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REVIEW

Multi-BRCT scaffolds use distinct strategies to support genome maintenance

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ABSTRACT

Genome maintenance requires coordinated actions of diverse DNA metabolism processes. Scaffolding proteins, such as those containing multiple BRCT domains, can influence these processes by collaborating with numerous partners. The best-studied examples of multi-BRCT scaffolds are the budding yeast Dpb11 and its homologues in other organisms, which regulate DNA replication, repair, and damage checkpoints. Recent studies have shed light on another group of multi-BRCT scaffolds, including Rtt107 in budding yeast and related proteins in other organisms. These proteins also influence several DNA metabolism pathways, though they use strategies unlike those employed by the Dpb11 family of proteins. Yet, at the same time, these 2 classes of multi-BRCT proteins can collaborate under specific situations. This review summarizes recent advances in our understanding of how these multi-BRCT proteins function in distinct manners and how they collaborate, with a focus on Dpb11 and Rtt107.

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Introduction

A critical aspect of genome maintenance is faithful DNA replication. It begins with the assembly of replisomes that are comprised of replicative enzymes and their cofactors. This multi-step process relies on a conserved cascade of protein-protein interactions involving more than 30 proteins.[1] Once formed, replisomes collaborate with another group of proteins that help to manage replication perturbation. These proteins, referred to as replication accessory factors, include DNA metabolism enzymes, protein modification enzymes, and scaffold proteins. DNA metabolism enzymes, such as nucleases and helicases, help eliminate DNA lesions, protein barriers, and secondary DNA structures that block replisome progression.[2,3] Protein modification enzymes conjugate substrates with small modifiers, such as phosphate and acetyl groups, or large modifiers, such as ubiquitin and SUMO, and can generate fast and diverse effects on many substrates in a coordinated manner to facilitate replication. Unlike DNA metabolism and protein modification enzymes, scaffold proteins usually do not possess enzymatic activities; rather they assemble protein complexes needed for sophisticated DNA transactions or signaling events. As such, scaffold proteins are central for the extensive network of interactions found among replisome members and replication accessory factors. Prominent scaffolds important for both replisome assembly and progression include proteins with multiple BRCT domains.

The BRCT (90–100 amino acids) domain was initially found in the tumor suppressor protein BRCA1, and has been subsequently identified in more than 2 dozen proteins in bacteria, archaea, and eukaryotes.[4-7] Biochemical and sequence analyses suggest that BRCT domains mostly support protein-protein interactions and that BRCT containing proteins act almost exclusively in genome maintenance, with many involved in replication.[6,7] For several of these proteins, 2 BRCT domains are connected by a linker of up to 60 amino acids to form tandem BRCT (or tBRCT). Some tBRCTs bind phospho-peptides wherein both units determine binding specificity.[8,9] As such, tBRCTs often act in response to kinase signals by establishing situation-specific protein-protein interactions that facilitate replication and response to DNA damage.

While most BRCT proteins contain only 1 or 2 such domains, 2 classes contain 4 or more.[6,7] The first includes the budding yeast Dpb11, the fission yeast Cut5/Rad4, and the vertebrate TopBP1. Members of this class share similar roles in DNA replication and repair as well as DNA damage checkpoint response, with TopBP1 acquiring more extensive functions compared to its yeast counterparts.[10] The second class includes the budding yeast Rtt107, the fission yeast Brc1, and vertebrate PTIP. Recent studies have begun to delineate the shared and distinct features of these of BRCT scaffolds, and how they collaborate with Dpb11 and related proteins to execute specific functions. In the sections below, we summarize these features with a focus on the budding yeast Dpb11 and Rtt107.

