancer.7 Despite this, the molecular basis for such defects remain undefined.

The MRN complex has critical DNA end-bridging and ATP-regulated endonucleolytic actions that initiate homologous recombination repair of DSBs8,9, yet high-resolution structures of the Mre11–Rad50 complex and its critical interfaces have eluded characterization. Crystal structures have shown that an 80-kDa Mre11 dimer can directly bridge DNA ends,9 have characterized the Rad50 ABC–ATPase monomer with and without adjacent coiled-coil regions10,11 and have defined a nucleotide-bound Rad50 dimer lacking coiled-coil regions (see Supplementary Fig. 1). Hints on the structure of quaternary assembly have also come from EM of Mre11–Rad50 that has revealed an ~100-Å diameter, four-lobed Mre11–Rad50 head (M2R2 head) with ~500-Å-long Rad50 coiled-coil protrusions.9,10,12,13 However, no Mre11–Rad50 co-complex structures have been found in either nucleotide-bound or free states, so how Mre11 is physically linked to Rad50, how Rad50 subunits assemble within the M2R2 head and how nucleotide binding to the ABC–ATPase may regulate Mre11–Rad50 structure and functions remain mysteries.3

Here we use Pyrococcus furiosus proteins and Schizosaccharomyces pombe genetics to define the key, conserved Mre11–Rad50 interface, the molecular basis of the interaction and the importance of this interface for DSBR in vivo. To elucidate Rad50 conformational changes that would affect Mre11, we solved four new structures of Rad50 containing critical coiled-coil regions in complex with either the Mre11–Rad50 binding domain (RBD), AMP:PNP, or both. Our new combined structural and mutational results define the ABC–ATPase signature helix motif and key basic-switch residues that drive and coordinate Rad50 domain rotations by toggling between specific, distinct salt-bridge networks. Our collective results help explain defects in cancer-linked Mre11 mutations and identify an underlying molecular basis, conserved across the ABC–ATPase superfamily, for coupling the ATPase nucleotide state to biological outcomes through conformational changes that affect interfaces and attached functional domains.

RESULTS
The Rad50 binding domain of Mre11
To map the Mre11 RBD, we generated a series of P. furiosus Mre11 (pMre11) deletion constructs and tested their ability...
to coexpress and copurify, using coiled-coil truncated pRad50 constructs (Fig 1a,b). Mre11 C-terminal truncations, which left the N-terminal core nuclease domain intact, revealed that the 342–379 region contained residues essential for binding His-tagged pRad50 (pRad50-NC). A C-terminal Mre11 construct (residues 348–426) also bound Rad50 (Fig 1b). To finely map the Mre11 RBD, we expressed and copurified Mre11–Rad50 complexes of untagged Minimal Mre11 polypeptide (Mre11(RBD)), bound tightly to Rad50 variants (I–V) shown in Fig. 1a. Bottom, His6-tagged Mre11 variants (V–VIII) were coexpressed with untagged pRad50-link1. Minimal Mre11 polypeptide (Mre11(RBD)) interacts with Rad50-link1. MW, molecular weight. (c) Sequence alignment of the linker region connecting the Mre11 RBD to the nuclease-capping domain in pfMre11, S. cerevisiae (scMre11), S. pombe (spMre11), Xenopus laevis (xlMre11) and human (hsMre11). Shaded regions show well-conserved residues. Disordered residues are shown in red, as seen in pfMre11 crystal structures or as predicted by Disopred2. (d) Mre11(RBD)–Rad50 interface, shown in orthogonal stereo views. The hydrophobic Mre11(RBD)–Rad50 interaction core is augmented by four flanking complementary salt-bridge interactions, with acidic residues from Mre11 RBD interacting with four positively charged Rad50 surface residues. (e) Superimposition of two nucleotide-free Mre11(RBD)–Rad50 crystal forms. The core Mre11(RBD)–Rad50 interface is maintained, but an ~45° rotation about the base of the Rad50 coiled coil identifies a flexible linkage to Rad50 ATPase domains. Residues equivalent to those mutated in rad50 yeast phenotypes are shown in space-fill representations.

