The BRCA2-Interacting Protein BCCIP Functions in RAD51 and BRCA2 Focus Formation and Homologous Recombinational Repair

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Received 17 August 2004/Returned for modification 13 September 2004/Accepted 29 November 2004

Homologous recombination repair (HRR) of DNA damage is critical for maintaining genome stability and tumor suppression. RAD51 and BRCA2 colocalization in nuclear foci is a hallmark of HRR. BRCA2 has important roles in RAD51 focus formation and HRR of DNA double-strand breaks (DSBs). We previously reported that BCCIPα interacts with BRCA2. We show that a second isoform, BCCIPβ, also interacts with BRCA2 and that this interaction occurs in a region shared by BCCIPα and BCCIPβ. We further show that chromatin-bound BRCA2 colocalizes with BCCIP nuclear foci and that most radiation-induced RAD51 foci colocalize with BCCIP. Reducing BCCIPα by 90% or BCCIPβ by 50% by RNA interference markedly reduces RAD51 and BRCA2 foci and reduces HRR of DSBs by 20- to 100-fold. Similarly, reducing BRCA2 by 50% reduces RAD51 and BCCIP foci. These data indicate that BCCIP is critical for BRCA2- and RAD51-dependent responses to DNA damage and HRR.

DNA double-strand breaks (DSBs) are induced by exogenous agents, such as ionizing radiation (IR), and arise spontaneously during normal DNA metabolism, such as at blocked or collapsed replication forks (9, 10, 39, 45). Defects in DSB repair confer genome instability associated with tumorigenesis. In mammalian cells, DSBs are repaired by nonhomologous end-joining and by homologous recombinational repair (HRR) (60, 62, 65). RAD51 binds single-stranded DNA (ssDNA) to form nucleoprotein filaments that are essential for strand transfer during HRR (23, 44, 61, 66). RAD51 is normally dispersed in the nucleus, but upon DNA damage induction, it redistributes to nuclear foci that are presumed sites of HRR (6, 7, 14, 20, 31, 46). RAD51 foci have been shown to be associated with ssDNA regions after DNA damage (46). Several HRR proteins, including XRCC2, XRCC3, RAD51B, RAD51C, RAD51D, and BRCA2, are important for RAD51 focus formation (1, 5, 7, 43, 55, 56).

BRCA2 has nine RAD51 binding regions, including eight BRC repeats encoded by exon 11 and a distinct RAD51 binding region encoded by exon 27 (8, 33, 69). Expression of individual BRC repeats interferes with RAD51 focus formation and HRR (5, 53, 70), indicating that RAD51-BRCA2 interactions are important for both processes. The C-terminal half of BRCA2 has three regions that are structurally related to the ssDNA binding region of RPA and bind ssDNA in vitro, suggesting that ssDNA binding is also important for BRCA2 function in HRR (71). These ssDNA binding regions occur in a region called conserved domain IV (30, 48, 73) or the BRCA2 C-terminal domain (71), which is the longest and most evolutionarily conserved BRCA2 domain (32, 57). This domain also has binding sites for several proteins including DSS1, BUBR1, ABP-280/filamin-A, and BCCIPα (16, 30, 34, 73).

BCCIPα is a BRCA2 and CDKN1A (p21, Cip1, and Waf1) interaction protein (30); it has also been called Tok-1α (42). A second isoform, BCCIPβ, shares an N-terminal acidic domain and a central conserved domain but has a distinct C-terminal domain (Fig. 1A). In this report, BCCIP indicates both proteins. The BCCIP proteins share no significant homology to other mammalian proteins. Although the interaction between BCCIPα and BRCA2 implicates BCCIP in RAD51-dependent processes, there is no prior direct support for this idea. In this report, we show that BCCIPβ interacts with BRCA2 in a region shared with the BCCIPα isoform. We further show that BCCIP colocalizes with BRCA2 in the nucleus and is associated with RAD51. To further analyze BCCIP function, we reduced BCCIP protein levels by RNA interference (RNAi) and found that this reduces RAD51 and BRCA2 focus formation in response to IR and sharply reduces HRR of DSBs. These results suggest a critical role for BCCIP in HRR regulation.

