Supplemental Information

Crystal Structure of Human Mre11:
Understanding Tumorigenic Mutations
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Inventory of Supplemental Information

Supplemental Experimental Procedures

Figure S1 is related to figures 1B and 1C.

Figure S2 is related to figures 1C, 3A, 3D and 6A-6F.

Figure S3 is related to figures 1C, 2A and 3A.

Figure S4 is related to figures 2, 3, 4 and 5.

Figure S5 is related to figure 2.

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Supplemental Experimental Procedures

DNA substrates

PAGE-purified DNA substrates were purchased from Bioneer Co. The dsDNA substrate consisted of 3′ [32P]-labeled TP124 (5′-CATCTGGCCTGTCTTACAC AGTGCTACAGACTGAAACACACCCTGCAG-T3′) annealed to TP580 (5′-CTGCAGGGTTTTTGTCCAGTCTGACTGTAAGACAGGCAsGsAs TsG-T3′). Phosphorothioate bonds are indicated by the “s” between the nucleotides. The 3′ overhang substrate consisted of TP811 (AACAACCTCGGGATTATGTCGGGCTCAGGGTCTGGTTCTGGTTTCAGGTTCTGGGTCTTACTACCATCACCATCAC CATC) annealed to TP812 (GTG ATGGTGATGGTGATGGTAGTAAGCACAGAACCTGAACC AGA ACC AGA CCC TGA GCC CGA CAT AAT CCC GAG GTT GTT). TP811 was labeled with 32P at the 5′ end. DNA substrates were prepared at the 3′ end with terminal deoxynucleotidyl-transferase (Roche) and [α-32P]dATP (PerkinElmer) or at the 5′ end with polynucleotide kinase (Roche) and [γ-32P]ATP. The labeled oligos were boiled for 5 min and then allowed to cool to anneal slowly. The dumbbell substrate consisted of DAR134 (CATCCATGCCTACCTGACAGCTTAGCCACATCGGACTGTCAGGTAGGCATG). DAR134 was labeled with [32P] at the 5′ end.

Protein expression and purification

The gene encoding residues 1–411 of human Mre11 was inserted into the pET28a vector. *Escherichia coli* Rosetta (DE3) containing the pET28a (hMre11 core) vector was cultured in LB broth. The hMre11 core was first purified by Ni-NTA affinity chromatography using a His-tag at the N-terminus of hMre11. The hMre11 core protein was subsequently eluted with 300 mM imidazole in the same buffer. Fractions containing the hMre11 core were subsequently purified using cation exchange and gel-filtration chromatography and concentrated by ultrafiltration (6 mg/ml). For Western blot analysis, a histidine tag was added to the N-terminus of the hMre11 core. GST-Nbs1 (residues 440–754) was expressed using *E. coli* Rosetta (DE3) and purified using a glutathione (GST)-Sepharose column. Protein concentrations were determined by the Bradford assay and confirmed by comparison with protein standards by Coomassie Blue stained SDS-PAGE gels. The
Se-Met-substituted hMre11 protein was obtained from *E. coli* strain B834 (DE3) containing the pET28a (hMre11 core) vector using a standard protocol. The Se-Met-substituted hMre11 protein was purified using the same method as that for the wild-type hMre11 protein.

**Mutagenesis**

All mutants used in this study were constructed by PCR-based mutagenesis. The hMre11 mutant proteins were purified using a Ni-column followed by cation-exchange and gel-filtration chromatography, as described above for the purification of the wild-type hMre11 protein.

**Analytical ultracentrifugation**

The molecular mass of the hMre11 core was analyzed with an Optima XL-A analytical ultracentrifuge (Beckman) using the sedimentation equilibrium technique. Sedimentation equilibrium data were evaluated using a nonlinear least-squares curve-fitting algorithm (XL-A data analysis software). Samples were analyzed in binding buffer containing 25 mM Tris (pH 7.4), 200 mM NaCl, 10 mM β-mercaptoethanol, 0.1 mM EDTA and 0.5 mM MnCl$_2$, and the protein concentration was 20 μM. Data were collected at 8,000 rpm and 15°C using an An60Ti rotor (Beckman) and by measuring the absorbance at 280 nm. The partial specific volume of hMre11 was estimated to be 0.734 cm$^3$/g from the protein sequence using the SEDNTERP program, and a rho value of 1.007 was used for the molecular mass calculation.