Figure 1 The Mre11(RBD)–Rad50 interface. (a) pRad50 and pfMre11 constructs schematics for domain mapping and crystallizations. pRad50-link constructs contain Gly-Ser repeat sequences to intramolecularly link Rad50 N and C lobes. (b) Mapping of Mre11 RBD. Top, His6-tagged Rad50 was coexpressed with Mre11 variants (I–V) shown in a. Bottom, His6-tagged Mre11 variants (V–VIII) were coexpressed with untagged pRad50-link1. Minimal pfMre11 polypeptide (Mre11(RBD)), residues 348–381 (Mre11(N)) and a corresponding Rad50 binding site within the first Rad50 coiled coil (~6 heptad repeats, proximal to the ATPase core. The four-helix architecture of the Mre11–Rad50 interface To define the Mre11–Rad50 interface, we solved two independent X-ray crystal structures of pfMre11(RBD) bound to pRad50-NC (Table 1), to 2.1-Å and 3.4-Å resolutions, which reveal the same interface. Our 2.1-Å structure provides high-resolution details about this interface (Fig 1). The Mre11 RBD consists of two helices (RBD-α and RBD-β), named sequentially from nuclease core labeling15) that interact with the Rad50 coiled-coil base through a conserved hydrophobic surface patch. This four-helix interaction differs from classical four-helix bundle interfaces, such as in human manganese superoxide dismutase14 and typical coiled-coil packing such as in bacterial pilI15,16, as Mre11 helices pack almost orthogonally to the two Rad50 coiled-coil helices. The Mre11(RBD)–Rad50 interface includes 72% of the 32 Mre11(RBD) residues and has an ~970-Å² buried surface area. Ten Mre11(RBD) hydrophobic core residues account for 75% of the total buried surface area, and this strong interface spans ~20 Å across and ~30 Å up the Rad50 coiled coils. The conserved interface in human MRE11–RAD50 probably involves MRE11 RBD residues Glu345–Lys475 and RAD50 coiled-coil regions around Arg184–Lys204 and Lys1098–Asp1129.

Mre11 is flexibly linked to the Rad50 coiled coils Our sequence alignments and Disopred2 (ref. 17) disorder predic-

TABLE 1

| Construct | Description | MW (kDa) |
|-----------|-------------|----------|
| pfRad50-NC | N-terminal | 20 |
| pfRad50-link1 | N-terminal | 20 |
| Mre11(RBD) | C-terminal | 20 |

Note: The table lists constructs used in the study, with their molecular weights (MW) in kilodaltons (kDa).
Mre11 nuclease coordinates$^{9,10}$ to residue 348 of our interface structure have high sequence divergence, suggesting intrinsic disorder (Fig. 1c). In fact, residues 334–342 were present and disordered in our previous structures (PDB codes 3DSC, 3DSD, 1II7)$^{9,10}$, but we missed the significance of this observation. Our past and present results thus reveal that a flexible tether links the Mre11 nuclease and RBD domains.

Comparison of Mre11$^{150}$–Rad50 structures from different crystal forms furthermore reveals that the Rad50 coiled coils can adopt dramatically variable orientations relative to the ATPase domains in the nucleotide free form (Fig. 2). These changes are highlighted by core Mre11$^{150}$–Rad50 superimpositions; this region superimposes well, but the Rad50 ATPase domain can rotate substantially with respect to the coiled coils. These domain motions uncover intrinsic flexibility in the hinge region at the base of the coiled-coil N-terminal α-helix, which is adjacent to the Mre11$^{150}$–Rad50 interface. In our structures, this region has limited contacts with the N-terminal half (N lobe) of the Rad50 ATPase domain and adopts dramatically different conformations, imparting a 30° twist and 15 Å shift relative to the coiled coils. Conversely, the base of the C-terminal helix of the coiled coil is rigid, and it makes extensive contacts with the C-terminal half (C lobe) of the Rad50 ATPase core.

### Interface mutants disrupt Mre11–Rad50 interactions in vivo

On the basis of sequence alignments of Mre11 orthologs (Fig. 2a), mutations were introduced into S. pombe Mre11 (also known as Rad32) to test the functional role of the Mre11 RBD. Hydrophobic Cys-Leu (CL) residues in RBD-αI and Cys-Val (CV) residues in RBD-αJ were changed to charged Arg-Arg (RR) residues, either separately or in combination. Collectively, these residues at the Mre11 RBD core mediate hydrophobic interactions between the Rad50 coiled-coil α-helices and also between RBD-αI and RBD-αJ. Their substitution to charged arginine residues thus should disrupt the interface. Indeed, two-hybrid analyses showed that Mre11 and Rad50 have a robust interaction, but this was severely diminished by the RR mutation in either RBD-αI or RBD-αJ (Fig. 2b). Importantly, our Mre11 RBD mutants did not impair Mre11 homodimeric or Nbs1 interactions (Fig. 2b), indicating that the RBD mutant phenotypes (see below) are the consequences of specific disruption of the Mre11–Rad50 interface.

### Mre11–Rad50 interface critical for double-stranded break repair

To test whether the Mre11 RBD mutants show increased DNA damage sensitivity, we examined responses to four genotoxins: ionizing radiation, which directly makes DSBs; UV light, which creates DNA photoproducts that can be processed into DSBs; camptothecin (CPT), a topoisomerase inhibitor that causes replication fork breakage when the replisome encounters a topoisomerase–CPT complex; and hydroxyurea, which stalls replication forks by inhibiting ribonucleotide reductase required for dNTP synthesis. The mre11 alleles replace genomic mre11 (mre11-WT) and encode a C-terminal Myc tag. These strains were compared to mre11Δ and Myc-tagged mre11-WT control strains. This Myc tag does not noticeably impair Mre11 function$^{9}$. Immunoblotting showed that the Mre11 RBD mutants were expressed at levels comparable to the wild type (Fig. 3a).