Dpb11 and its homologs function as molecular bridges and in other capacities

Dpb11 and Cut5 in replication initiation

The budding yeast Dpb11 and the fission yeast Cut5 contain 4 BRCT domains arranged as 2 pairs of tBRCT, named BRCT1-2 and BRCT3-4. During replisome assembly, S-CDK mediated

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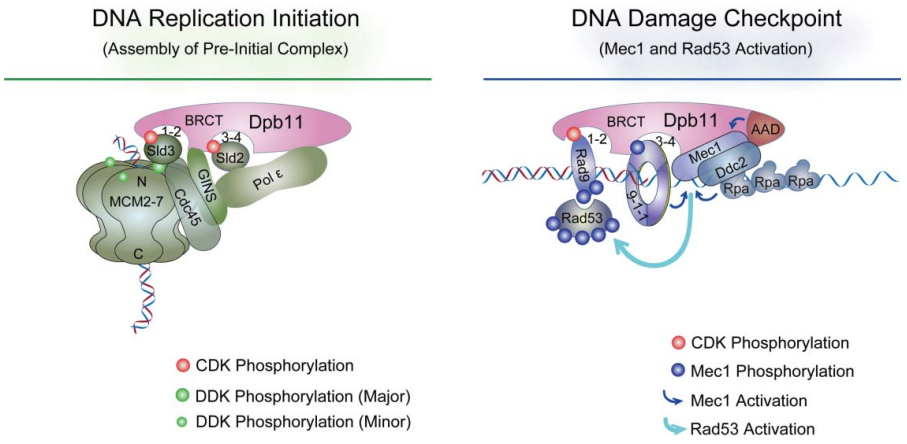

Figure 1. The roles of Dpb11 in DNA replication initiation and DNA damage checkpoint. The four BRCT domains of Dpb11 are depicted as 2 tBRCT pairs (BRCT1-2 and 3–4). As described in the text, Dpb11 binds Sld2 and Sld3, both phosphorylated by S-CDK, during DNA replication initiation (left panel).[13,14] Dpb11 also interacts with GINS and Pol epsilon. Sld3, in complex with Cdc45, recognizes and interacts with MCM that is phosphorylated by DDK (Dbf4-dependent kinase).[16,17] MCM, Cdc45 and GINS form the CMG complex that catalyzes template strand unwinding during replication.[129] In DNA damage checkpoint (right panel), Dpb11 binds 9-1-1 that is located at 5′ ss- and ds-DNA junction and phosphorylated by Mec1.[24,26] Dpb11 also binds to CDK-phosphorylated Rad9, which can associate with chromatin by interacting with γH2A and H3 with K79 methylation (not depicted here).[25,26] Subsequently Mec1 is activated by Ddc2, Ddc1 of the 9-1-1 complex, and the AAD (ATR-activation domain) of Dpb11.[29,30,130] Mec1 then phosphorylates and activates Rad53, which is recruited to DNA lesion sites by its association with phosphorylated Rad9.[31,32]

phosphorylation of the replication factors Sld3 and Sld2 (or Drc1) enables them to bind to BRCT3-4 and BRCT1-2, respectively (Fig. 1).[11-14] In addition, the region between the 2 tBRCTs in Dpb11 interacts with the replicative helicase cofactor GINS (Fig. 1).[15] A complex formed by Dpb11, GINS, Sld2, and the replicative polymerase epsilon is detected during S phase.[15,16] In the meantime, Sld3 binds to the DNA helicase MCM at replication origins (Fig. 1).[17,18] Consequently, Dpb11, in conjunction with Sld2 and Sld3, plays a major role in delivering polymerase epsilon and GINS to the origin-loaded MCM, enabling an important intermediate step during replisome assembly.

Dpb11 and Cut5 likely contribute to replication initiation by other means. For example, a recent study has implicated Dpb11 in delivering another MCM cofactor Cdc45 to MCM, as well as in ssDNA binding, which may facilitate the hand-off of GINS from Dpb11 to MCM.[19] The ssDNA binding feature appears to be conserved in TopBP1.[20] In addition, an association between Cut5 and the replication factor Mcm10 appears to facilitate the latter to be recruited to replication origins.[21] Understanding whether these additional interactions and functions are conserved and how they are temporally controlled will further clarify how various functions of Dpb11 and Cut5 are integrated during replication initiation.

