Published in final edited form as:

Biochemistry. 2017 July 05; 56(26): 3318–3327. doi:10.1021/acs.biochem.7b00184.

Dss1 Regulates Association of Brh2 with Rad51

Qingwen Zhou and William K. Holloman*

Department of Microbiology and Immunology, Weill Cornell Medical College, New York, New York 10065, United States

Abstract

Brh2, the BRCA2 ortholog in the fungus *Ustilago maydis*, mediates delivery of Rad51 to DNA during the course of homology-directed DNA repair. Rad51 interacts with Brh2 through the highly conserved BRC element and through a second region termed CRE located at the extreme carboxy terminus. Dss1, a small intrinsically unstructured protein that interacts with Brh2, is crucial for its activity in DNA repair, but the mechanism of this regulation is uncertain. In previous studies, we found that interaction of Brh2 with DNA was strongly modulated by association with Dss1. Here we report that CRE influences interaction of Dss1 with Brh2 and that Dss1 status markedly alters interaction of Brh2 with Rad51. While it appears that a single Rad51 protomer associates with Brh2 in complex with Dss1, loss of Dss1 is accompanied by a large increase in the number of Rad51 protomers that can associate with Brh2. Concomitant with this buildup of Rad51, Brh2 loses its ability to bind DNA. These observations suggest a feedback circuit in which release of Dss1 from Brh2 as it binds DNA triggers nucleation of a short Rad51 oligomer on Brh2, which in turn promotes dissociation of Brh2 from the DNA.

Graphical Abstract

Rad51 is an essential component of the homologous recombination and DNA repair system and is ubiquitous in eukaryotes. It catalyzes the search for DNA sequence homology and the DNA strand exchange process that is central in repair of DNA double-strand breaks by the homologous recombination pathway.^{1,2} Rad51 loads onto single-stranded DNA tracts at

*Corresponding Author: Telephone: 212-746-6510. Fax: 212-746-8587. wkholl@med.cornell.edu.

ORCID

William K. Holloman: 0000-0002-3372-4633

Author Contributions

Q.Z. conceived ideas for the project, conducted the experiments, and analyzed results. W.K.H. conceived ideas for the project, analyzed results, and wrote the paper.

Notes

The authors declare no competing financial interest.

break sites resected by nucleolytic degradation³ to form a polymerized nucleoprotein filament that constitutes the form of Rad51 active for DNA sequence searching and strand invasion. Loading of Rad51 onto single-stranded regions, however, is blocked by the ssDNA-binding protein RPA, which coats newly revealed single-stranded tracts quickly, thereby protecting it from adverse processing, but also preventing Rad51 from binding. Access of Rad51 to RPA-coated ssDNA must be enabled by a mediator,⁴ and with the exception of ascomycete yeasts, the primary mediator function in eukaryotes is provided by a member of the BRCA2 protein family,^{5,6} as was first demonstrated with Brh2, the BRCA2 ortholog in the fungus *Ustilago maydis*.⁷

BRCA2 family members in different taxa show considerable sequence divergence and size variation.⁸ Regardless of these differences, two defining features generally prevail. These are the BRC repeats that bind Rad51⁹ and the Dss1/DNA-binding domain (DBD), which is composed of a helix-rich region and a tandem array of oligonucleotide/oligosaccharide-binding (OB) folds, the latter being modules like those present in RPA with the potential for binding ssDNA.¹⁰ The number of BRC repeats is variable depending on the species and can range from one in the case of *U. maydis* Brh2 protein to eight in vertebrates and up to 15 in certain protozoans. There is also interspecies variation in the number of OB folds, from one in the worm *Caenorhabditis elegans* to two in *U. maydis* Brh2 and three in higher plants and animals. The helix-rich domain (HD) and adjacent OB1 module of the DBD is bound tightly by DSS1, a small intrinsically unstructured protein. The residues forming the DSS1-interacting interface comprise the most highly conserved region of the BRCA2 sequence.

In the *U. maydis* Brh2 protein, which has been studied in detail, there is another DNA-binding region present in the N-terminal region of the protein (termed NBD) in addition to the canonical DNA-binding domain located in the C-terminal domain (CTD).¹¹ The Brh2 NBD is capable of working in concert with the BRC motif to promote biological function in DNA repair.¹² It was localized by deletion mapping to a stretch of 144 residues between the BRC element and the canonical CTD, but that sequence stretch is poorly conserved and has no hallmark indicative of a DNA-binding motif. However, the binding activity is robust, and a side-by-side comparison of polypeptides Brh2^{106–551} and Brh2^{551–1075}, denoted Brh2^{NT} and Brh2^{CT}, containing NBD and CTD, respectively, revealed that the two different DNA-binding regions display remarkable similarities but differ sharply in response to Dss1.¹³ The DNA binding activity of the CTD is tightly regulated by Dss1, while the activity of NBD is independent of Dss1.¹⁴ When the CTD is associated with Dss1, it has no apparent DNA binding activity, but when freed of Dss1, it binds DNA as tightly as the NBD.

