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Review

Review

Factors forming the BRCA1-A complex orchestrate BRCA1 recruitment to the sites of DNA damage

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Abstract

Sustaining genomic integrity is essential for preventing onset of cancers. Therefore, human cells evolve to have refined biological pathways to defend genetic materials from various genomic insults. DNA damage response and DNA repair pathways essential for genome maintenance are accomplished by cooperative executions of multiple factors including breast cancer type 1 susceptibility protein (BRCA1). *BRCA1* is initially identified as an altered gene in the hereditary breast cancer patients. Since then, tremendous efforts to understand the functions of BRAC1 reveal that BRCA1 is found in distinct complexes, including BRCA1-A, BRCA1-B, BRCA1-C, and the BRCA1/PALB2/BRCA2 complex, and plays diverse roles in a context-dependent manner. Among the complexes, BRCA1-A is critical for BRCA1 recruitment to the sites of DNA damage. Factors comprising the BRCA1-A include RAP80, CCDC98/Abraxas, BRCC36, BRCC45, BARD1, BRCA1, and MERIT40, a RAP80-associated factor. In this review, we summarize recent findings of the factors that form the BRCA1-A complex.

Key words: BRCA1 complexes, BRCA1, RAP80, MERIT40, DNA damage

Introduction

BRCA1 (breast cancer type 1 susceptibility protein) is one of essential tumor suppressor genes, and mutations in the *BRCA1* gene increase the breast and ovarian cancer susceptibility. *BRCA1* was initially identified as a mutated gene in familial breast and ovarian cancers [1]. Since its discovery, extensive studies on the gene have revealed that BRCA1 plays critical roles in diverse cellular processes that ensure genome stability and control cell cycle checkpoints. Especially, BRCA1 is essential for repairing double strand DNA breaks (DSBs) through homologous recombination (HR). DSB could be induced by intrinsic factors, including reactive oxygen species and replication fork stalling, and extrinsic factors, including genotoxic chemicals, ultraviolet light, and ionizing radiation (IR) [2]. Inappropriate DSB repair results in genome instability and subsequent apoptosis or tumorigenesis [3]. To maintain genome integrity against deleterious DSBs, cells have evolved to have elegant two major categories of DSB repair. One is non-homologous end joining (NHEJ) and the other is HR. NHEJ mediates the direct ligation of the broken DNA ends without homologous template so it could be a low-fidelity pathway. In contrast, HR is homology-directed repair

with a sequence homology donor, and thus it is considered to be accurate and error-free [4]. Because BRCA1 is an indispensable component of HR repair mechanism, pathogenic mutations on *BRCA1* gene can lead to failure in proper DSB repair, which eventually develops cancers due to genomic instability [5]. As a tumor suppressor, the physiological importance of BRCA1 is supported by the evidence showing that 55%–65% of women with pathogenic BRCA1 mutations have a risk of developing breast cancer and 39% of women who have dangerous BRCA1 mutations may develop ovarian cancer by the age of 70 [6,7].

BRCA1 gene is located on chromosome 17q21.3 and encodes 1863 amino acids [1]. BRCA1 has two functional annotated domains at its termini, a RING (*R*eally *I*nteresting *N*ew *G*ene) domain at the N-terminus and two BRCT (*BR*CA1-*C T*erminal) domains at the C-terminus. Mutations in these two domains are clinically important. The N-terminal RING domain is implicated in protein ubiquitylation through its interaction with E2 enzyme and is also important for the interaction with BARD1 (BRCA1-associated RING domain protein 1) [8,9]. However, the clinical implication of the RING domain has not been clarified yet. E3 ligase dead I26A synthetic mutant of BRCA1, which cannot interact with E2 enzyme, merely affects tumor development frequency and genome stability [10]. On the other hand, a pathogenic C61G mutant that disrupts not only E3 activity but also the interaction with BARD1 leads to DNA repair deficiency and cancers in mice [11]. Given the discrepancy in the roles of the RING domain of BRCA1 in cancer development, the clinical implication of BRCA1 E3 ligase activity in DNA damage repair and tumor suppression remains to be elucidated. Next, the C-terminal of BRCA1 contains two BRCT domains, which bind to phosphopeptides by recognizing a phospho-SPxF motif [12–14]. To regulate multiple cellular processes, BRCA1 interacts with various DNA damage response (DDR) and repair factors through the BRCT domains [12,14]. Among the BRCT-mediated interacting proteins, three *bona fide* associated proteins, Abraxas, BACH1, and CtIP, are associated with BRCT domains of BRCA1 in a phosphorylation-dependent manner, and each of them comprises the distinct BRCA1-containing complex, which plays unique roles in DNA damage repair mechanism [12,14]. Although the exact roles of three different protein complexes harboring BRCA1 remain elusive, significant advancement has been made in recent studies, which will be discussed below.