Dpb11 and Cut5 in DNA checkpoint response

Upon replication perturbation or stress, Dpb11 and Cut5 engage 2 DNA damage checkpoint adaptor proteins: Rad9 (*S. p.* Crb2) and the 9-1-1 complex (Fig. 1).[22,23,24,25] Both adaptors transmit signals from the apical checkpoint kinase, Mec1 (*S. p.* Rad3), to a downstream effector kinase, Rad53 (*S. p.* Cds1).[26] Using budding yeast as an example, Mec1 and its partner Ddc2 localize to DNA lesion sites via interactions with RPA coated single strand DNA (ssDNA) (Fig. 1).[27] Mec1 can phosphorylate the Ddc1 subunit of the 9-1-1 complex that localizes to the ssDNA/dsDNA junctions at DNA lesions (Fig. 1).[26] Phosphorylated Ddc1 then binds to BRCT3-4 of Dpb11, helping to recruit the latter to DNA lesions.[24] In the meantime, S-CDK phosphorylation of Rad9 permits its association with BRCT1-2 of Dpb11 (Fig. 1).[25] In this manner, Dpb11 serves as a bridge between Rad9 and 9-1-1. Interestingly, both 9-1-1 and Dpb11 possess a region that can directly stimulate Mec1 kinase activity and is termed AAD (ATR activation domain).[28-30] One consequence of Mec1 activation is to hyper-phosphorylate Rad9, which then binds to and recruits Rad53 to the vicinity of Mec1 (Fig. 1).[31,32] Once there, Rad53 is phosphorylated and activated by Mec1 (Fig. 1), and diffuses to other cellular locations to phosphorylate a large number of targets, enabling a coordinated response to facilitate DNA replication and segregation.[26] In this sophisticated signaling transduction process, Dpb11 contributes to Rad53 activation by linking 2 checkpoint adaptor proteins through its tBRCT pairs and by stimulating the Mec1 kinase activity directly through a different region of the proteins.

A recent structural study has detailed the interactions between the 2 tBRCTs of Cut5 and corresponding checkpoint adaptor proteins.[23] It shows that although each BRCT repeat adopts a classical fold, the relative orientation of the 2 repeats within BRCT1-2 (perpendicular) is distinct from that in BRCT3-4 (anti-parallel), and both are distinct from canonical tBRCTs (parallel). Other unique features include the engagement of both BRCT1 and 2 with phospho-peptide, allowing association with a Crb2 dimer, and the interaction of BRCT4, but not BRCT3, with a phospho-peptide of Rad9. Some of these features appear to be conserved in TopBP1,[33,34] suggesting a common strategy by which this class of proteins engage ligands. Currently, it is unclear whether the same strategy is used for their interactions with replication factors, namely Sld2/Drc1 and Sld3, though it is worth noting that like Crb2, Sld3 also forms a dimer when in complex with its partner protein Sld7.[35] Further structural and *in vivo* studies will be needed to provide additional understanding of how different ligands are engaged with this family of scaffold proteins.

TopBP1 in replication initiation and checkpoint response, a comparison to its yeast homologs

Compared with Dpb11 and Cut5, 5 additional BRCT domains are present in TopBP1. Consequently, TopBP1 has a larger interactome, only a subset of which is conserved. For example, the N-terminal BRCTs of TopBP1, as seen for its yeast homologs, interact with the Sld3 homolog, Treslin, in a CDK-dependent manner and this interaction is critical for replication initiation.[36,37] On the other hand, while TopBP1 binds to RecQL4, a protein containing a Sld2-like domain, this binding is independent of CDK, unlike in yeasts.[38-40] During the DNA damage checkpoint response, TopBP1 deploys both conserved and vertebrate-specific interactions. As in yeasts, 2 sets of BRCT domains in TopBP1 bind to phosphorylated 9-1-1 and 53BP1 (*S.c.* Rad9) and its AAD directly stimulates ATR kinase activity.[28,41-44] Additionally, TopBP1 engages with RPA-coated single-stranded DNA and other checkpoint proteins, such as the MRN complex, BACH1/FANCJ, to be recruited to DNA damage site or to stimulate activity.[45-48] These additional interactions fit with the more complex requirements of DNA damage responses in higher eukaryotes, which depend on specific situations, such as tissue types, nutrient availability, and chromatin environment.