The BRC motif, consisting of a core sequence of ~35 amino acids, interacts with Rad51, and this interaction is vital for biological function.^{9,15} Mutations in BRC that abrogate interaction with Rad51 result in loss of DNA repair capacity.⁸ Crystallographic analysis revealed a β -hairpin structure of binding of BRC to the oligomerization interface of Rad51.¹⁶ The evidence supports the proposal that BRC mimics the Rad51 interaction surface. Within the BRC core sequence are two motifs.^{9,17} One comprises the FxxA consensus sequence that mimics the oligomerization interaction and contacts the catalytic domain of Ra51. A second domain in the core sequence within an α -helical region and containing the LFDE consensus sequence binds Rad51 through a different hydrophobic

pocket and is also essential for BRC–Rad51 interaction. In studies of isolated BRC repeats from human BRCA2, it seems clear that the function of the BRC elements is to govern Rad51's activity in forming an appropriate filament on single-stranded DNA.^{15,18}

In Brh2, a second Rad51-interacting region termed CRE (C-terminal Rad51-binding element) is located in the C-terminal 72 amino acid residues.¹⁹ This element is unrelated to BRC and appears to be functionally different in associating with Rad51.¹⁹ Both the BRC and CRE Rad51-binding regions are required for appropriately regulated Brh2 functional activity. Point mutation of key residues or deletion of either region abrogates Brh2 activity. However, these regions can contribute to function in trans, that is, from different molecules. Co-expression of a Brh2 variant without BRC and another without CRE can fully complement the DNA repair phenotype of a *brh2* null mutant.¹⁹ This cooperation requires an intact self-interaction interface, which is located medially in the Brh2 sequence. Dss1 appears to be critical for control of this Brh2 self-interaction. When Dss1 is present, Brh2 assumes a monomeric form, but when Dss1 is removed, then Brh2 forms dimers and possibly higher-order forms. As determined by a biological assay, there is no domain cooperation in the absence of Dss1. These findings suggest a model in which Dss1 controls a handoff of Rad51 from BRC to CRE (or vice versa). Such a dynamic interplay is probably crucial in the appropriate initiation of the Rad51 nucleoprotein filament.

Dss1 appears to play a leading role in mediating cooperation not only between the NBD and CTD, the two different DNA-binding regions of Brh2, but also in the communication between BRC and CRE, the two different Rad51-interacting sites.^{11,14} In the investigation presented here, we were interested in exploring the interaction between Rad51 and Brh2 CRE, but during the course of study, we came upon an unexpected role for Dss1 in regulating the Rad51–CRE interaction. This in turn led to the surprising finding that Rad51 appears to attenuate interaction of Brh2 with DNA.

EXPERIMENTAL PROCEDURES

Protein Preparations and Pull-Down Procedures

All Brh2 proteins and peptide derivatives were tagged with maltose-binding protein (MBP). Brh2^{106–1075} and Brh2^{551–1075} (Brh2^{CT}) proteins in complex with His-tagged Dss1 were purified after overexpression in *Escherichia coli* as described previously^{11,19} as Brh2 was stripped of Dss1 and Brh2^{106–551} (Brh2^{NT}). Coprecipitations or pull-down analyses to assess the association of Dss1 with Brh2 truncation fragments were performed using bacterial extracts prepared after co-expression of Brh2 peptides with His-Dss1.¹⁹ Pull-downs with purified affinity-tagged proteins alone or with biotinylated DNA were performed as described previously.^{11,14} Briefly, reaction mixtures (50 μ L) containing 50 mM Tris-HCl (pH 7.5), 10 mM MgCl₂, and protein combinations to be examined were incubated at 32 °C for 30 min and then mixed with Ni²⁺-NTA beads (5 μ L of settled slurry, Qiagen) or amylose resin (10 μ L of settled slurry, New England Biolabs). After 10 min on ice, beads or resin particles were collected by brief centrifugation, washed four times with 50 mM Tris-HCl (pH 7.5) and 200 mM KCl, and then eluted with 60 μ L of buffer containing 0.3 M imidazole or 10 mM maltose, respectively. When proteins bound to DNA were measured by coprecipitation, Brh2 or Rad51 was added to reaction tubes containing a suspension

(equivalent to 1 μL of a commercial stock) of streptavidin magnetosphere paramagnetic particles (Promega Biotech) that had been soaked in 150 nM 3'-biotinylated 60mer single strand preannealed with a tracer strand 3.3 nM 5'-IRD800 50mer to serve as a reporter. The 60mer was based on nucleotides 103–162 from bacteriophage ϕX174 , and the tracer strand was based on nucleotides 132–181. After incubation of protein with a particle suspension for 30 min at 32 °C to allow assembly of protein-bound complexes, particles were collected and washed three times (50 μL per wash) with 50 mM Tris-HCl (pH 7.5) and 200 mM KCl buffer and then resuspended a final time in 50 μL of buffer. Bound complexes were stripped off by heating at 100 °C in sodium dodecyl sulfate (SDS) sample buffer, and the composition was analyzed by electrophoresis in 10% polyacrylamide gels containing sodium dodecyl sulfate. Protein was visualized with Simply Blue Safe stain (Life Technologies) and quantified using the LI-COR Odyssey platform and ImageQuant TL software (GE Healthcare). DNA was tracked by fluorescence using the LI-COR Odyssey instrument.