Diverse BRCA1 Complexes

BRCA1 is a key component of at least four elusive complexes, which are RAP80-containing BRCA1-A, BACH1-harboring BRCA1-B, CtIP-holding BRCA1-C complex, and BRCA1/PALB2 (also known as FANCN)/BRCA2 complex (**Fig. 1**). BRCA1-A complex is composed of ubiquitin interacting motif (UIM) containing protein RAP80, adapter protein Abraxas, MERIT40 (mediator of RAP80 interactions and targeting 40 kDa, also known as NBA1), BRCC45, and deubiquitinylating enzyme (DUB) BRCC36 [15–22]. Abraxas acts as a central adapter linking BRCA1 to other components in the BRCA1-A complex [15,17,23]. C-terminal phosphoserine (p-S406) of Abraxas is responsible for the interaction with BRCT domain of BRCA1 [15,17,19,22]. BRCA1-A is thought to target BRCA1 to the sites of DSB through interaction with UIMs of RAP80, which recognize the Lys63 poly-ubiquitin chains of the H2AX [17,18,24]. The formation of Lys63-linked poly-ubiquitin chains is initiated by ATM-mediated H2AX phosphorylation near the DNA lesions [25,26]. Then, mediator of DNA damage checkpoint protein 1 (MDC1) is subsequently recruited to H2AX through its BRCT domains [25], and the DNA damage-induced phosphorylation of MDC1 on its three conserved TQxF clusters promotes assembly of RNF8 through its FHA domain [27]. Recruited RNF8 promotes histone H1 K63-linked ubiquitination [28] and this ubiquitination on the chromatin flanking DNA damage sites enables its recognition by a second E3 ubiquitin ligase RNF168, through its motif interacting with ubiquitin (MIU) and UIM-/MIU-related ubiquitin-binding domain, allowing amplification of the ubiquitination signal near the sites of DSB [29]. Lys63-linked ubiquitin chains play a role as binding sites for RAP80 through its two UIMs, and direct BRCA1-A complex to the sites of DSB [30–34]. Additionally, BRCA1-A has been shown to be required for proper G2/M checkpoint activation in response to DNA damage, and preventing over-resection of DSB ends [15–17,22,24,35].

BRCA1-B is composed of BRCA1, TopBP1, and BACH1, and is involved in DNA replication stress-induced checkpoint and DNA interstrand crosslink (ICL) repair [36]. BACH1 interacts with BRCT domain of BRCA1 through phosphoserine (p-S990) and its phosphorylation occurs in a cell-cycle-dependent manner, with the strongest interaction between BRCA1 and BACH1 during S-phase [14,37]. Defect in BACH1 shows reduced HR and delayed DNA repair [37]. And as a member of the Fanconi anemia (FA) proteins, BACH1 (also known as Fanconi anemia group J protein) also plays a role in ICL repair [38]. TopBP1, associated with BACH1 during the S-phase, is known to be required for DNA replication progress and replication checkpoint [39]. Since all three components form a complex in S-phase, and TopBP1 is important for proper replication, the BRCA1-B complex could work for sound S-phase progression by replication checkpoint control [39–41].

BRCA1-C comprises BRCA1, CtIP, and MRE11–RAD50–NBS1 complex (MRN complex). BRCA1-C complex plays a role in DSB end resection. Ser327 of CtIP is phosphorylated and subsequently interacts with BRCA1 through BRCT domain in the S/G2 phase [42]. Although CtIP S327 mutant, which fails to interact with BRCA1, does not inhibit the initiation of end resection, binding of CtIP with BRCA1 seems to modulate the speed of DNA end resection [43]. In addition, CtIP interacts with MRN and stimulates the DNA end resection [44]. DSB end resection is essential for early step of HR-mediated DNA repair. By cooperating with the 5′→3′ double stranded DNA exonuclease, ExoI, and the Bloom helicase [45–47], BRCA1-C complex is required for fine-tuning DNA end resection.

