Structure of C-terminal Tandem BRCT Repeats of Rtt107 Protein Reveals Critical Role in Interaction with Phosphorylated Histone H2A during DNA Damage Repair*\(^5\)

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**Background:** Rtt107 can be recruited to chromatin during the DNA damage response.

**Results:** Structures of C-terminal Rtt107 alone and in a complex with γH2A were determined.

**Conclusion:** Mutagenesis studies indicated that the phosphorylation-dependent interaction between Rtt107 and γH2A is important for the function of Rtt107.

**Significance:** Our work provides a structural basis for understanding the molecular mechanism of the recruitment of Rtt107 to chromatin.

Rtt107 (regulator of Ty1 transposition; 107; Esc4) is a DNA repair protein from *Saccharomyces cerevisiae* that can restore stalled replication forks following DNA damage. There are six BRCT (BRCA1 C-terminal) domains in Rtt107 that act as binding sites for other recruited proteins during DNA repair. Several Rtt107 binding partners have been identified, including Slx4, Rtt101, Rad55, and the Smc5/6 (structural maintenance of chromosome) protein complex. Rtt107 can reportedly be recruited to chromatin in the presence of Rtt101 and Rtt109 upon DNA damage, but the chromatin-binding site of Rtt107 has not been identified. Here, we report our investigation of the interaction between phosphorylated histone H2A (γH2A) and the C-terminal tandem BRCT repeats (BRCT\(_5\)-BRCT\(_6\)) of Rtt107. The crystal structures of BRCT\(_5\)-BRCT\(_6\) alone and in a complex with γH2A reveal the molecular basis of the Rtt107-γH2A interaction. We used *in vitro* mutagenesis and a fluorescence polarization assay to confirm the location of the Rtt107 motif that is crucial for this interaction. In addition, these assays indicated that this interaction requires the phosphorylation of H2A. An *in vivo* phenotypic analysis in yeast demonstrated the critical role of BRCT\(_5\)-BRCT\(_6\) and its interaction with γH2A during the DNA damage response. Our results shed new light on the molecular mechanism by which Rtt107 is recruited to chromatin in response to stalled DNA replication forks.

Maintaining genomic stability and integrity is very important for all living organisms. During S phase, eukaryotic cells unwind and duplicate billions of DNA base pairs. Three-dimensional DNA structures, such as replication forks that are formed during DNA synthesis, are very sensitive to both endogenous and exogenous insults (1). The repair of these lesions generally occurs in a stepwise manner. In *Saccharomyces cerevisiae*, the checkpoint kinase Mec1 is recruited to the break sites early during the damage response (2). Mec1 then phosphorylates a variety of proteins in the DNA replication and repair machineries to stabilize and repair the damaged replication forks (3, 4).

Rtt107 is one substrate of Mec1 phosphorylation and was originally identified in a genetic screen for increased Ty1 transposon mobility (5). The Rtt107Δ mutant is very sensitive to a wide spectrum of replication stress-inducing agents, such as the DNA-alkylating agent methyl methanesulfonate (MMS),\(^3\) the nucleotide reductase inhibitor hydroxyurea (HU), and the topoisomerase I poison camptothecin (CPT) (6–8). Furthermore, deletion of Rtt107 causes genomic instability, even in the absence of DNA-damaging agents (9). Rtt107 is required for normal DNA synthesis and to restart stalled replication forks during recovery from DNA damage in S phase (6, 8). In addition, two recent studies linked Rtt107 with double-strand break repair (10, 11).

Structurally, Rtt107 contains six BRCT homology domains. There are four tandem BRCT domains at the N terminus of Rtt107 and two more at the C terminus (12). The BRCT domain is a phosphoprotein interaction module frequently found in proteins involved in the DNA damage response, cell cycle control, and checkpoint-mediated DNA repair (13–16). It is believed that the multiple BRCT domains of Rtt107 create a scaffold that helps spatially coordinate different repair proteins during DNA damage repair (17). Consistent with this role, Rtt107 interacts with several repair proteins, such as the structure-specific endonuclease Slx4 (7), the recombination repair protein Rad55 (17), the ubiquitin ligase subunit Rtt101 (18), and the Smc5/6 complex (10, 11, 19). The N-terminal BRCT

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\(^3\)The abbreviations used are: MMS, methyl methanesulfonate; HU, hydroxyurea; CPT, camptothecin; r.m.s.d., root mean square deviation; Rtt107-C, Rtt107 C terminus.
domains of Rtt107 interact with Slx4 and the Smc5/6 complex (7, 10), but the binding partners of the C-terminal BRCT repeats during DNA damage repair have not yet been characterized.