DNA Gel Shift Assays

The DNA oligonucleotide (Eurofins/MWG/Operon) used as a substrate for DNA binding was a single-stranded oligonucleotide 60mer based on nucleotides 103–162 from a bacteriophage ϕX174 sequence with a 5'-IRD800 modification. Reaction mixtures (15 μL) containing 25 mM HEPES buffer (pH 7.7), 40 mM KCl, and 1 mM dithiothreitol were incubated at 32 °C for 30 min. Glutaraldehyde was added to a final concentration of 0.02%, and 10 min later, reactions were quenched with 0.1 M Tris-HCl (pH 7.5). Reaction mixtures were electrophoresed in 1% agarose gels and analyzed for DNA–protein complexes using the LI-COR Odyssey platform as described previously.^{13,14}

RESULTS

Association of Dss1 with Brh2 Requires CRE

In the crystal structure of the mammalian BRCA2 DBD in complex with DSS1, two acidic stretches in DSS1 are interwoven with two domains on apposing sides of the interface between the helix-rich domain and OB1.¹⁰ The structure of DSS1 extends part way around the circumference of OB1 and terminates within the interface between OB1 and OB2. This region of BRCA2 is highly conserved. In *U. maydis* Brh2, 36 of the 40 residues in the BRCA2 DBD that contact DSS1 are identical or conserved. However, because only a fragment of BRCA2 was crystallized in the co-complex with DSS1, we were curious to learn in the case of Brh2 if other regions of the protein might contribute to interaction with Dss1 or to the stability of the complex.

To approach this issue, we tested Brh2 truncated variants for the ability to form stable complexes with Dss1 (Figure 1A). Heterodimeric complexes of Brh2 and Dss1 can be monitored after co-expression in bacteria by use of a coprecipitation procedure to allow isolation of double-affinity-tagged complexes from cell-free extracts.^{11,19} Formation of a complex between Brh2 tagged at the N-terminus with maltose-binding protein (MBP) and Dss1 tagged at the N-terminus with a hexahistidine tract (His) can be readily detected by sequential coprecipitation with Ni^{2+} -NTA agarose beads followed by amylose resin (Figure

1B). MBP-Brh2 associated with His-Dss1 can be readily observed via the latter's interaction with Ni^{2+} -NTA beads (Figure 1B, Ni^{2+} -NTA panel). Then after elution with imidazole, the heterodimeric complexes can be recaptured with amylose resin via the MBP-Brh2 interaction and obtained in a substantially pure form freed of any unassociated His-Dss1 (Figure 1B, amylose panel). In the case of a Brh2 variant that cannot form a complex with Dss1, no MBP-Brh2 polypeptide would be expected after precipitation of His-Dss1 with Ni^{2+} -NTA agarose. In the experimentation described below, all versions of Brh2 were tagged at the N-terminus with MBP and Dss1 tagged at the N-terminus with His. For the sake of simplicity in the discussion, we will in general refer to MBP-Brh2, His-Dss1, and derivatives without mentioning the affinity tags.

On the basis of sequence alignment,²⁰ the residues in Brh2 corresponding to the DSS1-contacting residues in the BRCA2 DBD span amino acids 637–810.¹⁹ We found that three different Brh2 variants with increasingly large truncations of the N-terminal region could all form stable complexes with Dss1 (Figure 1A,B, samples 1, 3, and 5). It was notable that the Brh2^{645–1075} derivative truncated within the proximal sequence of the helix-rich domain to eliminate six Dss1-contacting residues²⁰ was still capable of forming stable complexes with Dss1 (Figure 1B, lanes 5). This indicates that the N-terminal region and even certain proximal Dss1-contacting residues are expendable in terms of the formation or stability of the Dss1 complex. However, derivatives of any of the constructs mentioned above in which the last 40 residues from the C-terminus of Brh2 were removed could not form complexes, which is evident in the absence of the expected Brh2 derivative in the Ni^{2+} -NTA pull-downs (Figure 1A,C, samples 2, 4, and 6). The fact that the Brh2 derivatives were expressed in a soluble form is evident by the appearance of the appropriate size fragment in the amylose pull-downs (Figure 1C, amylose). This 40-amino acid stretch, while well beyond the tract of Dss1-contacting residues, had been previously shown to include residues important for interaction of CRE with Rad51.¹⁹ This raised the possibility that this region might also be important for stable association of Brh2 with Dss1, although the caveat remains that the C-terminal truncation disturbs protein folding, thereby precluding Dss1 interaction. The smaller fragments eluting from the amylose resin could be indicative of degradation of misfolded protein. We note, however, that the Brh2^{106–1035} derivative, although inactive in DNA repair when expressed by itself,¹² is active when cooperating in trans with another inactive variant without the BRC region, suggesting that the C-terminally truncated protein folds properly in vivo.¹⁹