Lastly, BRCA1 has been identified in another complex containing PALB2 and BRCA2 [48,49]. The coiled-coil domain (CCD) of BRCA1 is responsible for its interaction with PALB2, which is associated with BRCA2 and RAD51 [48,49]. By forming the BRCA1/PALB2/BRCA2 complex, BRCA1 was proposed to guide the recruitment of BRCA2 to the sites of DSB. Then, BRCA2 recruits RAD51 through direct interaction and promotes the loading of RAD51 to ssDNA for efficient HR-mediated repair [48,49].

The molecular mechanism of BRCA1-A recruitment seems clear, but a number of questions still remain to be answered. One of remaining questions is about the involvement of SUMOylation (SUMO, Small ubiquitin-related modifier) in BRCA1 localization to DSB sites. Recent studies showed that protein inhibitor of activated STAT protein (PIAS1)/4-mediated SUMOylation at DSB sites is important for DDR [50,51] and another work revealed that RAP80 interacts with hybrid SUMO-ubiquitin chain 80 folds stronger than Lys63-linked ubiquitin via its SUMO-interacting motif (SIM) and two UIM domains [52]. PIAS1 and PIAS4 are localized at DNA damage

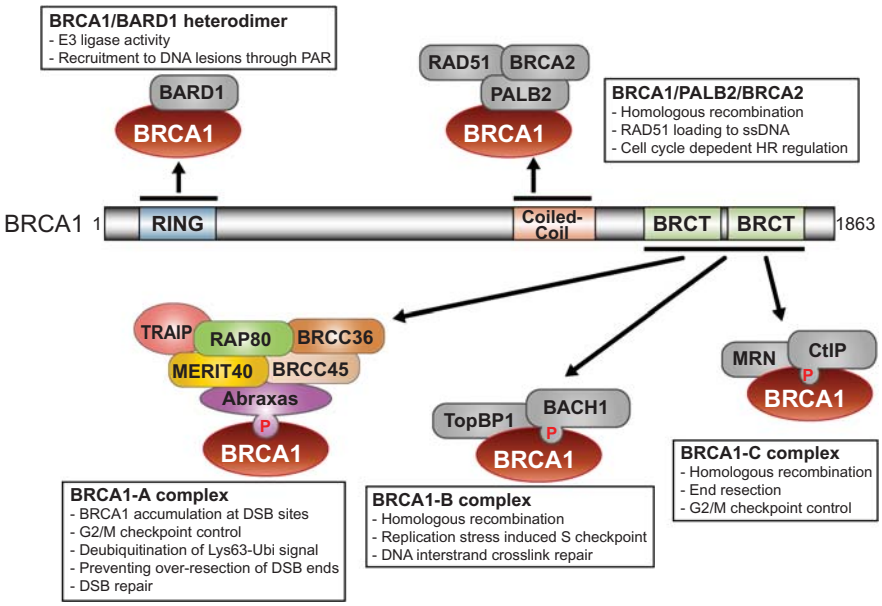

Figure 1. Schematic representation of BRCA1 domain structure and multiple BRCA1-containing complexes RING domain, coiled-coil domain (CCD), and BRCT domain of BRCA1 are illustrated. These domains are essential for the formation of multiple BRCA1 complexes. By forming heterodimer through each RING domain, BRCA1–BARD1 interaction is vital for ubiquitin E3 ligase activity of BRCA1. CCD is responsible for the formation of BRCA1/PALB2/BRCA2 complex. And the rest of complexes, BRCA1-A, -B, and -C, are connected with BRAC1 in a phosphorylation-dependent manner through BRCT domains. In response to DNA damage, BRCA1 associates with diverse factors to form BRCA1 complexes and travels to the sites of DNA damage to repair the lesions through HR. Diverse functions of BRCA1-containing complexes are depicted.