MATERIALS AND METHODS

Plasmids, cell culture, and plasmid transfection. Plasmid vectors expressing Myc-tagged BCCIPα, BCCIPβ, RAD52, UBC9, and RAD51 and the hemagglutinin (HA)-tagged fragment of BRCA2 (amino acids [aa] 2883 to 3194) were described previously (27, 29, 30, 36, 50, 73). Plasmid pMSGneo2S12His (54) carrying a neo direct repeat homologous recombination (HR) substrate was transfected into HT1080 cells, mycophenolic acid-resistant derivatives were isolated, and Southern hybridization and PCR assays were used to identify transfectants with a single, intact copy of the HR substrate as described previously (54). The I-SceI nuclease expression vector, pCMV(HA-3xNLS)I-SceI, was described previously (54). Plasmid pPUR/U6 carries a U6 promoter for short RNA
transcription and a puromycin resistance cassette (38). HT1080 cells (American Type Culture Collection) were cultured in αMEM (Gibco BRL, Grand Island, N.Y.) with 10% fetal bovine serum (Biowhitaker, Walkersville, Md.), 20 mM glutamine, and 1% penicillin–streptomycin (Gibco BRL). Plasmids were transfected into cells by using the Geneporter transfection kit (Gene Therapy Systems, Inc., San Diego, Calif.).

Antibodies, Western blotting, and immunoprecipitation. Rabbit anti-BCCIPα/β antibodies were reported previously (30). Other antibodies were purchased: anti-BRCA2 (Ab2) and anti-RAD51 from Oncogene Science (Cambridge, Mass.) and anti-Myc/anti-HA from Clontech Laboratories (Palo Alto, Calif.). Protein extracts were prepared from cells lysed with 50 mM HEPES (pH 7.6), 250 mM NaCl, 5 mM EDTA, and 0.1% Nonidet P-40. Proteins were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis, and Western blotting was performed as described previously (36, 38). For immunoprecipitation, cells were washed with phosphate-buffered saline (PBS), harvested by centrifugation, treated with lysis buffer (50 mM Tris-HCl [pH 7.5], 150 mM NaCl, 0.5% Tween 20, 0.25% NP-40, 1 mM Na3VO4, 100 μg of phenylmethylsulfonyl fluoride/ml, 20 μg of aprotinin/ml, 10 μg of leupeptin/ml), and precipitated proteins were detected by Western blotting with appropriate antibodies as noted in the figure legends.

Stable short hairpin RNA (shRNA) interference of BCCIP. Specific sequences targeting BCCIPα and/or BCCIPβ were inserted into pPUR/U6: 5'-GGG AAC TGT TGA TGT G TG-3' targets both proteins. The resulting vectors (pPUR/U6/BCCIPα, pPUR/U6/BCCIPβ, and pPUR/U6/BCCIPα/BCCIPβ) were transfected into cells, stable transfectants were selected in growth medium with 1.0 μg of puromycin/ml, and BCCIP expression was measured by Western blotting.

RESULTS

Mapping the BCCIP2-interacting domain of BCCIPα and BCCIPβ. We showed previously that BCCIPα interacts with amino acids 2883 to 3053 of BCCIP2, centering around amino acids 2973 to 3001 (30). However, the corresponding interaction region in BCCIPβ was not determined. To map this region, we constructed a series of plasmids that express Myc-tagged fragments of BCCIPβ (Fig. 1A). The BCCIPβ fragments were coexpressed with a HA-tagged BCCIP2 fragment (aa 2883 to 3194) that contains the BCCIP interaction region. We found that BCCIPα fragments corresponding to amino acids 1 to 258, 1 to 167, and 59 to 167 coprecipitated with BCCIP2 (Fig. 1B). Thus, the minimal region of BCCIPα required to interact with BCCIP2 resides in amino acids 59 to 167 in the N-terminal half of the internal conserved domain. The C-terminal half of this BCCIP domain (amino acids 161 to 259) interacts with p21 (42). Thus, BCCIP2 and p21 interact with distinct regions of BCCIPβ.