Interaction of CRE with Rad51 and Stimulation by Dss1

In previous studies, we mapped the Rad51 interaction site CRE in the carboxy terminus of Brh2 to the terminal 72 amino acids using coprecipitation or pull-down methodology with a set of Brh2 fragments.¹⁹ Interaction was evident by coprecipitation of Rad51 when the MBP-tagged CRE peptide was captured with amylose resin. Rad51 by itself did not bind to amylose resin. In the study presented here, we noticed that the Rad51–CRE peptide interaction was subject to marked temperature dependence. There was minimal binding of Rad51 to CRE peptide at low temperatures but then a jump in association after a threshold of ~22 °C was reached (Figure 2A,B). Because no temperature dependence was noted in the case of BRC–Rad51 interaction, we attribute the effect of temperature on binding of the

Rad51-CRE peptide to CRE and suggest there is a temperature-dependent conformational change within CRE that allows the interaction.

Increasing the level of Rad51 in reaction mixtures with the CRE peptide resulted in a corresponding linear increase in the amount of Rad51 bound to CRE (Figure 2C,D). This indicates that Rad51 bound to the CRE is not constrained in associating with additional Rad51 protomers. With the knowledge that residues within the CRE also could contribute to formation or stabilization of complexes with Dss1, we were curious about whether Dss1 could influence the interaction of the CRE peptide with Rad51. We noticed that when Rad51 was mixed with the CRE peptide at 0 °C, a temperature unfavorable for interaction, addition of Dss1 promoted Rad51–CRE association (Figure 2E,F). This finding supports the notion that, analogous to elevated temperature, Dss1 alters the CRE conformation into a state that favors interaction with Rad51. However, using the pull-down methodology, we were unable to demonstrate direct interaction between CRE and Dss1, so if there is physical interaction, it is weak.

In view of the temperature- and Dss1-promoted stimulation of association of Rad51 with CRE, it was important to determine if this interplay could be recapitulated in the context of the Brh2 domain harboring the Dss1 interaction region and CRE. Therefore, we tested the temperature dependence of association of Rad51 with the Brh2 CTD in complex with Dss1 or free of Dss1. We investigated this point by coprecipitation, mixing the MBP-Brh^{CT}/His-Dss1 heterodimer complex with a 4-fold molar excess of untagged Rad51 at 0 and 32 °C and then capturing complexes with Ni²⁺-NTA beads or amylose resin (see the schematic in Figure 2G). The amount of Rad51 associated with the Brh2^{CT}/Dss1 complex could be determined from the Ni²⁺-NTA-bound fraction. The amount of Rad51 associated with the Brh2^{CT} domain that had become dissociated from Dss1 and did not bind to Ni²⁺-NTA beads could be determined from the amylose-bound fraction. It was apparent that an elevated temperature stimulated association of Rad51 with Brh2^{CT} and that Brh2^{CT} complexed with Dss1 associated with Rad51 2–3 times more strongly than Dss1-free Brh2^{CT} did (Figure 2H,I).

We note that the Ni²⁺-NTA and amylose pull-down determinations described above are sensitive to the exact experimental conditions. As such, all experiments were performed independently at least three times and graphed with error bars showing standard deviations.

Dissociation of Dss1 from Brh2 Stimulates Association of Rad51 with Brh2

For the studies described below, we used as the Brh2 wild-type representative the derivative Brh2^{106–1075}, which is expressed from the third ATG in the open reading frame (see Figure 1A, sample 1). We have found no difference in biochemical or biological activity between this variant and full length Brh2^{1–107512} and refer to Brh2^{106–1075} as Brh2.

Given that Dss1 appeared to stimulate binding of Rad51 to the CRE peptide, we were curious to know if this response might extend to Brh2 protein. We investigated this point by the same pull-down procedure described above mixing the MBP-Brh/His-Dss1 heterodimer complex with a 4-fold molar excess of untagged Rad51 and increasing amounts of His-Dss1 and then capturing Brh2/Dss1 complexes with Ni²⁺-NTA beads (see the schematic in Figure

