Phosphorylation of ATR-Interacting Protein on Ser\textsuperscript{239} Mediates an Interaction with Breast-Ovarian Cancer Susceptibility 1 and Checkpoint Function

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Abstract

The signaling of DNA damage and replication stress involves a multitude of proteins, including the kinases ataxia-telangiectasia mutated (ATM) and ATM and Rad3-related (ATR), and proteins with BRCA1 COOH-terminal (BRCT) domains. The BRCT domain–containing proteins facilitate the phosphorylation of ATM/ATR substrates and can be coimmunoprecipitated with ATM or ATR. However, their mode of interaction with the ATM/ATR kinases remains elusive. Here, we show that breast-ovarian cancer susceptibility 1 (BRCA1) interacts directly with ATR-interacting protein (ATRIP), an obligate partner of ATR. The interaction involves the BRCT domains of BRCA1 and Ser\textsuperscript{239} of ATRIP, a residue that is phosphorylated in both irradiated and nonirradiated cells. Consistent with a role of BRCA1 in ATR signaling, substitution of Ser\textsuperscript{239} of ATRIP with Ala leads to a G\textsubscript{s3}-M checkpoint defect. We propose that a direct physical interaction between BRCA1 and ATRIP is required for the checkpoint function of ATR. [Cancer Res 2007;67(13):6100–5]

Introduction

Monitoring DNA replication is critical for maintaining genomic stability (1–3). DNA replication forks may stall as a result of DNA damage, low concentrations of nucleotides, or in the process of replicating specific DNA sequences (sequences that are “hard to replicate”). In eukaryotes, the DNA replication checkpoint prevents collapse of stalled forks (a term referring to dissociation of the replication machinery from DNA) and inhibits entry into mitosis, thus ensuring completion of DNA replication (2–6). The key transducing kinase of the DNA replication checkpoint is the ataxia-telangiectasia mutated (ATM) and ATM and Rad3-related (ATR) kinase (2, 3). ATR phosphorylates several substrates, including the kinase Chk1, which inhibits the Cdc25C phosphatase and, thereby, inhibits mitotic entry (7–10).

Evidence from yeast to human cells indicates that ATR localizes to sites of stalled DNA replication forks (11, 12). This localization is mediated by the ATR-interacting protein (ATRIP), an obligate partner of ATR (13–17); however, the mechanism is being debated. In Saccharomyces cerevisiae, Lcd1/Ddc2, the yeast orthologue of ATRIP, may be recruited to stalled forks by binding to DNA via evolutionarily conserved basic residues (15). Another, nonmutually exclusive, model suggests that ATRIP binds to replication protein A (RPA), which coats the ssDNA present at sites of stalled DNA replication forks (16, 17).

In addition to ATR, ATRIP, and Chk1, several other proteins are required for the integrity of the DNA replication checkpoint, such as breast-ovarian cancer susceptibility 1 (BRCA1) and topoisomerase II binding protein 1 (TopBP1; refs. 18–22). BRCA1 and TopBP1 colocalize with ATRIP/ATR and RPA at stalled DNA replication forks (11, 17, 20–23) and both contain BRCA1 COOH-terminal (BRCT) domains, which were recently shown to be phosphopeptide-binding domains (24, 25). The physiologic ligands of the BRCT domains of TopBP1 remain elusive, but the BRCT domains of BRCA1 are known to interact with BRCA1-associated COOH-terminal helicase 1 (BACH1) and with CtBP-interacting protein (CtIP; refs. 24–26). Three-dimensional structures of BRCA1 with phosphopeptides derived from BACH1 and CtIP have been determined by X-ray crystallography and explain at the atomic level the specificity of BRCA1 for its ligands (27–30).

Here, we identify Ser\textsuperscript{239} as a novel phosphorylation site in ATRIP and show that it is recognized specifically by the BRCT domains of BRCA1. Substitution of Ser\textsuperscript{239} with Ala compromises the interaction of ATRIP with BRCA1 and the DNA replication checkpoint. Thus, our studies provide a molecular handle to begin to understand the checkpoint function of BRCA1.