In this study, we performed sequence alignment and found that only the C-terminal tandem BRCT repeats (BRCT5–BRCT6) of Rtt107 contain phospho-recognition modules (20, 21). These data suggest that BRCT5–BRCT6 may interact with unidentified phosphoproteins during DNA damage repair. Roberts et al. (18) found that Rtt107 was recruited to chromatin in the presence of Rtt101 and the acetyltransferase Rtt109 in response to stalled replication forks. However, the chromatin-binding site of Rtt107 is poorly understood. An important clue is that Brc1, the fission yeast homolog of Rtt107, forms phosphorylation-sensitive nuclear foci in the presence of DNA damage (20). Without direct structural evidence, Leung et al. (10) proposed a model whereby Rtt107 is recruited to DNA break sites by binding to γH2A.

To explore the structure and function of the C-terminal tandem BRCT repeats of Rtt107 during DNA damage repair, we solved the crystal structures of BRCT5–BRCT6 alone and in a complex with γH2A. Fluorescence polarization assay data confirmed the phosphorylation-dependent interaction between Rtt107 and γH2A. An in vivo phenotypic analysis demonstrated that the interaction of Rtt107 with γH2A is functionally important during DNA repair. Our structural, biochemical, and functional studies reveal the structural basis of the Rtt107–γH2A interaction and shed new light on the interaction network of Rtt107 during DNA damage repair.

**EXPERIMENTAL PROCEDURES**

**Cloning, Expression, and Purification of Protein—**A DNA fragment of Rtt107-C (residues 820–1070) was amplified from yeast genomic DNA (S. cerevisiae S288C) by PCR. This fragment was ligated into plasmid pGEX-4T-1 (GE Healthcare) using NdeI/XhoI, yielding plasmid pGEX-Rtt107-C. The Rtt107-C mutations for L909M, L1028M, T842A, and K887M were introduced using the MutanBEST kit (Takara Co.). The proteins were produced in Escherichia coli BL21(DE3) (Merck). Protein expression was induced at 0.8–1.2 with 0.2 mM isopropyl β-d-thiogalactopyranoside, and the cells were grown at 16 °C for 18 h. The proteins were purified by GST-glutathione affinity chromatography and eluted with on-resin thrombin cleavage that removed the GST tag. Next, the proteins were further purified by Superdex 75 gel filtration chromatography (GE Healthcare) in buffer A (500 mM NaCl and 20 mM Tris-HCl, pH 7.5). The purified protein was then dialyzed into buffer B (50 mM NaCl and 10 mM Tris-HCl, pH 7.5) and concentrated to 20–40 mg/ml.

To prepare the SeMet-derivatized protein, Rtt107-C containing the L909M and L1028M mutations was expressed in E. coli strain B834 (Novagen) using LR (a minimal medium described by LeMaster and Richards, also called LMR) medium supplemented with SeMet and six amino acids (leucine, isoleucine, valine, phenylalanine, lysine, and threonine). The SeMet-derivatized protein was purified by a procedure similar to the one used to purify the native proteins.

**Crystallization and Data Collection—**Crystals of both native Rtt107-C and the SeMet-derivatized L909M/L1028M mutant were grown at 283 K by mixing 1 μl of 20 mg/ml protein in buffer B with 1 μl of well solution 1 (17.5% (w/v) mPEG 2000, 200 mM NaCl, and 100 mM HEPES, pH 7.0) using the hanging drop vapor diffusion method. Single crystals were obtained after 2 days.

All peptides used in this study were synthesized at GL Biochem (Shanghai) Ltd. The complex between Rtt107-C and γH2A was formed prior to crystallization by mixing the purified Rtt107-C protein with the γH2A phosphopeptide (ATKAP- SQE) in buffer B at a 1:1.5 protein/phosphopeptide molar ratio. The crystals of the complex were obtained by mixing 1 μl of the complex with 1 μl of well solution 2 (15% (w/v) PEG 1500, 200 mM NaCl, and 0.1 M MES, pH 5.8) and grown at 283 K for 2 days.