3A). In the control when no additional Dss1 was added, Rad51 associated with Brh2/Dss1 complexes at a molar ratio of ~0.8:1 (see Figure 3B, lane 1, no additional Dss1 added, corresponding to the zero Dss1/Brh2 added point in Figure 3C, Ni^{2+} -NTA curve). Because Rad51 protomers spontaneously self-assemble,²¹ the finding suggests that the single Rad51 associated with Brh2/Dss1 is constrained in such a way that it is shielded from interaction with other protomers free in solution. With increasing levels of Dss1, there was a marginal increase in the amount of Rad51 captured on the beads (molar ratio of ~1.3:1) (see Figure 3B, lanes 2–4, and Figure 3C, Ni^{2+} -NTA curve). This could indicate slightly strengthened association of Rad51 with the Brh2 complex, or alternatively, it could suggest that Rad51 associates weakly with Dss1.²² We investigated this point in more detail by examining whether Rad51 could be captured on Ni^{2+} -NTA beads upon being mixed with His-Dss1. The results indicate there was weak interaction between Rad51 and Dss1 (Figure 3G,H), and it was interesting to note that interaction was enhanced in the presence of DNA, suggesting that Dss1 was not competing with DNA for the same DNA-binding site on Rad51.

The findings described above notwithstanding, the striking observation from this determination was the high level of Rad51 that associated with Brh2 freed of Dss1 (Figure 3B, lanes 1–4, amylose-bound fraction; Figure 3C, amylose bound curve). We showed previously that although Brh2/Dss1 heterodimers were generally stable, they did dissociate over time under reaction conditions of moderate temperature in the presence of a divalent cation.^{13,14} On the other hand at least under the *in vitro* conditions tested, the reverse reaction, that is to say, the association of Dss1 with free Brh2, was barely detectable. Therefore, in standard binding reactions with a divalent cation, a fraction of Brh2 free of Dss1 accumulates over time. This Dss1-free Brh2 can be obtained by capturing the fraction that does not bind to Ni^{2+} -NTA beads (via Dss1) on amylose resin (see the schematic in Figure 3A). It should be emphasized that the Rad51 attributable to that associated with Brh2 is the fraction that specifically eluted from the amylose resin with maltose. Amylose resin does not retain any Rad51 by itself that can be specifically eluted with maltose (see Figure 3B, lane 5, amylose bound + maltose). There is some nonspecific binding of Rad51 to amylose (and Brh2), which is evident when the resin is boiled in SDS sample buffer (see Figure 3B, lane 5, amylose bound + SDS). We presume this represents aggregated, inactive forms. Only protein specifically eluted with maltose was considered in the determinations reported in this work.

As is apparent, the level of Rad51 associated with Brh2 that was free of Dss1 was almost 10 times higher than that associated with the Brh2/Dss1 heterodimer (Figure 3B, lane 1, amylose bound; corresponding to Figure 3C at zero additional Dss1 added, amylose bound curve). This suggests that Brh2 can act to nucleate a higher-order complex of Rad51 protomers, likely in the form of a short polymerized chain. When additional Dss1 was present, the amount of Rad51 associated with Brh2 increased even more, suggesting that Dss1 promotes or stabilizes the short polymer of Rad51 on Brh2 (Figure 3B, lanes 2–4, amylose-bound fraction; Figure 3C, amylose bound curve). Concomitantly, the overall level of Brh2 bound to amylose resin appeared to decrease with Rad51 association (note the Brh2 band in Figure 3B, amylose-bound fraction). This could be due to interference with the MBP tag binding to amylose resin perhaps as a result of steric hindrance imposed by the higher-order complex of Rad51. Previous findings supported the notion that Brh2 can self-associate

or dimerize in an antiparallel mode upon release of Dss1.¹⁹ Such action might also limit the spatial freedom of the MBP tag in associating with the amylose matrix.

As a control to address the concern that the enhanced association of Rad51 with Dss1-free Brh2 was not an artifact due to nonspecific aggregation, we tested a Brh2 variant with a single amino acid change (W1052A) in the CRE that was previously shown to reduce the level of Rad51 interaction.¹⁹ Brh2^{W1052A} poorly formed a complex with Dss1 (Figure 3D, Ni²⁺-NTA bound lanes) and was unresponsive to Dss1 in associating with Rad51 (Figure 3C,D, amylose bound curve). This control supports the notion that Dss1-enhanced association of Rad51 with Brh2 is not due to nonspecific aggregation.

Paradoxically, the observed elevated amount of Rad51 associating with Brh2 as Dss1 dissociates from Brh2 stands in contrast to the response noted in Rad51 binding reactions that start with Brh2 already stripped of Dss1, rather than the Brh2/Dss1 complex (see Figure 3E,F). In this case, Rad51 associates with Brh2 at a low level (molar ratio of ~2.2:1), and there is slight inhibition with added Dss1 (Figure 3E, lanes 1–4, amylose bound; Figure 3F). It would therefore appear that there are three states for Brh2's association with Rad51. (i) When Brh2 is in stable heterodimeric complex with Dss1, it can accept a single Rad51 protomer; we will refer to this as the primed state. (ii) When stripped of Dss1 under conditions of elevated temperature and ionic strength, it can accept two protomers. However, as Brh2 is not active *in vivo* in the absence of Dss1, this form represents an inactive dead-end complex or a spent state. (iii) In the transition period that occurs when Dss1 is being ejected, Brh2 can serve to nucleate a higher-order complex of a few dozen Rad51 protomers. We will refer to this as the transient state.