Because the BCCIP2-interacting domain of BCCIPβ was mapped to a region shared by BCCIPα and BCCIPβ (Fig. 1A), we tested whether BCCIPβ also interacts with BCCIP2. This was done by immunoprecipitating full-length BCCIP2 and then determining whether endogenous BCCIP2 was coprecipitated (Fig. 1C). Human RAD51 was used as a positive control for coprecipitating BCCIP2; RAD52 and UBC9 were used as negative controls. To ensure equal affinity for each protein during precipitation, proteins were Myc tagged and expressed in HeLa cells. In addition, DNA and RNA were removed by nuclease digestion to eliminate potential interference with protein complex formation. As expected, Myc-RAD51 coprecipitated endogenous BCCIP2. Although UBC9 and RAD52 interact with RAD51 (26, 50, 51), they did not coprecipitate BCCIP2. Both Myc-BCCIPα and Myc-BCCIPβ coprecipitated endogenous BCCIP2, and each isoform has approximately the same affinity toward BCCIP2. Furthermore, endogenous BCCIPα and BCCIPβ coprecipitate with endogenous BCCIP2 (Fig. 1D). Thus, BCCIPβ interacts with BCCIP2; the similar affinities of the two isoforms with BCCIP2 are consistent with the finding that BCCIP2 interacts with a region shared by BCCIPα and BCCIPβ.

BCCIP2 is in complex with RAD51. Because RAD51 and BCCIPαβ each interact with BCCIP2, we tested whether RAD51 also associates with BCCIP2. HA-tagged RAD51 was coexpressed with Myc-tagged BCCIPα, BCCIPβ, or RAD52 in HeLa cells. RAD52 is a positive control, since it binds to RAD51 (50). When these Myc-tagged proteins were precipitated, both endogenous and HA-tagged RAD51 coprecipitated with BCCIPα and BCCIPβ (Fig. 1E). These data indicate that BCCIP forms protein complexes with RAD51. BCCIP2 and RAD51 are known to be involved in HRR, thus the interactions between BCCIP and these proteins implicate BCCIP in HRR.

BCCIP2 colocalizes with BCCIP2 and RAD51 nuclear foci. RAD51 and BCCIP2 colocalize in nuclear foci after ionizing radiation (6, 8, 69, 72). Because BCCIP2 interacts with BCCIP2 and (perhaps indirectly) with RAD51, we tested whether BCCIP2 forms nuclear foci and, if so, whether these colocalize with BCCIP2/RAD51 foci. We previously showed that most BCCIP2 resides in the nucleus (30). When total cellular protein was examined by immunofluorescence, BCCIP2 and BCCIP2 nuclear foci were blurry (data not shown) and difficult to count accurately. To overcome this problem, cells were treated with Triton X-100 before paraformaldehyde fixation, which removes soluble nuclear proteins and retains chromatin-bound proteins (see Materials and Methods). Immunofluorescence revealed both BCCIP2 and BCCIP2 nuclear foci in unirradiated cells (Fig. 2A). Interestingly, ∼80% of cells showed colocalized BCCIP2 and BCCIP2 foci, but this was not altered by irradiation (Fig. 2C). Irradiation did not increase the number of BCCIP2 foci (see below) nor the percentage of cells with colocalized BCCIP2 and BCCIP2 foci, although BCCIP2 foci became more sharply defined. Among cells treated with 0, 2, or 8 Gy of...
gamma rays that showed BCCIP foci, 100% had colocalized BRCA2 foci (Fig. 2D) and vice versa (Fig. 2E). These results suggest that BRCA2 and BCCIP are associated in the absence of exogenous DNA damage and remain so after DNA damage.