sites and promote BRCA1 and 53BP1 recruitment and SUMOylation, suggesting that the signal cascade from H2AX to RAP80 is not simple and there will be additional players for the recruitment. Consistent with this, further studies showed that SUMO-targeted ubiquitin E3 ligase RNF4 is recruited to sites of DNA damage and required for DDR and repair [53,54]. RNF4 binds with PIAS1/4-dependent SUMO chain through its SIMs and promotes ubiquitination and subsequent proteasome-mediated degradation of DDR proteins, such as MDC1 and RPA. In addition, RNF4 is thought to induce the formation of hybrid SUMO-ubiquitin chain and the recruitment of RAP80 at DSB sites [52]. With the facts that the depletion of RNF4 causes enhanced accumulation of RPA and BrdU on damaged DNA [53] and the knockdown of RAP80 induces RPA, Rad51, and BrdU incorporation at damage sites due to excessive DSB end processing [35], each of RNF4 and BRCA1-A complex might play a role in DDR-terminating by its SUMO-targeted ubiquitination or its deubiquitination activity of BRCC36 [55]. Taken together, these data suggest that DDR and BRCA1 recruitment are controlled by complex interplay between ubiquitination and SUMOylation, and the roles of PIAS1/4 and RNF4 as well as the timing and substrate of modification could be the missing points of molecular mechanism in BRCA1 function.

The recruitment of BRCA1-B and -C complexes to DSB seems to be mediated by binding of poly(ADP)-ribose (PAR) [56]. BRCA1 is recruited to DSB end at the early stage of DDR and the depletion of RAP80 does not abolish DNA damage-induced BRCA1 foci formation completely, indicating that there are different types of BRCA1 complexes to form foci at the sites of DNA damage [35]. Recent studies revealed that BRCT repeats of BARD1 interact with PARP1-mediated PAR and this binding recruits BRCA1/BARD1 heterodimers to DSB end at early stage [56]. Compared with RAP80-mediated BRCA1 recruitment, which is H2AX-dependent and relatively late step of DDR, the PAR-dependent recruitment of BRCA1 is faster and H2AX-independent. More recent research showed that BARD1 BRCT repeats interact with K9-dimethylated histone H3 (H3K9me2) via HP1, and the interaction is important for the retention of BRCA1 and BARD1 at sites of DNA damage [57]. And in the case of BRCA1-C complex, the direct binding with MDC1 and NBS1 could be the way to BRCA1-C localization. NBS1, which is a component of MRN complex, directly interacts with MDC1, which mediates the retention of NBS1 at the DSB-flanking chromatin [58–60].

Whereas the BRCA1-A complex travels to sites of DNA damage by recognizing ubiquitinated H2AX, the BRCA1-B and -C complexes move through PARP1-dependent PARylation [poly(ADP-ribosyl)ation] at the sites of DNA damage [16,18,24,30,56]. Based on the research data showing that accumulation of PAR is observed earlier than H2AX phosphorylation, the BRCA1-B and -C complexes might come to the sites of DNA damage prior to the BRCA1-A complex [56]. Interestingly, it was reported that BRCA1-B and -C complexes promote end resection and thus boost HR, but BRCA1-A prevents end resection, resulting in reduced HR [35,43,61]. Considering the findings described above, BRCA1, which is found in distinct complexes, plays a key role in fine-tuning the degree of HR.

Factors Found in the BRCA1-A Complex

As previously mentioned, BRCA1-A complex is recruited to sites of DNA damage through the interaction between UIM domains of RAP80 and K63-linked ubiquitin chains on H2AX. These findings were initiated by the identification of components in RAP80-harboring BRCA1 complex, known as the BRCA1-A complex. Here, we will discuss each component of BRCA1-A complex in detail.