**Nuclease assays**

Cleavage reaction mixtures contained 20 nM $^{32}$P-labeled substrate DNA molecule(s) and enzyme (as indicated) in a reaction buffer comprised of 25 mM MOPS (pH 7.0), 50 mM NaCl, 1 mM DTT, and 1 mM MnCl$_2$. Reaction mixtures were incubated for 90 min or a specified time (for kinetics) at 37°C, and were stopped by adding 1/10 volume of a stop mixture (2% sodium dodecyl sulfate, 100 mM EDTA, and 0.5 mg/mL proteinase K), followed by a 10-min incubation at 37°C. Reaction products were boiled for 5 min and resolved on 15% denaturing polyacrylamide gels containing 7 M urea in TBE buffer. Gels were run for 360 min at 13 V/cm. After electrophoresis, the
gels were fixed in fixing buffer (30% methanol, 5% acetic acid, 5% glycerol), dried, and subjected to autoradiography and phosphoimage analysis. Three independent experiments were performed.

**Gel filtration analysis**
The hMre11 core (2 mg/ml) was loaded onto a Superdex 200 column (Amersham Biosciences) equilibrated with buffer containing 20 mM Tris-HCl (pH 8.0), 200 mM NaCl, 0.1mM EDTA, and 5 mM DTT.

**Mutant protein structural changes and stabilities**
Structural changes in the hMre11 mutant (10 µM) versus the wild type hMre11 were monitored by CD spectrophotometer (Jasco J-715) at wavelengths of 200-260 nm. All samples were prepared in the same buffer used for the gel filtration analysis. Conformational changes in the hMre11 mutant protein were monitored at various temperatures. The temperature was raised from 20 to 90°C over a 70-min period, with detection set at 220 nm.
Supplemental Information

Fig. S1, related to Figure 1. Predicted ordered domains of hMre11. The nuclease and capping domain (residues 1–411) is indicated in violet and green, respectively. Disordered regions were obtained by submitting the hMre11 sequence to disorder-prediction programs (IUPRED [http://iupred.ensim.hu]). A disorder value >0.5 indicates an unstructured protein region.

Fig. S2, related to Figures 1, 3 and 6. Size exclusion chromatography and analytical ultracentrifuge analyses reveal that the hMre11 core forms a dimer. (A) Size exclusion chromatography of the wild-type (blue), Cys146Ala (green) and NLD (orange, Nbs1-binding loop deletion) hMre11 core. The hMre11 core protein (0.5 mg, blue line) was loaded onto a Superdex200 HR 10/300 column. Other cancer-associated hMre11 mutant proteins, except for the Cys146 and NLD mutant proteins, were eluted as a dimer and are omitted for clarity. Standard molecular weights are marked on the graph. (B) Equilibrium fit results of the analytical ultracentrifuge for the wild type (left) and Cys146Ala (right) hMre11 core. The lower panel depicts the fitted overlay (red line) to the experimental data (blue circles). The upper panel depicts the residuals. The fitted parameter for the weight-average molecular mass (Mw, app) was estimated to be 97.3 kDa for the wild type hMre11 and 72.3 kDa for the hMre11 Cys146Ala mutant. For the hMre11 Cys146Arg mutant, the estimated molecular weight was 48.5 kDa, which corresponded to a monomer (data not shown).

Fig. S3, related to Figures 1, 2 and 3. Overall structure of the hMre11 core in an asymmetric unit. (A) Four hMre11 core molecules are observed in an asymmetric unit. Two hMre11 (hMre11-A and hMre11-B) molecules that form a dimer are shown in yellow, and the two hMre11 (hMre11-C and hMre11-D) molecules are shown in violet. (B) The superimposed structures of the two hMre11 dimers in an asymmetric unit. The two dimers exhibit a 0.8 Å root mean square deviation for the 749 Cα atoms.