As shown in Fig. 2B, significant numbers of RAD51 foci are apparent only after irradiation, and the majority colocalize with BCCIP. Among cells with BCCIP foci after a 2- or 8-Gy dose of gamma rays, 60 to 95% show colocalized RAD51 foci (Fig. 2D). Similar percentages of irradiated cells with BRCA2 foci show colocalized RAD51 foci (Fig. 2E). These results indicate an increased association of BCCIP with RAD51 in response to DNA damage. BRCA2 foci have not previously been detected in the absence of exogenous DNA damage. Note that we detected BRCA2 foci without exogenous damage only when soluble protein was removed, suggesting a certain level of constitutive association of BRCA2 with chromatin, perhaps to resolve endogenous DNA damage.

To gain further insight into the association of RAD51, BRCA2, and BCCIP, we scored the number of cells with at least three foci in HT1080 cells treated with 2 or 8 Gy of gamma rays. Eight hours after irradiation, we found that ~80% of cells with RAD51 foci also have BCCIP or BRCA2 foci (Fig. 2F). Thus, in ~20% of cells with at least three RAD51 foci, these foci apparently form independently of BRCA2 or BCCIP. This is consistent with a recent report showing some RAD51 foci forming independently of BRCA2 (58). In contrast, in both untreated and irradiated cells, 100% of cells with BCCIP foci have BRCA2 foci and vice versa (Fig. 2D and E). We obtained similar results 2 h after irradiation (data not shown). Together, these results suggest that chromatin-bound BRCA2 and BCCIP are constitutively associated, that RAD51 is recruited to these complexes after DNA damage, and that RAD51 self-associates at some sites independently of BCCIP and BRCA2.

RNAi silencing of BCCIP inhibits the formation of BRCA2 and RAD51 nuclear foci. Based on the results above, we hypothesized that BCCIP is required for most RAD51 and BRCA2 focus formation. To test this, we downregulated BCCIPα and/or BCCIPβ by expressing shRNAs targeted to shared or unique sequences in these isoforms by using adenovirus vectors that coexpress GFP. When soluble protein is removed by Triton X-100 treatment, the GFP signal indicative of adenovirus infection is destroyed. Therefore, we used sufficient adenovirus to infect an entire HT1080 cell population, irradiated the cells with 8 Gy of gamma rays or mock treated them 72 h later, and visualized chromatin-bound BRCA2 2 or 8 h after irradiation by immunostaining, as described above. After irradiation, cells infected with the control virus showed well-defined BRCA2 foci (Fig. 3A, top row). Reduced BCCIP expression was confirmed by Western blot analysis (Fig. 3B).

We scored BRCA2 foci in >300 cells per treatment group and found that the average number of BRCA2 foci per cell was reduced by twofold upon BCCIPα or BCCIPβ downregulation, and these reductions were apparent in both irradiated and unirradiated cells (Fig. 3A, bottom 3 rows, and C). In addition, BRCA2 foci were less intense in cells with reduced BCCIP (Fig. 3A, bottom 3 rows). The number of BRCA2 foci per cell varied widely within each treatment group; this is apparent in plots of the cumulative percentages of cells with more than a specific number of BRCA2 foci (Fig. 3D). For example, 50% of unirradiated cells had ~6 or more BRCA2 foci, and the number of foci per cell ranged from 0 to 15. Following irradiation, a wider distribution was seen: 50% of cells had 10 or more foci and ~10% had more than 15 foci. Downregulation of BCCIPα or BCCIPβ narrowed the distributions of foci in both unirradiated and irradiated cells to similar degrees. For example, 50% of irradiated cells with downregulated BCCIPα or BCCIPβ had 6 or more BRCA2 foci, compared to 10 or more foci in control cells (Fig. 3D, bottom panel). The similar effects of BCCIPα and BCCIPβ on BRCA2 foci distributions is consistent with our finding that BRCA2 interacts with a region common to the BCCIP isoforms (Fig. 1A and B).