Dpb11 and homologs function in recombinational repair

Apart from the roles in replisome assembly and checkpoint response as described above, Dpb11 and TopBP1 also promote early steps of recombinational repair and prevent anaphase ultrafine DNA bridges.[49-53] Though detailed mechanisms remain to be elucidated, recent studies suggest that TopBP1 executes these functions by interacting with specific partner proteins. One study found that human TopBP1 binds to Polo-like kinase and stimulates phosphorylation of the recombinase Rad51 at serine 14, a modification important for Rad51 recruitment to chromatin and recombination initiation.[53] Others studies have shown that TopBP1 aids the resolution of DNA non-disjunctions by recruiting the scaffold SLX4 and Topoisomerase 2A to chromatin.[52,54] Furthermore TopBP1 can promote DNA synthesis at under-replicated regions during mitosis by unknown mechanisms.[52] Both functions help prevent the formation of DNA bridges and reduce the transmission of damaged DNA to daughter cells. Whether similar strategies are used by Dpb11 and Cut5 remain to be determined.

In summary, Dpb11 and homologs have conserved functions in DNA replication initiation, DNA checkpoint responses, and recombinational repair. They function not only as scaffolding proteins through multiple BRCT domains and linker regions, but also act as DNA binding factors and co-activators for the ATR kinase. While some of these functions have the same underlying mechanisms among Dpb11 homologs, additional mechanisms have evolved as TopBP1 has acquired additional BRCT domains and sequences. A more detailed summary of TopBP1 and its homologs has been described in 2 recent reviews.[10,55] It is clear that further elucidation of the protein-protein and protein-DNA interactions involving the conserved versus species-specific BRCT domains and other regions of the proteins will be important for generating a more integrated picture regarding their functions, and for understanding how they help to coordinate different DNA metabolism processes.

Rtt107-like proteins interact with histones and distinct sets of proteins

The budding yeast Rtt107, the fission yeast Brc1, and vertebrate PTIP all contain 6 BRCT domains (BRCT1-6). Their C-terminal BRCT5-6 forms a tBRCT with residues critical for phospho-peptide binding and is the most conserved BRCT pair among these proteins.[56-58] Recent studies demonstrate that the BRCT5-6 of all 3 proteins adopt a similar structure and directly bind to γH2A, a phosphorylated form of H2A generated by Mec1 (hATR) or its paralog Tel1 (hATM).[59-63] The interaction between γH2A and BRCT5-6 helps recruit Rtt107 and Brc1 to sites where γH2A is enriched, such as DNA double strand breaks (DSBs) and regions behind replication forks (Fig. 2).[59,64-66] A similar role may also apply to PTIP, which has been detected at sites of DNA breaks and is required for complete genome replication.[61,67,68]

The more N-terminally located BRCT1-4 domains of Rtt107, Brc1, and PTIP appear to be more divergent from each other, though all lack the conserved residues for phospho-peptide binding.[5] Thus far, different sets of partners have been found to associate with these domains on PTIP vs. Rtt107, whereas interactors for Brc1 have not been reported. PTIP partners include histone methyltransferases MLL3 and 4, the DNA nuclease Artemis, and the yeast Rad9 homolog 53BP1; these proteins collaborate with PTIP in transcriptional regulation and DSB repair pathway choices.[69-73] The N-terminal BRCT domains of Rtt107 interact with a completely different set of proteins, all of which have roles in replication stress. These include the scaffold protein Slx4, a cullin ubiquitin E3 complex composed of Rtt101, Mms1, and Mms22, and an 8-subunit SUMO E3 complex, called Smc5/6 (Fig. 2).[56-58,74] Unlike Dpb11, which simultaneously interacts with 2 partners using its 2 tBRCT domains, Rtt107 forms different complexes with its partners.[75] Currently it is unclear how Rtt107 achieves such binding exclusivity and what the biological consequences of this mode of interaction are. However recent studies suggest that Rtt107, in collaboration with its partners, serves as a replication accessory protein that influences DNA repair, replisome progression and checkpoint signaling.[56,57,75-77] In these capacities, these proteins are critical for genome stability, and studies of their functions have provided important insights into how replication accessory proteins facilitate genome duplication. The remaining sections describe new developments regarding Rtt107 and its partners.