Fig. S4, related to Figures 2, 3, 4 and 5. Structure based sequence alignment of Mre11 homologues. Secondary structures of the nuclease and capping domains are indicated above the sequence. Strictly conserved residues in all Mre11 proteins
are boxed with green blue, and conserved residues in eukaryotic Mre11 proteins are boxed with green. The nuclease motifs are boxed with red. In TmMre11, residues marked with asterisks contain the GNHDWK (92–97) sequence, and the highly conserved His94 is equivalent to pfMre11 His85 or hMre11 His129. Every tenth residue is marked with a black circle. See the sequence alignment figure below for residue specific functional annotations. Abbreviations: h, human; mm, mouse; xl, frog; zf, zebra fish; dm, fly; at, Arabidopsis; sc, budding yeast; sp, fission yeast; Pf, *P. furiosus*; Tm, *T. maritima*.

**Fig. S5, related to Figure 2.** Structural superposition of hMre11 and PfMre11. A close-up view highlights the local structure of PfMre11 forming a three stranded sheet (light blue and green, residues 108–132) that overlays the Nbs1 binding loop (light pink, 94–103) of hMre11. A part of this region (green, 108–114,) in PfMre11 collided with a dimeric partner (blue and magenta, 132–138) of hMre11 in the superimposed structures.

**Fig. S6, related to Figure 2 and 3.** Close-up views of the hMre11 local structures that correspond to the dimeric interface of PfMre11 and TmMre11. (A) A region corresponding to the PfMre11 dimeric interface: Leu72 (Leu61 of PfMre11) makes van der Waals contacts with the side chains of Phe62, Pro67, Leu76, Ile143 and Leu144. Leu76 (Ile65 of PfMre11) is surrounded by Leu72, Leu144, Ala147 (Phe101 of PfMre11) and Phe149. Ile143 (Leu97 of PfMre11) is directed to the interior and establishes van der Waals contacts with Pro67, Leu72 and Ala140. (B) A region corresponding to the TmMre11 dimeric interface: Leu75 (Leu75 of TmMre11) is at the interior to interact with nearby residues. Ile143 and Cys146 (Phe102 and Phe105 of TmMre11) make hydrophobic interactions and a disulfide bond with Phe103, Phe106 (α2-β3), Leu138 and Cys146 from another hMre11.

**Fig. S7, related to Figures 6 and 7.** Overall structural changes of the hMre11 mutant proteins. Various hMre11 core mutant proteins (10 µM) and the wild type hMre11 core were monitored by CD spectrophotometer (Jasco J-715) at wavelengths of 200-260 nm.
Figure S1.
**Figure S2**

**A**

Elution volume (mL) vs. A280 (mAU)

**B**

Molecular weight of Human Mre11\(^{1-411}\) from equilibrium sedimentation

<table>
<thead>
<tr>
<th>Protein</th>
<th>Theoretical M.W. of dimer (kDa)</th>
<th>Estimated M.W. (kDa)</th>
<th>Estimated M.W./Theoretical M.W.</th>
</tr>
</thead>
<tbody>
<tr>
<td>hMre11 (WT)</td>
<td>98.4</td>
<td>97.3</td>
<td>0.99</td>
</tr>
<tr>
<td>hMre11 (C146A)</td>
<td>98.4</td>
<td>72.3</td>
<td>0.73</td>
</tr>
</tbody>
</table>

Model: Single ideal species
Speed: 8000 rpm
Temp: 15°C
V-bar: 0.7333
Rh: 1.0963
M.W.: 97260
Figure S3.
Absolutely conserved residues in eukaryotic, archaeal, and bacterial Mre11 proteins

Residues that interact with the Mn2+ coordinated residues

Mn2+ coordination

Legend

Nucleosome domain

Capping domain

Nde1 nucleosome motifs

Absolutely conserved residues in eukaryotic, archaeal, and bacterial Mre11 proteins

Disease related mutations

Absolutely conserved residues in eukaryotic, archaeal, and bacterial Mre11 proteins

Figure S4
Figure S5
Figure S6
Figure S7