For data collection, the crystals were flash-frozen in liquid nitrogen after being transferred into a cryoprotectant solution composed of 80% mother liquor and 20% glycerol. All single-crystal x-ray diffraction data were collected at the Shanghai Synchrotron Radiation Facility (SSRF) using beamline BL17U. The multiple-wavelength anomalous dispersion data set (λ_peak = 0.9805 Å, λ_inflection = 0.9807 Å, and λ_remote = 0.9506 Å) was collected from a single crystal of the SeMet-labeled protein at 100 K. X-ray data reduction and scaling were performed with the HKL2000 suite (23).

**X-ray Structure Determination and Refinement—**All four selenium positions within the crystallographic asymmetric unit (two selenomethionines per C-terminal polypeptide) were located and refined with SOLVE (24) and a three-wavelength SeMet multiple-wavelength anomalous dispersion data set. The initial phases were calculated by RESOLVE (25) with a resolution ranging between 30 and 2.30 Å, and an initial model was automatically built. The model was further built and refined at 2.30 Å resolution using Refmac5 (26) and COOT (27) by manual model correction until the crystallography R-factor and the free R-factor converged to 20.3 and 24.8%, respectively. The structure of the SeMet-labeled Rtt107-C L909M/L1027M mutant was used as an initial search model for determining the native structure of Rtt107-C by a standard molecular replacement method in the PHENIX package (28). The final crystallography R-factor and the free R-factor of the native Rtt107-C structure are 20.3 and 25.4%, respectively. The structure of the complex between Rtt107-C and γH2A was determined using the native Rtt107-C structure as the initial search model in the PHENIX package (28). The complex structure was further refined by procedures similar to those described above. Details regarding the data collection and processing of these crystal structures are presented in Table 1.

**Fluorescence Polarization Assay—**The FITC probe was conjugated to γH2A and H2A using a chemical reaction described in standard protocols. The labeled peptides were purified with an FPLC column. Fluorescence polarization assays were performed in buffer B at 293 K using a SpectraMax M5 microplate reader system. The wavelengths of fluorescence excitation and emission were 490 and 520 nm, respectively. Each well of a 96-well plate contained 100 nM FITC-labeled peptide and different amounts of Rtt107-C or the Rtt107-C mutant (concen-
trations ranged between 0 and 200 μM) in a final volume of 200 μl. For each assay, peptide-free controls (Rtt107-C or the Rtt107-C mutant alone) were included. The fluorescence polarization change (ΔP, in millipolarization (P/1000) units) was fit to Equation 2.

\[
P = \frac{l_\| - l_\perp}{l_\| + l_\perp}
\]  

(Eq. 1)

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\[
\Delta P = \frac{\Delta P_{\text{max}} \times \text{[Rtt107]}}{K_d + \text{[Rtt107]}}
\]  

(Eq. 2)

**CD Spectroscopy**—The CD spectra of Rtt107 and its mutant were recorded at 298 K on a Jasco J-810 spectropolarimeter. The spectra were recorded at wavelengths between 190 and 280 nm using a 0.1-cm path length cell and 0.2 mg/ml protein in PBS, pH 7.4. A buffer-only sample was used as the reference. The molar ellipticities ([θ]) were plotted against the wavelength, and the reference curve was subtracted from each curve.

**Yeast Strains, Plasmids, and Experiments**—To construct pRS316-Rtt107, the entire Rtt107 open reading frame plus 584 bp of upstream and 100 bp of downstream genomic sequence were cloned at the BamHI/XhoI sites of pRS316. The pRS316-Rtt107 mutations, including ABRCT5-BRCT6 and T842A plus K887M, were introduced using the MutanBEST kit. The pRS316-Rtt107 plasmids encoding wild-type or mutant constructs were transformed into yeast strain ΔRtt107 (BY4742, Invitrogen) by electroporation (29). For functional studies of Rtt107, the cells were grown to the exponential phase and then serially diluted 10-fold and spotted onto YPD (1% yeast extract, 2% peptone, and 2% dextrose) plates containing 0.03% MMS, 5 μg/ml CPT, 50 mM HU, or no DNA stress agent.