Mapping Additional Rad51 Contact Regions in Brh2

The increased level of association of Rad51 with Brh2 upon loss of Dss1 raises the notion that if a short polymerized chain of Rad51 protomers lies across the surface of Brh2 stretching from the BRC element to the CRE, then there could be additional sites along Brh2 for physical contact with Rad51. To test this idea, we examined additional fragments of Brh2 for association that did not include the BRC element or CRE region. In previous studies, genetic evidence indicated that three short FxTP sequences that are similar to the FQTG stretch in the BRC motif cooperated to promote interaction with Rad51.²³ Therefore, we tested interaction between a fragment (Brh2^{359–551}) containing the NBD and one active in DNA binding¹¹ that spanned the three FxTP sequences (Figure 4A). We also tested a fragment (Brh2^{645–1035}) containing the canonical C-terminal DNA-binding domain but lacking the CRE. This fragment was expressed and purified without Dss1. Rad51 associated with both fragments also in a temperature-dependent manner, suggesting conformation influenced interaction with Rad51 (Figure 4B,C). These findings are consistent with the notion that a large surface of Brh2 with multiple contact sites might open for interaction with a short chain of Rad51 protomers.

Rad51 Inhibits Brh2 Binding by DNA

Previously in DNA binding studies with the Brh2/Dss1 heterodimer, we observed that the DNA-bound protein complex that formed did not contain Dss1, indicating that Dss1

dissociated during the DNA binding process.¹⁴ We were unable to discern whether release of Dss1 from Brh2 was prerequisite for DNA binding or if contact between the Brh2/Dss1 complex and DNA promoted ejection of Dss1. Nevertheless, it was evident from those studies that Dss1 was not associated with the DNA-bound form of Brh2 and that the N-terminal region of Brh2 was important for triggering dissociation of Dss1 from the C-terminal region. These studies suggested a dynamic model in which ejection of Dss1 activates the full DNA binding potential of Brh2.

Here we were interested to know how Rad51 might influence Brh2/Dss1 in binding to DNA because Brh2 complexed with Rad51 would likely represent the more physiological situation *in vivo*.²⁴ The challenge experimentally was to focus only on the Brh2–DNA interaction because both Brh2 and Rad51 are capable of binding DNA. To eliminate complications in the interpretation of our experiments caused by DNA binding by both proteins, reactions were performed under conditions that support Brh2–DNA binding but do not support Rad51–DNA binding, that is, in the absence of ATP, which is a cofactor essential for Rad51's DNA binding activity²⁵ (Figure 5F,G). Therefore, any observed association of Rad51 with DNA in reactions without this cofactor would necessarily be indirect through interaction of Rad51 with Brh2.

Biotinylated DNA was used as a substrate so that the composition of the protein-bound fraction could be readily determined after capturing the protein-bound complexes on streptavidin beads (see the scheme in Figure 5A). No Brh2 or Rad51 was observed to bind to streptavidin beads in the absence of DNA (Figure 5B, lane 7, and Figure 4C). Remarkably, with increasing concentrations of Rad51, the level of Brh2 bound to DNA dropped precipitously (Figure 5B, lanes 1–6, and Figure 4C), suggesting that Rad51 decreases the affinity of Brh2 for DNA. When Rad51 was added to preformed Brh2/DNA complexes that were prepared with Brh2 stripped of Dss1, there was no obvious dissociation of Brh2 (not shown). The Rad51 inhibition of Brh2/DNA binding was apparent in the gel mobility shift assay, as well. At an input ratio of Rad51 to Brh2/Dss1 of 4:1, there was little detectable Brh2 binding (Figure 5D, left panel). The Brh2^{NT} and Brh2^{CT} fragments are both individually capable of binding DNA (see refs 11 and 14 and Figure 5D,E). It was evident that the Rad51 inhibitory effect was mediated through the C-terminal region of Brh2 with no contribution from the BRC-containing N-terminal region (Figure 5D,E, right and middle panels, respectively).

DISCUSSION

In studies with human BRCA2, it has been shown that DSS1 contributes to regulation by masking nuclear export signals²² and by promoting assembly of the RAD51 presynaptic filament²⁶ by serving as a DNA mimic to attenuate the affinity of RPA for ssDNA, providing access for Rad51 associated with BRCA2 to ssDNA.²⁷ The salient finding in this study could represent an additional mechanism by which BRCA2 family members are regulated by their cognate DSS1 partners.