In agreement with their poor abilities to interact with Rad50 in two-hybrid assays (Fig. 2b), the Mre11 RBD-αI (mre11-CL454RR) and RBD-αJ (mre11-CV479RR) mutants resembled mre11Δ in being very sensitive to ionizing radiation, UV, CPT and hydroxyurea (Fig. 3b,c). These mutants also formed smaller colonies than the wild type, indicating defects in repair of spontaneous DNA damage. Serial dilution assays done with UV, hydroxyurea or CPT show that the Mre11 RBD mutants are slightly more resistant than mre11Δ cells (Fig. 3b). This small difference might be because the Mre11 RBD-αI and RBD-αJ mutants retain residual interactions with Rad50 that were not detected by yeast two-hybrid analysis; thus, we did survival assays on an mre11$^{150}$–RRRR allele that should completely disrupt the interface. This allele appeared identical to the mre11-CL454RR and mre11-CV479RR alleles in serial dilution assays (Fig. 3b), and in ionizing radiation survival assays the mre11-RRRR strain is slightly more resistant than mre11Δ (Fig. 3c and Supplementary Fig. 2). Collectively, these data show that the Mre11 RBD forms an interface that is critical for DSBR, although weak function is maintained when it is mutated.

### Exol can compensate Mre11–Rad50 interface mutant phenotypes

To determine whether Mre11 interface mutants impact DNA end processing in fission yeast, we tested whether exonuclease I (Exol) can compensate for Mre11. In the ionizing radiation survival assays, the mre11-RRRR strain is slightly more sensitive than the previously
characterized mre11-H134S mutant (Fig. 3c). Genetic and biochemical studies indicate that the mre11-H134S genotoxin sensitivity is caused by a defect in ssDNA endonuclease activity that is suppressed by inactivating the Ku70–Ku80 complex, which can bind and block ends. This rescue requires Exo1, indicating that Mre11 endonuclease activity is critical for generating single-strand overhangs that are competent for homologous recombination repair. To assess whether the mre11-RRRR mutant is defective in DNA end processing, we created mre11-RRRR strains lacking Ku80, Exo1 or both. We found that the pks80A mutation suppressed the slow-growth phenotype as well as the ionizing radiation, CPT, UV and hydroxyurea sensitivities of mre11-RRRR cells (Fig. 3d). This supports a model in which Ku promotes nonhomologous end joining by binding to DSB ends, and inhibits Exo1-dependent resection. Accordingly, the extreme genotoxin sensitivity of the mre11-RRRR pks80A exo1Δ strain showed that this suppression was dependent on Exo1 activity. Indeed, the exo1Δ mutation substantially exacerbated the clastogen sensitivity of the mre11-RRRR mutant. These results indicate that the mre11-RRRR phenotypes are primarily caused by an inability of Mre11–Rad50 to process DNA ends for DSB repair by homologous recombination.

Architecture of the Mre11–Rad50 head
To test the Mre11RBD–Rad50 complex flexibility implied from crystal structures, we examined M₁-R₂ head solution conformations with small-angle X-ray scattering (SAXS). SAXS combined with crystal structure restraints can accurately define flexible conformations and ensembles in solution, and can also identify existing structures that most closely match the measured experimental scattering. Experimental SAXS curves of M₁-R₂ head preparations (Fig. 4a) show dramatic scattering curve changes, supporting a flexible-to-more-ordered transition: from featureless without ATP (−ATP) to defined peaks and troughs with ATP (+ATP). Further, the radius of gyration decreases from 46.5 to 41.0 Å upon ATP binding, dimensions resembling M₁-R₂ head regions within intact pMre11–Rad50-EM images. These results, along with a compaction observed in the pair distribution p(r) plot (Supplementary Fig. 3), show that the M₁-R₂ head transitions from a more open to a compacted state upon ATP binding. This supports our hypothesis that ATP binding in the M₁-R₂ head leads to Rad50 dimerization, which would close the M₁-R₂ head to form a globular, toroidal structure.

To model the conformational flexibility implied by the −ATP data by the featureless curve and overall architecture of the M₁-R₂ head in the absence and presence of ATP, we used molecular dynamics (MD) and minimal ensemble searches (MES) to find M₁-R₂ head structural models that best fit the data. We find the predominant M₁-R₂ head architecture without ATP is a partially open state; yet, improved fit to the data by a mixture of open, partially open and closed conformations shows the inherent flexibility of the complex without nucleotide (Fig. 4a). With ATP we expected to see mixed ATP-bound and free states in solution. So, to accurately model M₁-R₂ head complex with ATP, we used MES with closed ATP-bound M₁-R₂ head models, based on our crystal structures described below, combined with models identified for the −ATP data (Fig. 4a). The results suggest that 89% of the M₁-R₂ head is in a closed state, with the M₁ dimer and ATP-induced Rad50 forming a toroidal, globular structure.