The Rtt107 and Slx4 association and its functions in conjunction with Dpb11

Slx4 and the associated Slx1 protein interact with Rtt107 constitutively, and this interaction appears to be required for Mec1-mediated phosphorylation of Slx4 and Rtt107 under replication stress and DNA damage situations (Fig. 2).[57,78] Interestingly, upon Mec1-and S-CDK-mediated phosphorylation of Slx4,

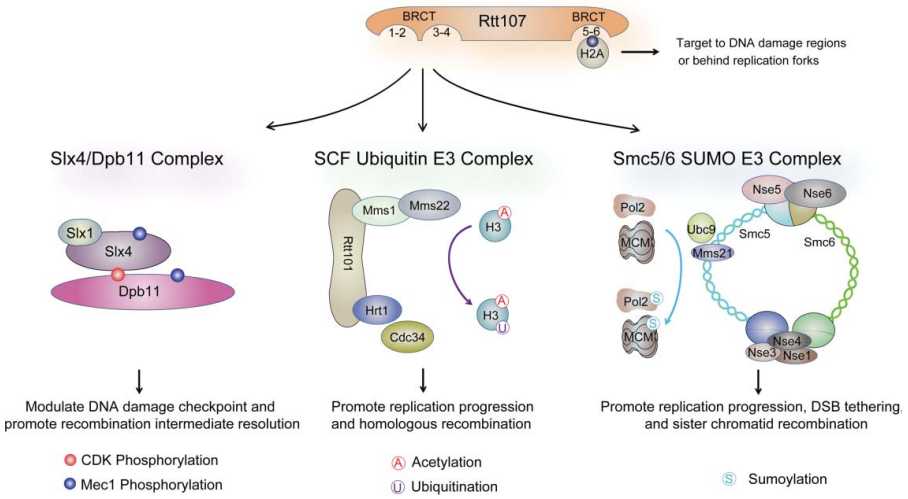

Figure 2. The Rtt107 interactome and functions. As described in the text, Rtt107 uses its BRCT5-6 to interact with γH2A for targeting at DNA breaks and regions behind replication forks. The Rtt107 N-terminal region containing BRCT1-4 interact with 3 different complexes as depicted. The main functions conferred by each of the interactions are indicated. Note that in the middle panel, the Rtt101 complex, but not Rtt107, is involved in histone ubiquitination.

Slx4 and Rtt107 become associated with Dpb11 (Fig. 2).[74] The latter modification, particularly at Serine 486 of Slx4, contributes to its interaction with Dpb11.[77,79] These studies also suggest that phosphorylated Slx4 likely directly binds to Dpb11, while Rtt107 stabilizes this interaction. The Slx4-Dpb11 interaction is conserved as phosphorylated human SLX4 also associates with TopBP1.[79]

The complex composed of Rtt107, Slx4, and Dpb11 can diminish Dpb11 interaction with Rad9, thus reducing Rad53 checkpoint activation, a process termed checkpoint dampening (Fig. 3).[77,80,81] An underlying mechanism may be that Slx4 and Rad9 compete for binding to BRCT1-2 of Dpb11, though Slx4 can also associate with BRCT3-4 of Dpb11.[77,79] It appears that BRCT1-2 and BRCT3-4 of Dpb11 can engage Slx4 and the 9-1-1 complex.[77,79,80] As such, Dpb11 can bridge Slx4 and 9-1-1 (Fig. 3). Future biochemical studies dissecting the precise manner by which Rtt107, Slx4, Dpb11, and 9-1-1 associate will provide in-depth understanding on how their association dampens Rad9 functions. As another way to reduce Rad9 function, Rtt107 also disfavors the Rad9-γH2A association, an interaction that helps recruit Rad9 to damaged chromatin (Fig. 3).[80,82] It is possible that the affinity of the tBRCT of Rad9 toward γH2A is less than that of Rtt107, a hypothesis worthy of additional testing. Despite this potential difference, BRCT5-6 of Rtt107 and tBRCT of Rad9 are interchangeable and the domain swapped constructs can partially support the functions of either protein.[83]