Here we observe that Brh2 in complex with Dss1 appears to bind a single Rad51 protomer but upon release of Dss1 is able to accommodate multiple protomers, enough to constitute a

short polymer. As demonstrated previously, the full DNA binding potential of Brh2 is not realized until Dss1 is released,¹⁴ but as reported here, also concomitant with release of Dss1, multiple Rad51 protomers are loaded on Brh2, causing a loss of affinity of Brh2 for DNA. Therefore, in addition to the role of Dss1 in controlling the DNA binding status of Brh2, Dss1 also appears to regulate association of Rad51 with Brh2, which in turn counteracts association of Brh2 with DNA. These findings suggest a Dss1-directed feedback loop in which Brh2 mediates loading of a preformed Rad51 oligomer on DNA and then is itself dissociated from DNA. The Dss1 status orchestrates both DNA binding and Rad51 interaction.

The BRC motif located in the N-terminal region provides an essential docking site for Rad51 and is required for Brh2's functional activity. CRE, the unrelated second Rad51-interacting region located within Brh2's C-terminal 72 amino acid residues, also is essential for Brh2's functional activity and appears to be directly influenced by Dss1. Truncations of Brh2 that remove the last 40 residues from the C-terminus do not form complexes with Dss1 even though this region is well beyond residues in the medial region of Brh2 corresponding to those in the BRCA2 DBD crystal structure known to contact DSS1. This observation suggests that there is communication between Dss1 and CRE, and this notion is supported by the finding that Dss1 promotes interplay between Rad51 and CRE under conditions where there is little interaction between the two by themselves.

In solution, Rad51 self-interacts to form chains of protomers polymerized end to end.²¹ When Rad51 is associated with Brh2/Dss1, however, only a single Rad51 protomer is accommodated in the heterotrimeric complex. This implies that the bound protomer is sequestered in such a manner that its interaction interface is blocked from contact with other Rad51 protomers. Both the BRC motif and the CRE are capable of associating with Rad51,¹⁹ but it is not clear if their engagement with Rad51 is temporally different, reflecting the nonequivalent functional activity of these two different elements. As DNA repair proficiency depends on all three components (Brh2, Dss1, and Rad51), we presume this complex is in a state primed for action but on standby, awaiting instructions so to speak as illustrated in the model. However, as we have shown before, Brh2 in this state is not yet under a condition that is optimal for DNA binding because the CTD is impeded in interaction with DNA by association with Dss1.¹⁴ With loss of Dss1, the impediment of CTD to DNA is lifted, as is the constraint against association of additional Rad51 protomers. Therefore, it appears that just as Brh2 reaches its maximal potential for binding DNA, it is concomitantly opened to nucleate a tract of Rad51 protomers. This open form of Brh2, however, must be transient and in a higher-energy state compared to that of Brh2 freed of Dss1 by physicochemical treatment because the latter is again limited in the number of Rad51 protomers it can accommodate. Thus, it would appear that Brh2 in a dynamic transition state of losing Dss1 is functionally different from the static form of Brh2 obtained after stripping Dss1. This could well explain why cells lacking Dss1 are completely defective in homologous recombination even though Brh2 remains present and capable of forming subnuclear foci in response to DNA damage.¹²

A surprising consequence of loading multiple Rad51 protomers on Brh2 is the apparent loss of its ability to bind DNA. We imagined that this inhibition might result from steric

hindrance as more and more Rad51 protomers become associated with Brh2, and we presumed this polymerization would be initiated on the BRC-bound Rad51 present in the primed Brh2/Dss1/Rad51 complex. However, this inhibitory action of Rad51 on Brh2 appears to be mediated through the C-terminal region, not the N-terminal BRC-containing region of Brh2, because the inhibition is evident when Rad51 is added to DNA binding reaction mixtures with the Brh2^{CT} polypeptide, not Brh2^{NT}. On the basis of this finding, it would seem to be logical to conclude that association of Rad51 with the CRE interferes with DNA binding by the CTD, but this raises the question of what the functional significance of such inhibition might be.

We speculate that the observed Rad51-promoted inhibition of DNA binding by Brh2 is part of a feedback loop by which Brh2 serves as a vessel to assemble and transport a short Rad51 polymer to DNA and in turn is cast off once the cargo of Rad51 is delivered (see Figure 6). The process is put into motion by ejection of Dss1 (Figure 6, steps 1 and 2). This triggers assembly of the nascent Rad51 polymer on Brh2 and activates Brh2 for DNA binding (Figure 6, steps 2 and 3), which is subsequently counteracted once the Rad51 polymer reaches a certain size. If conditions are permissive for Rad51 to bind DNA, then as Brh2 is jettisoned the Rad51 nascent polymer transfers to the DNA (Figure 6, step 4). We hasten to add this scenario is highly speculative given the caveats noted in the individual experiments and the lack of direct evidence for the last step. However, it does provide a rough framework for additional experimentation. The process of Brh2-mediated transport of Rad51 to DNA likely proceeds in a smooth flowing dynamic cycle. Unfortunately, we are limited by our bulk phase experimentation to view static representations or snapshots of only a few discrete steps. A deeper understanding and clearer visualization would necessarily involve a more powerful approach such as a single-molecule analysis that would allow us to follow the protein choreography in real time.