Materials and Methods

Recombinant plasmids. A plasmid expressing human ATRIP in mammalian cells was constructed by cloning a PCR fragment of human atrip into the NotI and NotI restriction sites of the pIRE2 bicistronic vector (Clontech Laboratories). Ser\textsuperscript{239} of ATRIP was substituted with Ala in the context of the pIRE2-ATRIP plasmid by site-directed mutagenesis (Quick-Change mutagenesis kit, Stratagene). Small interfering RNA (siRNA)–resistant pIRE2-ATRIP wild-type and A239 plasmids were generated also by site-directed mutagenesis using the oligonucleotide 5′-GATCA-TAAGGTCCATCGTTTGTTAGATGGCATGTC-3′, which introduces silent nucleotide substitutions in the sequence of atrip that is targeted by the siRNA. Fragments of human BRCA1 or TopBP1 were expressed in Escherichia coli as glutathione S-transferase (GST) fusion proteins by cloning corresponding PCR fragments into the NcoI and NotI restriction sites of the pGEX4T1 vector (Pharmacia).

Antibodies. Primary antibodies recognizing the following proteins were used for immunoblotting, immunoprecipitation, and/or immunofluorescence: p53 binding protein 1 (53BP1) and ATRIP (Wistar Hybridoma Facility), ATRIP phosphorylated at Ser\textsuperscript{68} or at Ser\textsuperscript{239} (PhosphoSolutions), BRCA1 (Calbiochem), RPA (Lab Vision Corp. and GeneTex), TopBP1 (Abcam, Inc.), actin (Calbiochem), GST (Calbiochem), and HA.11 epitope (Covance). Secondary antibodies conjugated to alkaline phosphatase recognizing mouse IgG (heavy and light chains), rabbit IgG (Fc; Promega), or mouse κ light chains (Southern Biotechnology) were used for immunoblotting. For immunofluorescence, the secondary antibodies were conjugated to AlexaFluor 488 or 568 (Molecular Probes).
Plasmid and siRNA transfections. U2OS cells were obtained from the American Type Culture Collection. To generate stable pools, 1 × 10^6 U2OS cells/100 mm dish were transfected by calcium phosphate precipitation with 5 μg of pIRESN2 plasmid encoding siRNA-resistant hemagglutinin-tagged wild-type ATRIP or the A239 point mutant. Stable transfectants were selected by adding 0.5 mg/mL G418 in 60-mm plates were incubated with mixtures of Oligofectamine medium containing 0.25 mg/mL G418. For siRNA transfections, cells seeded in 60-mm plates were incubated with mixtures of Oligofectamine (Invitrogen) and 400 to 800 pmol control (luciferase) or one of the following siRNAs (Dharmacon): 800 pmol atrip (GGUCCAGAUAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGAUAUGA
proteins were released from the beads by boiling in 2× SDS loading buffer, resolved by SDS-PAGE, and immunoblotted.

**GST pull down assay.** GST-BRCA1 or GST-TopBP1 fusion proteins or GST alone were bound to glutathione Sepharose beads (Pharmacia). After washing, the beads were incubated with 500 ng cell extract at 4°C for 1 h in cell lysis buffer. After three washes with cell lysis buffer, proteins bound to the beads were resolved by SDS-PAGE and were immunoblotted.

**Cell cycle checkpoint assay.** Parental U2OS cells or stably transfected U2OS cells expressing hemagglutinin-tagged wild-type or Ala239 mutant ATRIP were seeded on 60-mm culture dishes. A day later, the cells were irradiated (9 Gy), and 4 h later 1 mmol/L hydroxyurea (HU) was added. 53BP1 foci were evident 5 min after irradiation in practically all cells and their number decreased thereafter, whereas ATRIP foci formed later (Fig. 1B). The fraction of these cells increased with time (from 1 to 6 h), probably reflecting a larger fraction of cells in S phase. At all time points after irradiation (5–60 min), but at later time points some cells exhibited a significant number of ATRIP foci (Fig. 1B). The number of these foci did not increase at early time points after irradiation (5–60 min), but at later time points some cells exhibited a significant number of ATRIP foci (Fig. 1A). The fraction of these cells increased with time (from 1 to 8 h), probably reflecting a larger fraction of cells in S phase. At all time points examined, the ATRIP foci also stained positive for ATRIP phosphorylated on Ser239 (Fig. 1A).