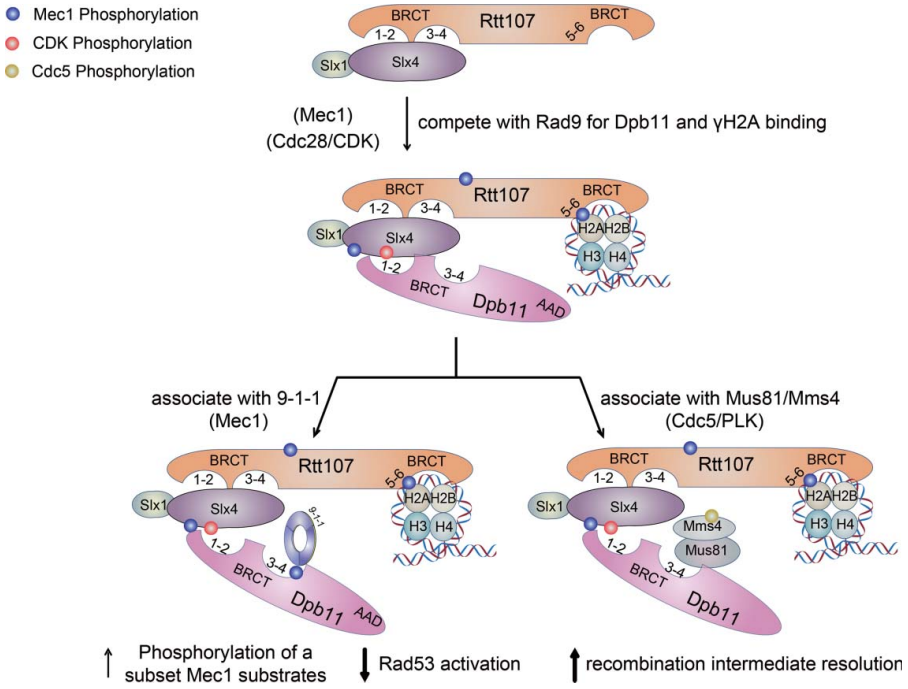

Figure 3. The Rtt107-Slx4 interaction and its functions. Three kinases promote specific interactions as depicted. Several functions of the Rtt107-Slx4-Dpb11 complex are indicated. See text for details.

Taking into account the newly found role of Dpb11 in checkpoint dampening and its classical role in checkpoint activation, how might Dpb11 juggle these 2 functions? This is related to a more general issue of how cells transit from a "pro-checkpoint" state, established upon initial exposure to stress, to an "anti-checkpoint" state following alleviation of stress. One clue has emerged from studies showing that Dpb11, Slx4 and Rtt107 can uncouple Mec1 activity from that of Rad53.[77,84,65] The Slx4-Rtt107-Dpb11 complex can actually increase Mec1-mediated phosphorylation of Rtt107, H2A and Dpb11, presumably due to the Mec1-activation function of Dpb11 (Fig. 1 and 3).[65] Such selective increase of Mec1 activity in principle can reduce Rad53 functions, because Rtt107 phosphorylation disfavors Rad53 activation (see above). As such, Slx4-Rtt107-Dpb11 plays a dual role in tuning down Rad53 activity: through dampening Rad9 and through selective activation of Mec1. It is likely that the formation of Dpb11-Slx4-Rtt107 complex marks an important turning point for Rad53 inactivation. Interestingly, Mec1 and Rad53 uncoupling also occurs in normal S phase, where Mec1, but not Rad53, is highly active.[85] It will be interesting to explore whether this uncoupling also requires the complex containing Slx4-Rtt107-Dpb11.

A second role of the Slx4-Dpb11 interaction is to facilitate the removal of recombination intermediates (RIs).[79] A recent study found that the Slx4-Dpb11-Rtt107 complex interacts with the RI resolvase Mus81-Mms4 only in G2/M phase of the cell cycle (Fig. 3).[79] Unlike mammalian SLX4, which binds directly to Mus81-Mms4 homologs,[86] yeast Slx4 interacts with this nuclease through Dpb11 (Fig. 3).[79] In addition, Mms4 phosphorylation by the polo-like kinase Cdc5 is required for Mms4-Dpb11 association, although whether this is through a direct interaction is not yet known.[79] It remains to be ascertained how Slx4-Dpb11 influences Mus81-Mms4 functions. The physical interaction between Slx4-Dpb11 and Mus81-Mms4 suggests a direct effect, perhaps through regulation of enzymatic activities or RI association. In addition, since Cdc5-mediated Mms4 phosphorylation is disfavored by Rad53, suppression of Rad53 functions by Slx4-Dpb11 may in turn boost Mms4 phosphorylation.[79,87-89] These two models are not mutually exclusive and further *in vivo* and *in vitro* experiments should provide more insight into how the association of Slx4 and Dpb11 can influence RI resolution.