Signature helices couple nucleotide state to domain movements
To test whether this Mre11RBD–Rad50 interface is affected upon nucleotide binding in the Rad50 ABC–ATPase core, we used our Rad50 constructs that intramolecularily link the ABC–ATPase N and C lobes with adjacent coiled-coil regions (Fig. 1a); these constructs facilitated ternary structure solution of Mre11RBD–Rad50-link1–AMP:PNP–Mg²⁺ to 3.3 Å (Fig. 4b, c) and Rad50-link2–AMP:PNP–Mg²⁺ to 1.9 Å resolution (Table 1). Superposition of our nucleotide-free and
nucleotide-bound structures of Rad50 containing coiled-coil regions and morphing between crystallographically defined states reveals both global conformational changes (Supplementary Movies 1–3) and their underlying basis (Fig. 3). As Rad50 AMP:PNP structures with and without bound Mre11RBD superimpose well, we used the higher resolution structure for most analyses.

Our Mre11RBD–Rad50-link1–AMP:PNP–Mg2+ structure defines the nucleotide-bound state of the unknown half of the M1/R2 head, with molecular dimensions of 120 × 74 × 62 Å. Globally, the Rad50 ABC–ATPase core dimerizes with AMP:PNP–Mg2+ sandwiched at the crystallographic two-fold interface (Fig. 4b), inducing an ~35° rotation of the C lobe relative to the N lobe, supporting and extending changes proposed from core structures lacking all coiled-coil regions11. However, our new nucleotide-bound structures reveal new positions of two helices, which we term the signature-coupling helices, immediately C-terminal to the Rad50 Q loop. These helices, which are absent from the original nucleotide-bound Rad50 structure, connect the Q loop to the base of the N-terminal helix of the coiled coil. Upon nucleotide binding, Rad50 N-lobe rotation drives a π-helix element (π-helix wedge) between the signature-coupling helices to splay them apart (Fig. 5a–c and Supplementary Fig. 4). The movement of the signature-coupling helices resembles the opening of an arm at the elbow and acts as a lever, exerting force on the base of the N-terminal coiled-coil helix, which our nucleotide-free structures show is a point of flexion. This force repositions the coiled coils with respect to the ATPase core, affecting the Mre11 RBD position. As shown by structure-based animation (Supplementary Movie 3), the ATP-driven domain rotation is transduced to an ~30 Å linear pull on the Mre11 linker by the Rad50 coiled-coil movement at the Mre11 interaction interface (Fig. 5d).

Basic switches and alternating salt bridges control ATPase rotations

Underlying the global conformational changes described above is an extensive network of >20 charge pairs that switch upon nucleotide binding (Fig. 5b). These changes provide a mechanism to physically couple ATPase conformational rotation to coiled-coil and attached Mre11 RBD repositioning. Basic-switch residues (Arg797 and Arg805 in pRad50) immediately adjacent to the conserved signature motif, which defines the ABC–ATPases superfamily, occupy a conserved helix. We term this the signature helix, as it encodes a molecular conformational switch that links signature-motif nucleotide recognition to subdomain rotation.

Arg797 hydrogen-bonds to main chain signature motif atoms in the nucleotide-free state. Upon nucleotide binding, the signature motif moves to contact the nucleotide. As a consequence, Arg797 detaches from the signature motif and moves to form interaction networks with Glu148 and Asp144 on signature-coupling helix-α1 (Fig. 5c and Supplementary Fig. 4). These new Arg797 interactions can only form after opening and translation of the signature-coupling helices has occurred following N-lobe rotation, and these interactions limit further rotation of the first helix. Arg797 movements thus directly link nucleotide recognition by the signature motif to movements of the signature-coupling helices that control coiled-coil positioning.

Signature helix Arg805 integrates the signature motif Q loop and domain rotations. Nucleotide binding breaks Arg805 hydrogen bonds to the Asn134 main chain. Arg805 then moves toward the protein surface with concomitant rearrangements of the Q loop. Arg805 rotates into the signature-coupling helices and guides the π-helix to wedge open signature-coupling helix-α1 and form new hydrogen bonds to Gln142 and Ile143 main chain carboxyl atoms (Fig. 5c). This switch at the junction between the N lobe, C lobe and coiled-coil base implicates Arg805 as a key coordinator of Rad50–ATPase lobe rotation and its coupling to coiled-coil rearrangements.

Rad50 basic switches are critical for DSBR in fission yeast

Rad50 ortholog sequence alignments reveal the conservation of basic residues corresponding to pRad50 Arg797 and Arg805 (Fig. 6a). To test the functional role of these basic-switch residues for DSBR in vivo, we made K1187A, K1187E (pRad50 Arg797 equivalent), R1195A and R1195E (pRad50 Arg805 equivalent) mutations in S. pombe Rad50. The rad50 alleles replace genomic rad50 (rad50-WT) and encode a TAP tag. These strains were compared to rad50Δ and TAP-tagged rad50-WT control strains. This TAP tag does not noticeably impair Rad50 function, and immunoblotting showed that the Rad50 basic-switch mutants were expressed at wild-type levels, with the exception of R1195A, which has reduced expression (Fig. 6b).