Further, at all time points examined (up to 8 h after irradiation), the vast majority of ATRIP and 53BP1 foci did not colocalize, consistent with ATRIP and 53BP1 recognizing distinct lesions (Fig. 1B and data not shown), as previously described (11, 17, 20, 22, 31).

**Phosphorylation of ATRIP on Ser239.** ATRIP contains an evolutionarily conserved sequence starting at Ser239 that shares homology proteins respond differently to irradiation. 53BP1 foci were evident 5 min after irradiation in practically all cells and their number decreased thereafter, whereas ATRIP foci formed later (Fig. 1B). Further, at all time points examined (up to 8 h after irradiation), the vast majority of ATRIP and 53BP1 foci did not colocalize, consistent with ATRIP and 53BP1 recognizing distinct lesions (Fig. 1B and data not shown). 53BP1 recognizes DNA DSBs, whereas ATRIP is likely to recognize DNA replication forks that have encountered IR-induced DNA damage lesions.

**Phosphorylation of ATRIP on Ser239.** ATRIP contains an evolutionarily conserved sequence starting at Ser239 that shares homology
with the sequences of BACH1 and CtIP that are recognized by the BRCT domains of BRCA1. Indeed, all the residues of BACH1 and CtIP that are seen in the three-dimensional structures of the BRCA1-BACH1 and BRCA1-CtIP complexes to make sequence-specific contacts with BRCA1 are present in human ATRIP (Fig. 2A; refs. 27–30). This raised the possibility that ATRIP would bind to BRCA1 with the interaction between these two proteins being mediated by an ATRIP fragment containing phospho-Ser239. To test this hypothesis, we first examined whether ATRIP is phosphorylated on Ser239. We generated a polyclonal antibody specific for phospho-Ser239 and confirmed its specificity by blotting extracts prepared from cells expressing hemagglutinin-tagged wild-type ATRIP or ATRIP containing a single substitution of Ser239 with Ala (Fig. 2B). Analysis of endogenous ATRIP using this phosphospecific antibody indicated that ATRIP was phosphorylated on Ser239 in both nonirradiated and irradiated cells and in cells exposed to hydroxyurea (Fig. 2C). The level of phosphorylation increased at late time points after exposure to IR or hydroxyurea. This may represent a cell cycle effect, as the related sequence in BACH1 is phosphorylated predominantly in the S and G2 phases of the cell cycle by cyclin-dependent kinases (25) and exposure of cells to IR or hydroxyurea leads to accumulation of cells in S and G2. Immunofluorescence analysis further indicated that the pool of ATRIP that localized at nuclear foci was also phosphorylated on Ser239 (Fig. 2D).

**Interaction of ATRIP with BRCA1.** We next examined whether ATRIP interacts with BRCA1. In a GST pull down assay, the BRCT domains of BRCA1, but not GST protein alone, interacted with endogenous ATRIP extracted from untreated cells or cells exposed to IR or hydroxyurea (Fig. 3A). Ectopically expressed hemagglutinin-tagged wild-type ATRIP also interacted with BRCA1 in the pull down assay, but an ATRIP protein with Ala at position 239 interacted very weakly, suggesting that the interaction between ATRIP and BRCA1 involves Ser239 of ATRIP (Fig. 3B). Finally, polypeptides encompassing the BRCT domains of TopBP1 failed to interact with ATRIP, suggesting specificity for the BRCT domains of BRCA1 (Fig. 3C).

**Defective checkpoint in cells expressing ATRIP Ala239.** Because both BRCA1 and ATRIP are required for maintenance of G2 arrest after irradiation (14, 25), we examined whether the interaction between these two proteins is also required for G2 arrest. We generated cells stably expressing hemagglutinin-tagged wild-type or mutant (Ala239) ATRIP and studied the integrity of the DNA replication checkpoint. To negate the effect of endogenous ATRIP, the cells were examined after being transfected with siRNA targeting the endogenous *atrip* gene, whereas the plasmids expressing the ectopic ATRIP proteins had silent nucleotide substitutions that rendered them resistant to the transfected siRNA (Fig. 4A). Clones with similar levels of wild-type and A239 mutant ATRIP (Fig. 4B) were transfected with control or *atrip*-specific siRNA and examined for maintenance of G2 arrest 24 h after irradiation. When endogenous ATRIP was depleted by siRNA, the ectopically expressed wild-type ATRIP effectively inhibited mitotic entry, whereas the A239 mutant was defective (Fig. 4C). These results suggest that the interaction between BRCA1 and ATRIP is required for checkpoint function.