Roles of Rtt107 when partnered with Rtt101 cullin ubiquitin ligase

Rtt107 also interacts with the SCF ubiquitin ligase composed of the cullin subunit Rtt101 (mammalian Cul4) and the substrate adaptor subunits Mms1 and Mms22 (Fig. 2).[56,90-92] Within this ubiquitin ligase (referred to as the Rtt101 E3 complex), Mms1 serves as a bridge between Rtt101 and Mms22 (Fig. 2).[91,92] Mms22 interacts with Rtt107 directly and this interaction requires the region of Rtt107 encompassing its N-terminal BRCTs.[75,91,92] Several lines of evidence suggest that the Rtt101 complex facilitates histone H3 modification and nucleosome assembly. During replication, newly synthesized histone H3, in complex with H4 and the histone chaperone Asf1, is acetylated at lysine 56 (H3K56ac) by Rtt109.[93,94] The modified H3 is then ubiquitinated by the Rtt101 complex at 3 lysines, weakening its association with Asf1 and allowing its transfer to downstream histone chaperones for deposition on DNA.[95,96] As H3 lysine 56 is located at the DNA entry site of the nucleosome, H3K56ac favors nucleosome removal, a step thought to enable recombinational repair behind replication forks.[97] Indeed, removing Rtt109 or the Rtt101 complex impairs recombinational repair and replication, and sensitizes cells to replication stress.[93,94,98-101] Once replication is completed, histone deacetylases Hst3 and Hst4 remove the acetyl group from H3K56ac to establish a more stable nucleosome state.[102-104] Cells lacking Hst3 and Hst4 or containing the acetylation mimetic H3 mutation (H3K56Q) exhibit defects such as persistent checkpoint and sensitivity to higher temperature and genotoxins.[102,103,105,106] The fact that H3K56 acetylation and deacetylation are both important suggests that the 2 states must be carefully regulated. Indeed, the removal of Rtt109 or the Rtt101 complex suppresses several *hst3Δ hst4Δ* defects.[93,94,107,108]

The role of Rtt107 in the above process is not completely understood. Like Rtt109 and the Rtt101 complex, Rtt107 loss also suppresses *hst3Δ hst4Δ* defects; however, Rtt107 does not affect H3 ubiquitination.[96,107] It is therefore thought that Rtt107 acts downstream of Rtt109 and Rtt101. The close relationship among these factors is also evidenced in their chromatin association. Stable association of Rtt107 with chromatin requires Rtt109 and Rtt101; conversely, Rtt101 chromatin association requires Rtt107 and Rtt109.[90] Considering that the E3 has multiple substrates, Rtt107 may collaborate with it in several functions.[91,109-111] Our recent findings suggest that when cells are under replication stress, Rtt107 and the Rtt101 complex favor the synthesis of large replicons or regions far from fired origins that occurs in the late stages of S phase.[75] This conclusion is consistent with a *Xenopus* study that implicates PTIP (or Swift) in later stages of replication.[67] Another study implicates the Rtt101 complex in regulating recombinational repair during replication stress.[112] This study and our recent work found that removal of Mrc1, which suppresses late origin firing and recombination, rescues replication stress sensitivity of mutants of Rtt107 and/or the Rtt101 complex. The suppression could be due to the combined effects of increasing recombination and reducing large replicons.[75,112] It is likely that Rtt107, in association with the Rtt101 E3 complex, promotes replisome progression and recombinational repair at stalled replication forks, ultimately contributing to replication recovery and large replicon synthesis. Future studies will be required to test these models and examine the molecular mechanisms by which H3 modification and chromatin assembly influence these processes.