To test whether the Rad50 basic-switch mutants show increased DNA-damage sensitivity, we examined their responses to genotoxins. Serial dilution assays show that Rad50 basic-switch mutants are more sensitive to the clastogen agents than the rad50-WT control strain (Fig. 6b). The K1187A variant phenotype is seen mainly with higher doses of clastogens. In contrast, the K1187E, R1195A and R1195E variants are unusually sensitive to clastogen agents and are as deleterious as rad50Δ. These assays thus reveal the importance of Rad50 signature helix basic-switch residues for DSBR in vivo.
DISCUSSION

Implications for Mre11–Rad50 functions

Long-range allostery in Mre11–Rad50 is implied by coupling Rad50 nuclease states to differential impacts on Mre11 endonuclease and exonuclease activities\(^1\), Rad50 Zn-hook region mutations that disrupt binding to Mre11 over a distance of ~500 Å\(^2\) and DNA binding at the M\(_2\)R\(_2\) head that straightens Rad50 coiled coils\(^3,23\). This allostery has been enigmatic, but the results presented here illuminate a chemomechanical conduit coupling the Rad50 state to Mre11–DNA interactions. Combined crystal structures and SAXS solution results show the Mre11–Rad50 complex undergoes open-to-closed conformational changes upon ATP binding, appropriate to load Mre11–DNA complexes on to DNA but also be transmitted by means of the Mre11 RBD interface to effect positioning of Mre11 nuclease-capping domains, to regulate DNA access and nuclease activities at the active site. Nucleotide-induced dimerization of Rad50 ABC–ATPase within Rad50 coiled-coil base. Right, schematic of the structure. (c) Orthogonal views of the complex as in b. See Table 1 for data processing and refinement statistics.

Similarly, ATP-driven movement in Rad50 could not only control Mre11–Rad50 loading onto DNA but also be transmitted by means of the Mre11 RBD interface to effect positioning of Mre11 nuclease-capping domains, to regulate DNA access and nuclease activities at the active site. Nucleotide-induced dimerization of Rad50 ABC–ATPase within the M\(_2\)R\(_2\) head has key architectural consequences (Figs. 4 and 5 and Supplementary Movies 1–3): the M\(_2\)R\(_2\) head loses degrees of freedom, moving from a conformationally flexible, open state to a closed, toroidal architecture, and nucleotide binding fixes subdomain rotation within each Rad50 ABC–ATPase, resulting in Rad50 coiled-coil and attached Mre11-RBD repositioning, which pulls on the nuclease-capping domain (Fig. 5d and Supplementary Fig. 5). Saccharomyces cerevisiae mutations resulting in rad50s phenotypes, which include persistent DNA-damage signaling and defects in processing covalent protein–DNA adducts,24 map primarily to the Rad50 N-lobe surface both distal from and—in the nucleotide-free state—flexible with respect to the Mre11 RBD (Fig. 1e). Our results show that these sites undergo flexible-to-fixed conformational switching upon nucleotide binding, suggesting that rad50s mutations either affect this locked conformation or disrupt transmission of conformational changes to partners.

The Mre11–Rad50 mechanical linkage provides an elegant mechanism to couple Rad50 nuclease states to Mre11 endonuclease and exonuclease activities, and helps explain results from the S. cerevisiae mre11-6 allele. mre11-6 lacks linkage between the Mre11 nuclease-capping domain and RBD through the deletion of the RL6 DNA-binding loop, yet retains the RBD and Rad50 binding\(^24\). Notably, mre11-6 affects Mre11–Rad50 activities in vivo and in vitro, as indicated by deficiencies in meiotic DSB processing, moderate sensitivity to the DNA alkylating agent MMS, and decreases in DNA binding, 3’–5’ exonuclease and ssDNA exonuclease activities. The flexible Mre11 nuclease-capping domain connection to the RBD thus appears critical for multiple Mre11–Rad50 catalytic and DNA-binding functions. Notably, this key Mre11–Rad50 linkage is evidently impaired in human cancers. Both a truncation between the Mre11 RBD-c
Implications for the ABC–ATPase superfamily

ABC–ATPases function in DNA repair and chromosomesegregation through Rad50, MutS and SMC proteins, and in ABC transporters, where nucleotide binding regulates transmembrane domain opening and closing to control cellular import and export.\(^5\) ABC–ATPase superfamily members are also associated with human disease and bacterial pathogenicity, and are thus of extreme biomedical interest.\(^6\) For example, dysfunction of the cystic fibrosis transmembrane conductance regulator (CFTR) results in cystic fibrosis, a glycoprotein ABC transporter acts in multidrug resistance of cancer cells, and inherited mutations in MRN or MutS result in cancer-predisposition diseases.