**Discussion**

Several proteins with BRCT domains, such as BRCA1, TopBP1, 53BP1, NBS1, and MDC1, function in DNA damage and replication block checkpoint pathways (1–3). These proteins are thought to facilitate the function of the key checkpoint kinases, ATM and ATR/ATRIP, either by activating them or by facilitating the phosphorylation of their substrates (32, 33). Consistent with such a function, 53BP1 and NBS1 interact with ATM, whereas BRCA1 and TopBP1 interact with ATR/ATRIP (13, 33–35). The interaction between NBS1 and ATM involves the COOH-terminal 20 amino acids of NBS1 (13). The interaction of TopBP1 with ATR/ATRIP requires both ATM and ATRIP and involves a segment of TopBP1 between BRCT domains 6 and 7, whereas the domains mediating the interactions between endogenous ATM and 53BP1 and between endogenous ATR and

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**Figure 3.** Phospho-Ser239–dependent interaction of ATRIP with the BRCT domains of BRCA1. **A,** GST pull down (GSTpd) assay using GST protein or GST fused to the BRCT domains of human BRCA1 (residues 1,649–1,859) and extracts from untreated cells or cells exposed to IR or hydroxyurea. Endogenous ATRIP bound to BRCA1 was detected by immunoblotting with a monoclonal antibody against ATRIP or the phospho-Ser239–specific antibody. **B,** GST pull down assay using parental U2OS cells (−) or U2OS cells expressing hemagglutinin-tagged wild-type ATRIP or ATRIP with a Ser239 to Ala substitution. **C,** GST pull down assay of endogenous ATRIP from U2OS cells using GST proteins fused to fragments containing the indicated residues of human TopBP1 (these fragments encompass all the BRCT domains of human TopBP1) or to the BRCT domains of BRCA1.

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BRCA1 proteins had not been previously mapped (33–35). Thus, the interaction of BRCA1 with ATRIP described here is, to our knowledge, the first interaction reported between ATM or ATR/ATRIP and a BRCT domain–containing protein that involves the BRCT domains of the latter protein. It remains to be determined whether other BRCT domain–containing proteins interact with ATM or ATR/ATRIP in a similar manner.

What is the physiologic significance of the BRCA1-ATRIP interaction? In meiotic prophase I, localization of ATR to the unsynapsed chromosomes is dependent on BRCA1, suggesting that BRCA1 may recruit ATR/ATRIP to blocked DNA replication forks or sites of DNA damage (35). However, in our hands, depletion of BRCA1 by siRNA did not inhibit ATRIP focus formation in U2OS cells exposed to IR or hydroxyurea, and, further, the ATRIP A239 mutant was also not defective in forming foci in response to DNA damage (data not shown). These results argue that in interphase cells the interaction between BRCA1 and ATRIP does not recruit ATR/ATRIP to stalled DNA replication forks. However, we cannot rule out the possibility that BRCA1 has some role in ATR/ATRIP recruitment, because such recruitment may be mediated by multiple mechanisms, only one of which may involve BRCA1. Indeed, RPA can mediate recruitment of ATR/ATRIP to DNA damage sites (16, 17). Interestingly, the region of S. cerevisiae Lcd1/Ddc2 that shares homology to the sequence surrounding Ser239 of human ATRIP is required for targeting Lcd1/Ddc2 to DNA damage sites (15). A clear homologue of human BRCA1 is not present in either S. cerevisiae or S. pombe. Nevertheless, it remains possible that another BRCT domain–containing protein interacts with this region of Lcd1/Ddc2.

In conclusion, we report here a novel interaction between human ATRIP and BRCA1 mediated by phosphorylated Ser239 of ATRIP and the BRCT domains of BRCA1. This interaction is critical for the function of ATR/ATRIP in the DNA damage checkpoint and may therefore form the foundation for understanding the molecular mechanism by which BRCA1 exerts its checkpoint function.

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