**RESULTS**

**Crystal Structure of C-terminal BRCT Domains of Rtt107**—The crystal structures of Rtt107-C (residues 820–1070; BRCT5-BRCT6) were determined by multiple-wavelength anomalous dispersion phasing and molecular replacement (Protein Data Bank codes 3T71 and 3T7J). The crystallographic statistics are summarized in Table 1. There are two copies of Rtt107-C packed against each other to compose one asymmetric unit of the crystal. As shown in Fig. 1, the overall structure illustrates the tandem BRCT repeats as expected. Surprisingly, the N terminus of BRCT5 contains an additional helix, named αN. From the structure, we found that some residues (Ala-822, Ile-825, Leu-826, Phe-829, and Leu-832) of the N helix and (Val-852, Glu-855, and Ile-856) of the I helix (Val-852, Glu-855, and Ile-856) and the α3 helix (Ile-913) of BRCT5 (Fig. 1B). These three α helices form a three-helix bundle stabilized predominantly by hydrophobic interactions. We were not able to obtain any stable proteins without the αN helix, presumably due to its essential position within the three-helix bundle. Despite the N-terminal extension, BRCT5 consists of a four-stranded parallel β sheet (β1, β2, β3, and β4) flanked by two α helices (α1 and α3) on one side and one α helix (α2) on the other side; this structure is similar to other BRCT domains.

BRCT5α, similar to BRCT5, shows a remarkable variation to the canonical BRCT domain. The β sheet core consists of four parallel β strands (β1′, β2′, β3′, and β4′) and one antiparallel β strand (β5′), which is unique among the BRCT domains. The BRCT5α sequence is not homologous to any other structures.
recorded in the Protein Data Bank, further defining its unique role among BRCT domains. There are two disordered regions (residues 922–926 and 1007–1014) on the outer face of these two BRCT domains that are missing in the electron density map.

The two BRCT domains adopt a head-to-tail arrangement, and the interface of these tandem BRCT domains contains both core BRCT structural elements and the interdomain linker. Helix \( \alpha_2 \) packs against helices \( \alpha_1 \prime \) and \( \alpha_3 \prime \), forming a helix bundle with helix \( \alpha L1 \). The interface is stabilized by the hydrophobic interactions of residues within the helix bundle. Polar contacts between Arg-884 and Asn-1044 in addition to the hydrophobic interactions between Lys-942 and Leu-1053 further stabilize the interface (Fig. 1C). The tandem BRCT domains form a rigid body due to the stable interface between them.

**Structural Comparison with Other Tandem BRCT Repeats**—

There is very low sequence identity between BRCT\(_5\)-BRCT\(_6\) of Rtt107 and the tandem BRCT repeats of other proteins. (There is only 19% identity with the homolog Brc1 (supplemental Fig. S1).) A structural comparison may further our understanding of the BRCT domains of Rtt107. We used the BRCT\(_5\)-BRCT\(_6\) structure of Rtt107 as the search model in the Dali server (30). Not surprisingly, the root mean square deviation (r.m.s.d.) between BRCT\(_5\)-BRCT\(_6\) of Rtt107 and other tandem BRCT repeats for C\(_{\alpha}\) atoms are all above 3 Å. BRCT\(_5\)-BRCT\(_6\) of Rtt107 shows the highest similarity to the four tandem BRCT repeats of Brc1 (fission yeast homolog of Rtt107, r.m.s.d. of 3 Å) (20), MDC1 (r.m.s.d. of 3.4 Å) (31), BRCA1 (r.m.s.d. of 3.4 Å) (32) and 53BP1 (r.m.s.d. of 3.8 Å) (33). All of these proteins play important roles in DNA damage repair. As shown in Fig. 2, the positions and orientations of the secondary structures in the C-terminal tandem BRCT repeats of Rtt107 deviate considerably from those of the homolog Brc1. In Rtt107, the \( \alpha_1 \) helix tilts \( \sim 30^\circ \) compared with the \( \alpha_1 \) helix in Brc1, and the \( \alpha_3 \) helix extends 6 Å longer along its helical axis (Fig. 2B) than the same structure in Brc1. These changes cause these two helices to pack more tightly with the \( \alpha N \) helix in Rtt107. The orientation of the main \( \alpha_2 \prime \) helix in the C terminus of the BRCT domain changes as much as \( 80^\circ \) from the main helix in Brc1 (Fig. 2C). Another remarkable difference is that the linker regions of BRCT\(_5\)-BRCT\(_6\) in Rtt107 are shorter and packed more tightly than the other three BRCT repeats (supplemental Fig. S2). A previous study has shown that the linker regions between BRCT repeats are highly variable both in sequence and in length (34). Therefore, BRCT linker regions may provide specificity for binding interactions. For example, the \( \beta \) hairpin of 53BP1 is involved in the interaction with the tumor suppressor protein p53 (35, 36). It would be interesting to investigate the function of the short linker region in BRCT\(_5\)-BRCT\(_6\) of Rtt107.