RAP80

RAP80 is a key protein in the DDR because it recruits BRCA1-A complex to DNA damage sites by recognizing Lys63 poly-ubiquitin chains via its UIM motifs [17,18,24]. RAP80 was originally found as a retinoid-related testis-associated protein using yeast two hybrid screen [62]. Later, several groups demonstrated RAP80 as a BRCA1-associated protein, which regulates DDRs [16–18,24,30]. Human RAP80 protein consists of 719 amino acids and has tandem UIMs and SIM at the N-terminus and tandem zinc finger domains at the C-terminus [63]. Translocation of RAP80 to the sites of DNA damage is well defined [16–18,63]. RAP80 recognizes Lys63-linked ubiquitination and SUMOylation at DNA damage sites via UIMs and SIM of RAP80 [63]. The length between tandem UIMs of RAP80 should be precise to recognize Lys63-linked ubiquitin chain [64,65]. In consistent with the function of RAP80 to recruit BRCA1-A complex to DSB sites, BRCA1-A complex foci formations are significantly decreased in RAP80-null cells [66–68]. Recent studies demonstrated the relationship between FA pathway and RAP80 [69,70]. It was reported that FA deficiency increases foci formation of RAP80, 53BP1, and RIF1, and promotes NHEJ repair induced by ICLs [69]. Another research group showed that RAP80 interacts with Lys63-linked poly-ubiquitin chain of FANCG, which is one of the FA core complex [70]. Additionally, RAP80–BRCC36 complex mediates deuibiquitination of FANCG and impedes HR repair. However, the underlying mechanisms of RAP80 functions in NHEJ and HR remain largely elusive.

Abraxas/CCDD98

Abraxas was identified by affinity purification using the BRCT domain of BRCA1 and subsequent mass spectrometry analysis in three different groups [15,17,22]. Abraxas is a central adapter protein in the BRCA1-A complex, as it has several domains necessary for the interactions between BRCA1 and other components. Mpr-1/Pad-1 N-terminal (MPN) domain at the N-terminus of Abraxas is important for the interactions with MERIT40, BRCC45, and RAP80. In the C-terminus, Abraxas contains pSPxF motif, which is responsible for the interaction of the BRCT domain with BRCA1. In addition, CCDs in both Abraxas and BRCC36 mediate Abraxas/BRCC36 dimer formation [36]. Association of Abraxas with BRCA1 is crucial for the recruitment of BRCA1 to DNA damage sites, and downstream BRCA1 functions as a tumor suppressor. Even though viability and birth rate do not change in Abraxas-null mice, Abraxas-null and heterozygous mice display over 60% tumor incidence, suggesting the role of Abraxas in tumor suppression [71]. Abraxas-null cells showed increased spontaneous DNA breaks and IR-induced chromosome aberrations, and the recovery of Abraxas deficiency with mutants lacking BRCA1 binding (S406A) fails to rescue genomic integrity. Identification of BRCA1 binding-abolished Abraxas mutants in human cancer patients indicates the importance of interaction between BRCA1 and Abraxas in tumor suppression. Additionally, cells from Abraxas-knockout mice displayed hypersensitivity to DNA-damaging agents, such as IR, mitomycin C, and PARP inhibitor. As demonstrated in all works with mice and human patients, Abraxas is required for efficient DNA repair and plays a critical role in maintaining genome stability and tumor suppression [71].

BRCC36/45

BRCC36 and BRCC45 were isolated as components of BRCA1 complex with affinity purification, followed by mass spectrometry analysis [72]. In early studies, these two BRCC proteins were thought to enhance E3 ligase activity of BRCA1/BARD1 heterodimer, and depletion of both BRCC proteins resulted in enhanced sensitivity to IR and defects in G2/M checkpoint. In further studies, BRCC36 was identified as a member of the JAMM/MPN$^+$ family of zinc metalloproteases, which are Lys63-specific DUBs, and Abraxas and BRCC45 are vital for BRCC36 DUB activity within the RAP80 complex [23,55,73]. Despite unclear role of BRCC36 DUB in the increment of BRCA1 E3 ligase activity, BRCC36 within RAP80 complex is thought to eliminate or edit chromatin-associated Lys63-linked ubiquitin chains at DSB sites to terminate DDR pathway. However, the molecular basis of BRCC36 functions as a DUB remains elusive. Another interesting feature of BRCC36 is that the BRCC36 interacts with Abraxas-like protein KIAA0157 in cytoplasm. Although the binding of Abraxas to BRCC36 is not sufficient to activate BRCC36, the association of KIAA0157 with BRCC36 seems to fully activate BRCC36, and the DUB activity is higher than that of BRCC36-harboring RAP80 complex [73]. Abraxas and KIAA0157 determine the subcellular localization of BRCC36, and the depletion of KIAA0157 enhances the assembly of BRCC36-containing BRCA1-A complex to DNA damage sites. *BRCC45* is identified as the brain- and reproductive organs-specific gene and its transcription is regulated by several stresses, such as DNA damage agent and retinoic acid [74]. The interaction of BRCC45 with p55 TNF receptor may modulate signal transduction initiated by TNF-α [75]. In the case of BRCA1-A complex, BRCC45 interacts with MERIT40 through a C-terminal ubiquitin E2 variant domain of BRCC45 and the C-terminal conserved PxxR motif of MERIT40, which mediates the binding with MERIT40 and Abraxas [19,76]. BRCC45 is critical for maintaining the integrity of BRCA1-A complex and forming IR-induced foci of BRCA1 [19].