All ABC–ATPase machines have a very similar heterotetrameric assembly, composed of attached ABC–ATPase and substrate-or function-specific dimers. Rad50 structures have provided prototypical information on the ABC–ATPase superfamily by revealing that the signature motif acts in trans across the dimer.\(^11\) However, an unanswered fundamental scientific question has been how ABC–ATPases communicate nucleotide binding and ligand states across long distances and among protein partners to effect diverse functions. We show here that nucleotide sensing by the signature motif in Rad50 is connected to the ABC–ATPase subdomain rotation through basic-switch residues encoded on the adjacent \(\alpha\)-helical subdomain, equivalent to the Rad50 C lobe, rotating \(\sim15^\circ\) with respect to a region topologically equivalent to the Mre11–Rad50 interface and \(\alpha\)-helix, which we call the signature helix. In ABC transporters, similar movements occur between nucleotide states with the \(\alpha\)-helix, which we call the signature helix. In ABC transporters, similar movements occur between nucleotide states with the \(\alpha\)-helix, which we call the signature helix. In ABC transporters, similar movements occur between nucleotide states with the \(\alpha\)-helix, which we call the signature helix. In ABC transporters, similar movements occur between nucleotide states with the \(\alpha\)-helix, which we call the signature helix.
exporter SAV1866 (ref. 30) and a metal-chelate-type transporter31, also reveal critical conserved features of the ABC–ATPase domain that are important for propagating the subdomain rotation to conformational changes in the divergent functional domain. An α-helix from the transmembrane domain that inserts into the interface of the ATPase lobes is topologically equivalent to the Rad50 signature-coupling helix-02 (Fig. 7). In ABC transporters, this transmission helix is important for the concerted conformational changes that occur within the intact transporter between different states, undergoing a rotation and translation upon the rotation of the ABC–ATPase domain (also called the nucleotide-binding domain)29. In contrast to ABC transporters, the Rad50 ABC–ATPase subdomain rotation is much larger, ~35° rather than ~15°. Although this results in a similar rotation and translation of the transmission interface equivalent helix, its transmission to the attached functional domain Mre11 is indirectly propagated through repositioning of the coiled coils.

Direct observation and quantification of Rad50 structures here show the importance of Rad50 basic switches for DSBR and their conservation in ABC transporters. Yet, convergence has probably resulted in adaptations that couple conformational changes to diverse functional domains. For example, in CFTR, there is an essential role for signature helix Arg555, which falls between the basic switches identified here, in coordinating dimer interactions during ATP-driven conformational changes32. Also, mutation of CFTR Arg1303, present on the signature helix of the second ABC–ATPase domain but mapping past pF550 Rad50 Arg805, results in misregulation of ATP-mediated allostery; promoting spontaneous, ATP-independent opening of CFTR33.

Collectively, our results redefine a structurally and functionally relevant extended ABC–ATPase superfamily signature motif (Fig. 6a). This newly discovered loop-helix motif encodes the means for propagating the subdomain rotation and translation upon the rotation of the ABC–ATPase domain (also called the nucleotide-binding domain)29. In contrast to ABC transporters, the Rad50 ABC–ATPase subdomain rotation is much larger, ~35° rather than ~15°. Although this results in a similar rotation and translation of the transmission interface equivalent helix, its transmission to the attached functional domain Mre11 is indirectly propagated through repositioning of the coiled coils.

Figure 7 Topologically equivalent signature helices connect nucleotide binding to conformational changes in the ABC–ATPase superfamily. Rad50 ABC–ATPase molecular surface with attached coiled coil and mapped Mre11 RBD compared to MalF ABC–ATPase with interacting MalF transmembrane protein. The extended signature helix (purple) and signature-coupling helices or helix (cyan) connect nucleotide binding to movements of attached functional domains and proteins.
METHODS

Methods and any associated references are available in the online version of the paper at http://www.nature.com/nsmb/.

Accession codes. Protein Data Bank: Coordinates and structure factors for Mre11Rad50 crystal form 1 (3QKS), Mre11–Rad50 crystal form 2 (3QKR), Mre11Rad50–link1–AMP:PNP–Mg²⁺ (3QKU) and Rad50-link2–AMP:PNP–Mg²⁺ (3QKT) have been deposited under the accession codes indicated in parentheses.

Note: Supplementary information is available on the Nature Structural & Molecular Biology website.

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AUTHOR CONTRIBUTIONS

G.J.W. analyzed results, did SAXS experiments and wrote the manuscript. J.S.W. and O.L. did S. pombe experiments and analysis. G.M. and R.S.W. solved crystal structures. A.S.A. refined structures. S.S. and G.G. purified proteins. M.H. assisted and O.L. did collection assistance. A.S.A. refined structures. S.S. and G.G. purified proteins. M.H. assisted and O.L. did collection assistance.