We next investigated whether BCCIP silencing affected RAD51 focus formation in irradiated cells. Unlike BRCA2 and BCCIP, RAD51 foci can be visualized without removing soluble nuclear protein. Therefore, the GFP signals in adenovirus-infected cells can be preserved. This allowed us to score RAD51 foci in BCCIP silenced cells (GFP') and in cells with normal BCCIP levels (GFP) simultaneously on the same slide. We treated HT1080 cells with an appropriate virus titer to infect ~50% of the cells and then fixed cells with paraformaldehyde before treating them with Triton X-100, which pre-
serves the GFP signal. In cell populations infected with a control vector, endogenous BCCIP protein was readily detected whether cells were infected (GFP/H11001) or not (GFP/H11002) (Fig. 4A, top row). Infection with viruses expressing BCCIP shRNAs substantially reduced BCCIP protein levels in GFP/H11001 cells but not in GFP/H11002 cells (Fig. 4A, bottom three rows), confirming the efficacy of these silencing vectors. As expected, RAD51 focus formation was unaffected by infection with a control vector (compare RAD51 signals in GFP/H11001 and GFP/H11002 cells in Fig. 4B, top row). However, downregulation of BCCIPα and/or BCCIPβ (GFP+ cells) resulted in substantially fewer RAD51 foci than in neighboring, uninfected (GFP+) cells (Fig. 4B, bottom 3 rows). We scored RAD51 foci after 8-Gy gamma ray exposures and found that BCCIP downregulation reduced the average number of RAD51 foci by ~2-fold when scored 2 or 8 h after irradiation (Fig. 4C). As seen with BRCA2 foci above, downregulation of BCCIPα and/or BCCIPβ had similar effects on the distribution of RAD51 foci (Fig. 4D).
Role of BRCA2 in BCCIP and RAD51 focus formation. The data above indicate that BCCIP plays a role in the formation of BRCA2 and RAD51 foci. It was previously established that BRCA2 is required for RAD51 focus formation (58, 72). To determine whether BCCIP focus formation also depends on BRCA2, we downregulated BRCA2 by RNAi and examined BCCIP foci. Consistent with BRCA2 being essential for cell viability (49), we were able to reduce BRCA2 to only \( \leq 50\% \) of wild-type levels (Fig. 5A), yet this was sufficient to reduce the average number of BCCIP foci per cell by \( \approx 40 \) to \( \approx 50\% \) in unirradiated cells and \( 8 \) h after irradiation (Fig. 5B and C); similar results were obtained when foci were scored 2 h after irradiation (data not shown). Thus, BRCA2 downregulation reduced but did not eliminate BCCIP focus formation. However, unlike BRCA2 foci, which increased after irradiation when BCCIP was downregulated (Fig. 3C and D), BCCIP foci did not increase after irradiation in BRCA2 downregulated cells (Fig. 5B and C). Consistent with prior reports (58, 72), BRCA2 downregulation sharply reduced RAD51 focus formation, evident by the fourfold reduction in the average number of RAD51 foci per cell (Fig. 5D) and by the dramatic change in the distribution of RAD51 foci (Fig. 5E). These results suggest a degree of mutual dependence among BRCA2, BCCIP, and RAD51 for focus formation. However, because the amount of RNAi reduction varied among the BCCIP isoforms and BRCA2, it is difficult to quantify the magnitude of the various dependencies.

Role for BCCIP in HRR of DNA DSBs. Based on the results above, we hypothesized that BCCIP is involved in HRR. We tested this by using a derivative of HT1080 called HT256, which carries a single integrated copy of a \( \text{neo} \) direct repeat HR substrate. One of the \( \text{neo} \) repeats is driven by the mouse mammary tumor virus promoter but is inactive due to the insertion of an I-SceI recognition site, and the second allele (\( \text{neo12} \)) has 12 single-base polymorphic markers but codes for wild-type protein and lacks a promoter (54) (Fig. 6A). Transfection of HT256 cells with an I-SceI expression vector creates DSBs at the I-SceI site. Repair of these DSBs by HRR can produce a functional \( \text{neo} \) that confers G418 resistance and are...
distinguishable by a PCR assay (see Materials and Methods). As in CHO cells carrying this same HR substrate (54), spontaneous HR in HT256 cells is below the limit of detection (<10⁻⁷) and expression of I-SceI increases HR by several orders of magnitude (data not shown).