MERIT40

Although BRCA1-A complex recruitment to the sites of DNA damage is well defined, the functions and mechanisms of BRCA1-A complex are not well understood and controversial [77]. Some researchers suggest that BRCA1-A complex decreases end resection activity in processes of HR repair [35,61]. On the contrary, under different experimental conditions, BRCA1-A complex increases HR repair activity [17,18,78]. One possible explanation would be that the BRCA1-A complex can be regulated by different factors in different context. Here, we will discuss about MERIT40, which is identified as a factor of BRCA1-A complex.

Several research groups concurrently identified MERIT40 as one factor of BRCA1-A complex by using tandem immunoaffinity purification [19–21]. Human MERIT40 protein consists of 329 amino acids and has a von Willebrand factor A domain at the N-terminal, which mediates proteasome protein processing [79]. MERIT40 functions as a scaffolding protein between BRCA1–RAP80 complex and mediates BRCC36-dependent K63 deuibiquitination activity at the sites of DNA damage [20,76]. In addition, it was reported that MERIT40 maintains protein stability of factors forming BRCA1-A complex. As a result, MERIT40 is essential for proper DNA damage repair, G2/M checkpoint, and cell survival [19,20]. Recently, it was suggested that MERIT40 is required for ICLs repair pathways, which is independent of BRCA2 [78]. Jiang and colleagues [78] demonstrated that end resection and HR are reduced after ICLs damage in MERIT40-null cells. Another study suggested that MERIT40 might be a drug target because MERIT40 was phosphorylated by Akt, which is activated by cancer drug doxorubicin [80]. Phosphorylated MERIT40 increases the accumulation of BRCA1-A complex at sites

of DNA damage induced by doxorubicin, which is a cytotoxic chemotherapy drug. Consequently, MERIT40 positively regulates cancer cell viability by promoting DNA damage repair.

Conclusion

Here, we summarize the BRAC1 and its complexes, and take a closer look at components of BRCA1-A complex. Since the discovery of BRCA1, multiple roles of BRCA1 in DNA repair and tumor suppression have been identified through the studies not only on the BRCA1 itself but also on its interacting proteins. BRCA1 and its interacting proteins form several distinctive complexes and these combinations are crucial for the biological functions of BRCA1. And additional studies about each complex give us broad insight on how BRCA1 is recruited to DNA damage sites and suppresses tumors. But there are still many questions to be answered. Is the recruitment of each complex to damage sites based on either competition or consecutive order? Do those BRCA1 and the binding partners exist as a complex constantly? Or are those complexes shaped at the sites of damage in response to DNA damage? If so, which factors or forces drive the change of combination at each step of DNA repair? To clarify these issues, it will be important to identify unknown components in the BRCA1 complexes. There are common features in those BRCA1 complexes: the interaction between BRCA1 and its major components is mediated by phosphorylation and the recruitment of BRCA1 complexes to damage sites is regulated by other post-translational modifications (PTMs), such as ubiquitination, SUMOylation, and PARylation. Furthermore, recent study showed that the interaction change in BRCA1 and PALB2 is regulated by ubiquitination of PALB2 in a cell cycle-dependent manner [81]. Interestingly, a number of components, including BRCA1 itself, have the RING domain, which activates the E3 ligase activity of ubiquitin-like modifiers (UBLs) or acts as substrates of such modifiers. The PTMs of UBLs, in addition to phosphorylation and PARylation, occurred in BRCA1 pathway are time-specific and reversible, which controls the protein–protein interaction, localization, and stability [82]. But the exact roles played by such PTMs in the biological functions of BRCA1 are still obscure. We expect that finding new players and establishing the function of PTMs will shed light on clarifying the diverse functions of BRCA1 complexes and result in a meaningful impact on disease treatment.

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