The roles of Rtt107 in collaboration with Smc5/6 SUMO ligase

Among the 3 partners associated with the N-terminal BRCTs of Rtt107, the roles of the Smc5/6 complex are the least understood and the most perplexing. The core of the complex includes the Smc5 and Smc6 proteins that can adopt 3-partite configurations common to SMC proteins (Fig. 2).[113-115] The Smc5-6 proteins use their globular head and hinge domains and long coiled coil arm to interact with 6 Non-SMC elements, Nse1-6: the Nse1-3-4 subcomplex interacts with the Smc5/6

head regions, the Nse5-6 complex with the Smc5/6 hinge regions, and Nse2 (or Mms21) binds to the arm of Smc5.[114-117] Besides the ATPase activity of the Smc5/6 head regions, thought to regulate the loading of Smc5/6 onto chromatin,[113,118,119] the SUMO E3 ligase activity of the Mms21 subunit is also conserved.[115,120,121]

Rtt107 appears to interact with the Smc5/6 complex through the Nse6 subunit based on yeast 2-hybrid analysis.[122] As mutants of the Smc5/6 complex do not show increased Rad53 levels or defects associated with reduced H3K56ac levels, its association with Rtt107 unlikely affects checkpoint dampening or the histone modification functions assigned to Slx4 and the Rtt101 complex, respectively.[75] This conclusion concurs with the physical separation of the Rtt107-Smc5/6 complex from that of Rtt107-Slx4 and Rtt107-Rtt101.[75] One function of the Rtt107 and Smc5/6 association appears to DSB targeting (Fig. 2).[64,76,122] Their inter-dependence in DSB localization implicates both protein entities in recombinational repair and DSB tethering to the nuclear envelope [56,123] (Fig. 2).

The Rtt107 and Smc5/6 interaction also contributes to replication under replication stress (Fig. 2). They both affect the sumoylation of DNA polymerase epsilon and MCM helicase subunits (Fig. 2).[75] As Rtt107 interacts with these substrates, it may facilitate Smc5/6-mediated transfer of SUMO to these proteins.[75] Whether and how the observed sumoylation events affect replication remains to be addressed, though several lines of evidence suggest this may be related to the replication of large replicons. For example, replication stress sensitivity of *smc5/6* mutants is suppressed by reducing the size of large replicons achieved by firing hundreds of repressed origins.[75] We envision that Rtt107 partners with both Smc5/6 and Rtt101 complexes to promote replication progression using SUMO and ubiquitin modifications, respectively. Testing this model will likely shed light on the roles of Rtt107 and the 2 E3s in replication.

Conclusions and perspectives

Studies of multi-BRCT scaffolds and their partners have elucidated the various strategies used by cells to duplicate the genome. In the above sections, we have mainly summarized recent findings detailing the roles of Dpb11 and Rtt107, and some of their interactors. While both contribute to DNA replication, Dpb11 plays a critical role in initiation and Rtt107 promotes progression in collaboration with its ubiquitin and SUMO E3 partners. On the other hand, Dpb11 and Rtt107 cooperate to prevent checkpoint over-activation. Finally, both Dpb11 and Rtt107 regulate DNA repair, but through different mechanisms. While these findings highlight their importance in genome maintenance and the complexity of their actions, many mechanistic questions remain to be addressed as exemplified above. Answering these questions will provide insight into how Dpb11 and Rtt107 execute diverse, coordinated and antagonistic functions in genome maintenance.

As described earlier, a good degree of functional conservation has been observed for Dpb11 and homologs. In comparison, there appears to be fewer similarities between Rtt107 and its homologs, though some common features have been noted. In particular, BRCT5-6 targets these proteins to γH2A at DNA lesions. In addition, Brc1 acts in the same pathway as Mms22[124] and its overexpression suppresses the DNA damage sensitivity of *smc6* mutants.[125] Furthermore, like Rtt107, Brc1 and PTIP are implicated in replication fork stability or replication stress responses, and Brc1 influences nuclease pathways.[66-68,126,127] Despite these similarities, differences have been noted among Rtt107, Brc1 and PTIP. For example, unlike Rtt107, Brc1 does not have a checkpoint-dampening function and the genetic interaction profiles of Brc1 and Rtt107 mutants are quite different.[66,128] Moreover, unlike the positive influence of Rtt107 in homologous recombination, PTIP disfavors this repair pathway through its interaction with 53BP1 and the Artemis nucleases.[71,73] These diverse functions may have evolved to suit the specific needs of each particular organism. Future studies in multiple organisms will better elucidate the similarities and differences among these multi-function proteins, and their roles in genome maintenance and human diseases.

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

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