We transfected HT256 cells with plasmid vectors that express a puromycin-resistance gene and shRNAs to downregulate BCCIPα, BCCIPβ, or both isoforms or with an empty vector as a control. Stably transfected derivatives were selected with puromycin, and BCCIP expression was measured by Western blotting with anti-BCCIP antibodies. As shown in Fig. 6B, BCCIP downregulation varied among these derivatives, with BCCIPα reduced by ~90% and BCCIPβ reduced by ~50%. These lower BCCIP levels were stable for 15 to 20 population doublings (three to four passages), and all HRR assays were performed within the first three passages. RNAi knockdown of BCCIP did not affect the expression of HA-tagged I-SceI (Fig. 6B, bottom panel). It is worth noting that several alternative strategies to further reduce BCCIP expression were unsuccessful, probably because one or both BCCIP isoforms are essential for viability. As shown in Fig. 6C, downregulation of BCCIPα reduced HRR by ~20-fold, and downregulation of BCCIPβ reduced HRR by ~100-fold. We analyzed genomic DNA isolated from 44 G418-resistant products from the control strains with PCR (see Materials and Methods for details) and found that 42 arose by gene conversion without crossover, 1 arose by deletion, and 1 had a complex pattern. Similarly, among 17 G418-resistant products from cells with reduced BCCIP expression, 15 arose by gene conversion, 1 arose by deletion, and 1 had a complex pattern. The marked reduction in HRR efficiency with no change in HRR product spectrum suggests that BCCIP plays a critical role early in HRR.

**DISCUSSION**

The present study revealed several critical links between BCCIP and the HRR proteins BRCA2 and RAD51. We found that a region shared by BCCIPα and BCCIPβ interacts with BRCA2, that BCCIP is in complex with RAD51, that chromatin-bound BRCA2 consistently colocalizes with BCCIP, and that the majority of radiation-induced RAD51 nuclear foci colocalize with BCCIP. Furthermore, downregulating BCCIP reduces both BRCA2 and RAD51 focus formation and markedly reduces HRR of DNA DSBs. Although the molecular mechanism by which BCCIP regulates HRR remains to be elucidated, our results establish BCCIP as a critical component of the DNA damage response network and HRR pathway.

Two key findings are that BCCIP is present in chromatin-bound nuclear foci even in the absence of induced DNA damage and that all BRCA2 foci colocalize with BCCIP (Fig. 2E). Although RAD51 foci also colocalize with BRCA2 (5, 7, 69) and BCCIP foci (Fig. 2B), RAD51 foci appear only after induced DNA damage. These results suggest the possibility that BCCIP and BRCA2 are normally associated in chromatin and that RAD51 is recruited to these repair-complex centers when DNA is damaged.

Studies of mouse embryonic stem cells expressing BRCA2 lacking exon 27 revealed twofold (64) and five- to sixfold (40) reductions in HRR. In human Capan-1 cells, which express truncated BRCA2 lacking BRC repeats 7 and 8, conserved region IV, and exon 27, HRR is reduced by 2- to 12-fold (70). In the present study, moderate decreases in BCCIP levels reduce HRR by up to 100-fold (Fig. 6). This surprising result may reflect a threshold effect, i.e., BCCIP-BRCA2-RAD51 function in HRR requires proper BCCIP stoichiometry. We note also that reducing BCCIP reduces both the number and intensity of BRCA2 foci (Fig. 3A), raising the possibility that the foci that do form are not fully functional. In any case, our results indicate a critical role for BCCIP in HRR. BCCIP most likely functions in HRR through its direct interaction with BRCA2 in or near the BRCA2-ssDNA interaction domains and through its indirect interactions with RAD51 (discussed further below). However, we cannot exclude the possibility that BCCIP regulates HRR indirectly because BCCIP is also involved in cell cycle control (36, 37, 42).