Williams et al. (20) found that there are conserved electronegative patches in MDC1 and Brc1; this region may contribute to the recognition of exposed histone core surfaces during DNA damage repair. We found that the electrostatic potential of this

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**Figure 1.** Crystal structure of BRCT\(_5\)-BRCT\(_6\) of Rtt107. A, overall three-dimensional structure of BRCT\(_5\)-BRCT\(_6\). The two BRCT domains are colored cyan and green. The linker region is orange. Disordered regions are shown as dotted lines. B, the three N-terminal helices (\( \alpha N \), \( \alpha_1 \), and \( \alpha_2 \)) of BRCT\(_5\) form a helix bundle. The labeled residues are involved in hydrophobic interactions within the helix bundle. C, interface between BRCT\(_5\) and BRCT\(_6\). The interactions between the labeled residues render the tandem BRCT repeats a rigid body.
region in Rtt107 has been changed (Fig. 3). It contains several hydrophobic residues, and these residues split the electronegative patch of Rtt107. Whether Rtt107 can interact with the surface of histone core particles remains a question.

**BRCT<sub>5</sub>-BRCT<sub>6</sub> of Rtt107 Binds to γH2A**—Rtt107 is believed to act as a scaffold during DNA damage repair and can be recruited to chromatin in the presence of Rtt109 and Rtt101 in response to stalled replication forks (18). However, the chromatin-binding site of Rtt107 has not been identified. Tandem BRCT repeats have been found to act as phosphopeptide-binding modules in a wide range of systems (16, 37). Sequence analysis indicates that there are phospho-recognition modules in the fifth BRCT domain of Rtt107; the C1 (TG) motif is located at the end of β1, and the C2 (TEK) motif is located at the beginning of α2. The electrostatic potential of this surface shows that this region forms a positively charged binding pocket (Fig. 4A). C-terminal phosphorylation of H2A (γH2A) is very important during DNA damage repair and chromatin packing (38), and the docking of Brc1 to γH2A is critical for the chromatin-specific response to replication-associated DNA damage (20). Thus, Leung et al. (10) proposed γH2A as a possible target of Rtt107. To test whether BRCT<sub>5</sub>-BRCT<sub>6</sub> in Rtt107 can bind γH2A, we synthesized a phosphorylated tail of H2A and conjugated FITC to the N terminus of this peptide. A fluorescence polarization binding assay showed that BRCT<sub>5</sub>-BRCT<sub>6</sub> in Rtt107 can directly interact with γH2A, and the binding affinity is ~8 μM, which is similar to other tandem BRCT repeats. The mutations within the C1 and C2 motifs (T842A and K887M) block most of the interaction, and the CD spectra indicate that these mutations do not alter the overall protein structure (Fig. 4C). No interaction was detected between Rtt107 and unphosphorylated H2A (Fig. 4B). These data suggest that Rtt107 binds H2A in a phosphorylation-dependent manner.