COMPETING FINANCIAL INTERESTS

The authors declare no competing financial interests.

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ONLINE METHODS

Protein expression and purification. Recombinant pfMre11 constructs I–V (Fig. 1a) were coexpressed and copurified with pfRad50-NC as previously described. The His6-tagged Mre11RBD variants (constructs VI–VIII, Fig. 1a) were cloned with a thrombin-cleavable His6 tag and expressed from pET15b. Untagged intramolecularly linked Rad50 constructs were made by PCR to introduce a Gly–Gly–Ser–Gly–Gly bridge between pfRad50 residues Lys177 and Thr726 (pfRad50-link2) or between residues Tyr187 and Ile716 (pfRad50-link1). His6–Mre11RBD constructs (VI–VIII) were coexpressed with either pfRad50-link1 or pfRad50-link2 in Escherichia coli Rosetta2 (DE3) cells (Novagen) grown in Terrific Broth plus 0.4% (v/v) glycerol and induced with IPTG at 16 °C overnight.

For coexpressed constructs, copurification of thermostable pfMre11–Rad50 complexes was achieved by sequential Ni-affinity chromatography, heat denaturation of E. coli proteins at 65 °C, Superdex 200 gel filtration and cation-exchange chromatography. For crystallization, the N-terminal His6 tag of pfMre11RBD (construct VI, Mre11 residues 348–381) was removed by thrombin digestion. Untagged pfRad50-link2 was purified by heat denaturation of pfRad50 constructs at 65 °C, Superdex 200 gel filtration and cation-exchange chromatography. Proteins concentrated to 10 mg ml⁻¹ in protein buffer 1 (50 mM Tris, pH 7.5, 500 mM NaCl) were buffer-exchanged by dialysis into protein buffer 2 (20 mM Tris pH 7.5, 150 mM NaCl) before crystallization.

Crystallization. Crystals were grown by hanging-drop vapor diffusion. Crystals of the Rad50-link2–AMP–PNP–Mg²⁺ complex were grown by mixing 1 µl of 10 mg ml⁻¹ Rad50-link2 in protein buffer 3 (200 mM Tris, pH 7.5, 150 mM NaCl, 10 mM MgCl₂, 2.5 mM AMP–PNP (Sigma)) with 1 µl of crystallization solution 1 (100 mM Tris, pH 9.0, 100 mM NaCl, 16–18% (w/v) PEG 550 MME). Mre11RBD–Rad50-link1–AMP–PNP–Mg²⁺ complex crystals were grown by mixing 1 µl of protein buffer 3 with 1 µl of crystallization solution 2 (100 mM Tris pH 8.5, 200–300 mM LiSO₄, 12–13% (w/v) PEG 3350). Nucleotide-free Mre11RBD–Rad50-NC crystals were grown at 20 °C in crystallization solution 3 (100 mM Tris, pH 7.5, 200 mM NaCl, 2 M ammonium sulfate; crystal form 2) or at 4 °C in crystallization solution 4 (100 mM Tris, pH 7.5, 200 mM MgCl₂, 20% (w/v) PEG 3350; crystal form 1). Crystal cryoprotection was done by rapid soaks in Paratone-N (Hampton Research) for all crystal forms except Mre11RBD–Rad50-link1–AMP–PNP–Mg²⁺ complex crystals that were gradually soaked into crystallization solution 2 supplemented with 26% (v/v) ethylene glycol before flash cooling in liquid nitrogen.

X-ray diffraction data collection, structure determination and processing. Data were collected and processed in HKL2000 (ref. 42). Structures were solved by molecular replacement with MOLREP and refined in REFMAC4 and PHENIX with rounds of manual rebuilding in O and COOT (Supplementary Methods). Refined models of Mre11RBD–Rad50 crystal form 1 (2.1 Å), Mre11–Rad50 crystal form 2 (3.4 Å), Mre11RBD–Rad50-link1–AMP–PNP–Mg²⁺ (3.3 Å) and Rad50-link2–AMP–PNP–Mg²⁺ (1.9 Å) all have good statistics and geometry (Table 1).

Protein pull-down assays. Constructs were coexpressed as described above (Fig. 1a,b) and following lysis in 50 mM Tris, pH 7.5, 500 mM NaCl, soluble extracts were incubated with Ni-NTA beads (Qiagen). Bound protein was eluted with lysis buffer containing 300 mM imidazole after washing with 50 mM imidazole and analyzed by SDS-PAGE.