The BCCIPα and BCCIPβ isoforms are 80% identical, and our biochemical results indicate that these isoforms interact with BRCA2 through a shared domain of BCCIPα and BCCIPβ (Fig. 1A and B). The very similar effects of BCCIPα and/or BCCIPβ downregulation on BRCA2 and RAD51 focus formation (Fig. 3 and 4) suggest that both isoforms are capable of mediating focus formation, probably through this specific BRCA2 interaction. Note also that there was an apparent decrease of BCCIPα protein when BCCIPβ was silenced and vice versa (Fig. 6B). Thus, silencing of one isoform may affect the stability of the other, and this may account for the similar effects when either isoform is reduced.

There is only one BCCIP homolog in *Saccharomyces cerevisiae*, *Arabidopsis thaliana*, *Caenorhabditis elegans*, and mice. The C terminus of human BCCIPα (aa 259 to 322) has no homology with BCCIP homologs in other organisms, but the C terminus of human BCCIPβ (aa 258 to 314) shows various degrees of homology with all known BCCIP homologs (data not shown). Thus, BCCIPβ appears to be the conserved isoform. The *S. cerevisiae* BCCIP homolog, Bcp1 (open reading frame number YDR361C), is essential for viability (17) (Saccharomyces Genome Database at http://www.yeastgenome.org). Silencing of the *C. elegans* 2H343 gene (the ortholog of human BCCIPβ) leads to embryonic lethality (24). In addition, BCCIPα is expressed at lower levels than BCCIPβ, and we were able to silence BCCIPα much more than BCCIPβ. These results suggest that BCCIPβ is essential in human cells. RAD51 and BRCA2 are both required for cell viability (28, 49, 52), and RAD51 defects in particular result in proliferation-dependent defects and altered DNA replication (22, 52). Given that HR proteins function in restarting blocked replication forks (11, 12, 47) and the importance of BRCA2, RAD51, and BCCIP in HRR, it is possible that these proteins function together in recombinational restart of blocked replication forks and that this function is essential for cell viability.

Biochemical and structural studies revealed several features of BRCA2 critical for HRR. The interactions between RAD51 and the BRCA2 BRC repeats and/or exon 27 may promote RAD51 loading onto ssDNA (68). BRCA2 appears to interact with ssDNA via three OB domains that mimic the structure of the ssDNA binding domain of RPA. Protruding from the second ssDNA binding region, OB2 is a 3-helix bundle domain that has structural similarity to proteins that bind double-stranded DNA (71). Interestingly, BCCIP interacts with a...
2973 to 3001 in the OB2 region, but this binding site lies opposite the ssDNA binding surface (30). Thus, BCCIP may play a critical role in regulating ssDNA binding by BRCA2, perhaps by stabilizing the OB2-ssDNA interaction. BCCIP may also influence RAD51 function indirectly through its interactions with BRCA2. We recently found that expression of an OB2 peptide has strong dominant-negative effects on HRR (M. A. Brenneman, X. Guo, Z. Shen, D. J. Chen, and J. A. Nickoloff, submitted for publication), indicating that OB2 is critical for HRR. This result, together with the present results indicating a critical role for BCCIP in HRR, further supports the notion that BCCIP functions in HRR through its interaction with OB2. Note, however, that BCCIP downregulation strongly inhibits HRR but has only a moderate effect on RAD51 focus formation. Thus, it appears that BRCA2 can promote RAD51 focus formation in the absence of BCCIP, but such foci may be very inefficient repair centers.

Genome instability, manifested as aneuploidy, broken chromosomes, translocations, and chromosome fusions, is common in cells with defects in HRR proteins, including RAD51, RAD51 paralogs, BRCA1, and BRCA2 (2, 3, 13, 56, 59). Defects in genes encoding FANCA, XRCC3 is required for efficient repair of chromosome breaks by homologous recombination. Mutat. Res. 459/460:1-97.