**Complex Structure of BRCT<sub>5</sub>-BRCT<sub>6</sub> of Rtt107 with γH2A**—To investigate the molecular basis of the interaction between Rtt107 and γH2A, we co-crystallized BRCT<sub>5</sub>-BRCT<sub>6</sub> of Rtt107 with γH2A.
was determined by molecular replacement using the native structure of BRCT<sub>5</sub>-BRCT<sub>6</sub> as the search model. The phosphopeptide corresponding to the five C-terminal residues of H2A exhibits clear electron density (Fig. 5B). The phosphate group of phosphorylated Ser-129 of H2A binds to a positively charged pocket and makes polar contacts with the side chains of Thr-842 and Lys-887 (Fig. 5C). Glu-131 is also well ordered and forms a hydrogen bond with the main chain atoms (Fig. 5D). The side chain of Leu-132 is recognized by a hydrophobic groove located at the interface between the two BRCT domains. The acidic C-terminal carboxyl group of H2A forms a salt bridge with the amino group of Arg-884 (Fig. 5D). The surface electrostatic potential of BRCT<sub>5</sub>-BRCT<sub>6</sub> in this region is positively charged, complementary to the strong negative charge of the H2A C-terminal peptide (Fig. 5E).

To better understand the Rtt107 BRCT<sub>5</sub>-BRCT<sub>6</sub> phosphopeptide-binding properties and specificity, we compared the phospho-binding pocket and pSer<sup>13</sup> (Leu-132) recognition pocket of Rtt107 with those in the structures of other proteins (Brc1, BRCA1, and MDC1) containing three similar tandem BRCT repeats. The phospho-binding pockets are similar among these structures, but the pSer<sup>13</sup> recognition pockets contain two distinct regions, termed D1 (comprising the β1′-α1′ connecting loop and the α1′ helix of the second BRCT of the BRCT pair) and D2 (comprising the α3′ helix of the second BRCT of the BRCT pair) (Fig. 6). The D1 regions show remarkable structural flexibility and determine the pSer<sup>13</sup> recognition specificity. Although both Rtt107 and Brc1 recognize pSer<sup>13</sup> Leu, their D1 regions are quite different. In Brc1, three residues (Asn-788, Ala-791, and Ile-795) in the β1′ helix form a hydrophobic pocket that is capped by a 3<sub>10</sub> helix. In Rtt107, the 3<sub>10</sub> helix is replaced by a flexible loop (Gly-966 and Gly-967), and the pSer<sup>13</sup> recognition pocket is formed by residues (Gly-966 of the β1′-α1′ connecting loop, Thr-970 of α1′, and Glu-886 of α2) from three different secondary structures. These changes make the hydrophobic recognition pocket of Rtt107 similar to that of MDC1. However, the α1′ helix in MDC1 tilts to make the pocket large enough to fit the pSer<sup>13</sup> Tyr side chain. In contrast with Rtt107 and Brc1, another residue (Gln-2008) in the extended β1′-α1′ connecting loop in MDC1 also contributes to the recognition pocket. Hence, the structural flexibility of the D1 regions in these tandem BRCT repeats shows how these pSer<sup>13</sup> recognition pockets are adapted for binding specificity, and our structural data provide an additional evidence to support this idea.

Critical Role of Binding of BRCT<sub>5</sub>-BRCT<sub>6</sub> to γH2A during DNA Damage Resistance—A previous report has shown that the Rtt107Δ mutant is sensitive to a wide range of DNA-damaging agents (6). Based on the structural basis of the Rtt107-γH2A interaction, plasmids pRS316-Rtt107, pRS316-Rtt107(ΔBRCT<sub>5</sub>-BRCT<sub>6</sub>) and pRS316-Rtt107(T842A/K887M) were introduced into the ΔRtt107 (BY4742) background yeast.
strain, and they were tested for sensitivity to various genotoxins to evaluate the genetic epistasis relationship between Rtt107 and H2A. As is shown in Fig. 7, both mutants of Rtt107 were sensitive to MMS, CPT, and HU. This phenomenon indicates that BRCT5-BRCT6 of Rtt107 is critical for its DNA damage resistance, and its binding to H2A is very important during this process. However, these two mutant strains showed less sensitivity than the parental Rtt107 strain. This finding suggests that the Rtt107 mutants may be recruited to damaged sites by another factor but that they cannot function properly as a scaffold like wild-type Rtt107 and that H2A may have both Rtt107-dependent and Rtt107-independent functions in the DNA damage repair pathway during S phase.