Small-angle X-ray scattering data collection and processing. M₉₂₃ reservoirs were purified following coexpression and purification of Rad50-NC and Mre11 (1–379) constructs (Fig. 1) and SAXS data collected and analyzed at the Advanced Light Source SIBYLS beamline (BL12.3.1) as described. Briefly, data were collected at a wavelength of 1.0 Å and sample-to-detector distance of 1.5 m. Purified M₉₂₃–head protein at 6 mg ml⁻¹ was dialyzed into SAXS buffer (50 mM Tris, pH 7.5, 150 mM NaCl). Protein was diluted 1:1 in SAXS buffer (−ATP data) or SAXS buffer with 2.5 mM ATP and 2.5 mM MgCl₂ (for +ATP). Following heating at 55 °C, designed to trap ATP-bound states, short (0.5 s) and long (2 s) SAXS exposures were collected at 20 °C for protein and relevant buffer. Scattering profiles were generated by subtracting buffer from sample exposures, followed by merging of short and long exposures in PRIMUS to generate SAXS data including the entire scattering spectrum. Guinier analysis (Supplementary Fig. 3) revealed the absence of aggregates. SAXS scattering data analysis using molecular dynamics and MES was done using BILBOMD and FoXS software (see Supplementary Methods).

Strain construction, survival assays and yeast two-hybrid analysis. Strain genotypes are listed in Supplementary Table 1. Growth media and methods for S. pombe were developed and done as described. Spot assays were done by plating five-fold serial dilutions of exponentially growing cells onto rich-medium plates in the absence or presence of the indicated DNA-damaging agents. Plates were incubated at 30 °C and scanned after 2–3 d of growth. The ionizing radiation survival was assayed by counting cells plated in triplicate onto rich medium after exposure to indicated ionizing radiation doses. Normalization was to untreated samples. Yeast two-hybrid analysis was done as described with S. cerevisiae reporter strain AH109 (Clontech Matchmaker system). Immunoblotting. Anti-Myc (9E10: Santa Cruz Biotechnology), Pstair and PAP (Sigma) antibodies were used for western blotting as described.
**Erratum:** ABC ATPase signature helices in Rad50 link nucleotide state to Mre11 interface for DNA repair

Gareth J Williams, R Scott Williams, Jessica S Williams, Gabriel Moncalian, Andrew S Arvai, Oliver Limbo, Grant Guenther, Soumita SilDas, Michal Hammel, Paul Russell & John A Tainer

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In the version of this article initially published, the acronym ENIGMA was spelled out incorrectly in the Acknowledgments section. The error has been corrected in the HTML and PDF versions of the article.

**Corrigendum:** Genome-wide identification of Ago2 binding sites from mouse embryonic stem cells with and without mature microRNAs

Anthony K L Leung, Amanda G Young, Arjun Bhutkar, Grace X Zheng, Andrew D Bosson, Cydney B Nielsen & Phillip A Sharp

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In the version of this article initially published, the blue curve in Figure 2c was mistakenly replaced with a duplicate of that in Figure 2a. The error has been corrected in the HTML and PDF versions of the article.

**Erratum:** Genome-wide CTCF distribution in vertebrates defines equivalent sites that aid the identification of disease-associated genes

David Martin, Cristina Pantoja, Ana Fernández Miñán, Christian Valdes-Quezada, Eduardo Moltó, Fuencisla Matesanz, Ozren Bogdanović, Elisa de la Calle-Mustienes, Orlando Domínguez, Leila Taher, Mayra Furlan-Magaril, Antonio Alcina, Susana Cañón, Maria Fedetz, María A Blasco, Paulo S Pereira, Ivan Ovcharenko, Félix Recillas-Targa, Lluís Montoliu, Miguel Manzanares, Roderic Guigó, Manuel Serrano, Fernando Casares, & José Luis Gómez-Skarmeta

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In the version of this article initially published, the affiliation for authors at the Department of Molecular and Cellular Biology, Centro Nacional de Biotecnología, Madrid, Spain, was incomplete. The full affiliation is "Department of Molecular and Cellular Biology, Centro Nacional de Biotecnología, CSIC, Madrid, Spain." The error has been corrected in the HTML and PDF versions of the article.

**Corrigendum:** The resistance of DMC1 D-loops to dissociation may account for the DMC1 requirement in meiosis

Dmitry V Bugreev, Roberto J Pezza, Olga M Mazina, Oleg N Voloshin, R Daniel Camerini-Otero & Alexander V Mazin

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In the version of this article initially published, the legend for Figure 2d,e did not include the source of the data in those panels. These data originally appeared in ref. 20. The error has been corrected in the HTML and PDF versions of the article.
Erratum: ABC ATPase signature helices in Rad50 link nucleotide state to Mre11 interface for DNA repair

Gareth J Williams, R Scott Williams, Jessica S Williams, Gabriel Moncalian, Andrew S Arvai, Oliver Limbo, Grant Guenther, Soumita SilDas, Michal Hammel, Paul Russell & John A Tainer
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In the version of this article initially published, panels in Figures 2b (middle row, Dex-LWH), 3b (0.1μM CPT) and 3d (0.1μM CPT) were mistakenly replaced with duplicates of adjacent panels. The errors have been corrected in the HTML and PDF versions of the article.