**DISCUSSION**

**C-terminal Tandem BRCT Repeats of Rtt107 Binds to γH2A**

To maintain genomic integrity and stability, cells integrate and control various DNA damage responses by assembling specific repair factors and enzymes into multiprotein repair foci. The γH2A foci, which are established by phosphorylation of histone H2A (H2AX in mammalians) flanking DNA damaged sites, are usually considered as a biomarker during the DNA damage response (39 – 41). Formation of the foci is likely mediated by special scaffolding proteins. Binding to these scaffolding proteins is mediated post-translationally by protein phosphorylation in most cases. In this study, we characterized the phosphorylation-dependent interaction between the multiple BRCT-containing protein Rtt107 and γH2A.

Although several partners of Rtt107 have been identified (7, 10, 17, 19), how it is recruited to chromatin during the DNA damage repair process is unclear. By sequence analysis, we found that the C-terminal tandem BRCT repeats (BRCT5-BRCT6) of Rtt107 contain phospho-recognition modules. The fluorescence polarization binding assay indicated that Rtt107 interacts with the H2A C terminus in a phosphorylation-dependent manner. Our crystal structure of BRCT5-BRCT6 in complex with γH2A provides structural insight into the interaction mechanism. The tandem BRCT repeats harbor a positively charged pocket for pSer interaction and a hydrophobic pocket for pSer+3 (Leu-132) recognition. The key residues for pSer interaction are Thr-842 (in the C1 motif) and Thr-885 (in the C2 motif), just as predicted in sequence analysis. Mutation of Thr-842 and Lys-887 dramatically decreases the interaction between Rtt107 and γH2A. The pSer+3 recognition hydrophobic pocket is located in a groove formed at the interface between the two BRCT domains. To achieve high selectivity for pSer+3 residues, the hydrophobic pockets in tandem BRCT repeats show structural
Our structural data provide extra evidence to support this idea. In vivo yeast phenotypic analysis defined the critical role of BRCT5-BRCT6 during the DNA damage response. Its interaction with γH2A is very important not only for resistance to MMS but also for resistance to HU and CPT. These findings suggest that Rtt107 may be the effector of γH2A and that Rtt107 is recruited to chromatin near stalled replication forks by γH2A during all types of lesions.

Multiple BRCT Domains in Rtt107 Act as Scaffold to Recruit Different Binding Partners during DNA Damage Repair Process—The structural diversity of BRCT repeats makes them particularly attractive as scaffolding elements at the heart of large multiprotein complexes. In mammalian cells, several mediator proteins, such as 53BP1 (35, 42) and TopBP1 (43–45), employ BRCT domains to coordinate the activities of various DNA repair and recombination proteins. Multiple BRCT repeats could bring together several different target proteins to a single repair focus. There are three pairs of BRCT repeats in Rtt107. It is believed that Rtt107 may act as a scaffold during DNA damage repair (17, 19, 46). In support of this hypothesis, Rtt107 was found to interact with various factors and enzymes involved in the DNA damage response, such as Slx4, Rad55, Rtt101, and Smc5/6.

One of the best characterized binding partners of Rtt107 is Slx4. The interaction between Rtt107 and Slx4 is particularly important for the resistance to DNA alkylation damage (7). Recent studies have shown, however, that Rtt107 also has Slx4-independent functions. The Smc5/6 complex links Rtt107 with double-strand break repair. Rtt107 can localize on double-strand breaks and affects the efficiency of sister chromatid recombination (10, 11). Intriguingly, the four N-terminal BRCT domains in Rtt107 are sufficient for the interactions with both Slx4 and Smc5/6. These data suggest that the Rtt107 scaffold may bind and exchange different partners in response to different types of DNA lesions.

Additionally, there are no phospho-recognition modules in the four N-terminal BRCT domains of Rtt107. This finding indicates that some BRCT domains can interact with binding partners by using surfaces other than the phospho-recognition pockets. The best characterized examples are the interactions between 53BP1 and p53 (35) and those between DNA ligase IV and XRCC4 (22). Both of these interactions involve large flexible structures that are not well conserved in the BRCT family. Our data show that each BRCT domain in C-terminal Rtt107 shows considerable differences from canonical BRCT domains. The structural flexibility of BRCT5 and BRCT6 suggests that they may interact with additional partners using surfaces other than the γH2A-binding pocket during DNA damage repair. Identifying these partners may provide new insight to better understand the functions of Rtt107 during DNA repair. Future work should focus on answering...
the question of how the multiple BRCT domains in Rtt107 coordinate to recruit binding partners during the DNA damage repair process.

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