It has been suggested on the basis of tracking fluorescent derivatives of BRCA2 and RAD51 in live cells that the pool of freely diffusing RAD51 is limited and that there is nearly complete association between BRCA2 and mobile RAD51.²⁴ The stoichiometry of association of RAD51 with BRCA2 was estimated to be 6.7–33:1. Given that the stoichiometry as determined *in vitro*⁵ was ~6:1, higher than in the case of Brh2/Dss1 due to the presence of eight BRC repeats in the mammalian BRCA2, we wonder whether the elevated level *in vivo* might reflect the presence in the mix of some DSS1-free complexes in the transient state carrying short polymers of RAD51 as we describe. It is intriguing to consider the possibility that RAD51 already preformed as a short oligomer in association with BRCA2 after loss of DSS1 might be the preferred form for delivery to DNA.

Acknowledgments

Funding

This work was supported in part by National Institutes of Health Grant GM079859.

We thank Dr. Lorraine Symington (Columbia University, New York, NY) and Dr. Jeanette Sutherland, this laboratory, for discussion and comments on the manuscript.

ABBREVIATIONS

BRC	Rad51-binding element
CRE	C-terminal Rad51-binding element
CT	carboxy-terminal
DBD	DSS1/DNA-binding domain
ds	double-stranded
HEPES	<i>N</i> -(2-hydroxyethyl)-piperazine- <i>N'</i> -(2-ethanesulfonic acid)
His tag	hexahistidine tag
MBP	maltose-binding protein
NT	amino-terminal
NTA	nitrilotriacetate agarose
OB	oligonucleotide/oligosaccharide binding
oligo dT	oligothymidylate
ss	single-stranded
UV	ultraviolet

References

1. Kowalczykowski SC. An Overview of the molecular mechanisms of recombinational DNA repair. *Cold Spring Harbor Perspect Biol.* 2015; 7:a016410.
2. Renkawitz J, Lademann CA, Jentsch S. Mechanisms and principles of homology search during recombination. *Nat Rev Mol Cell Biol.* 2014; 15:369–383. [PubMed: 24824069]
3. Symington LS. Mechanism and regulation of DNA end resection in eukaryotes. *Crit Rev Biochem Mol Biol.* 2016; 51:195–212. [PubMed: 27098756]
4. San Filippo J, Sung P, Klein H. Mechanism of eukaryotic homologous recombination. *Annu Rev Biochem.* 2008; 77:229–257. [PubMed: 18275380]
5. Jensen RB, Carreira A, Kowalczykowski SC. Purified human BRCA2 stimulates RAD51-mediated recombination. *Nature.* 2010; 467:678–683. [PubMed: 20729832]
6. Holloman WK. Unraveling the mechanism of BRCA2 in homologous recombination. *Nat Struct Mol Biol.* 2011; 18:748–754. [PubMed: 21731065]
7. Yang H, Li Q, Fan J, Holloman WK, Pavletich NP. The BRCA2 homologue Brh2 nucleates RAD51 filament formation at a dsDNA-ssDNA junction. *Nature.* 2005; 433:653–657. [PubMed: 15703751]
8. Shin DS, Pellegrini L, Daniels DS, Yelent B, Craig L, Bates D, Yu DS, Shivji MK, Hitomi C, Arvai AS, Volkmann N, Tsuruta H, Blundell TL, Venkitaraman AR, Tainer JA. Full-length archaeal Rad51 structure and mutants: mechanisms for RAD51 assembly and control by BRCA2. *EMBO J.* 2003; 22:4566–4576. [PubMed: 12941707]
9. Rajendra E, Venkitaraman AR. Two modules in the BRC repeats of BRCA2 mediate structural and functional interactions with the RAD51 recombinase. *Nucleic Acids Res.* 2010; 38:82–96. [PubMed: 19875419]

10. Yang H, Jeffrey PD, Miller J, Kinnucan E, Sun Y, Thoma NH, Zheng N, Chen PL, Lee WH, Pavletich NP. BRCA2 function in DNA binding and recombination from a BRCA2-DSS1-ssDNA structure. *Science*. 2002; 297:1837–1848. [PubMed: 12228710]
11. Zhou Q, Kojic M, Holloman WK. DNA-binding domain within the Brh2 N terminus is the primary interaction site for association with DNA. *J Biol Chem*. 2009; 284:8265–8273. [PubMed: 19182269]
12. Kojic M, Zhou Q, Lisby M, Holloman WK. Brh2-Dss1 interplay enables properly controlled recombination in *Ustilago maydis*. *Mol Cell Biol*. 2005; 25:2547–2557. [PubMed: 15767662]
13. Zhou Q, Holloman WK. Dual DNA-binding domains shape the interaction of Brh2 with DNA. *DNA Repair*. 2014; 22:104–111. [PubMed: 25128760]
14. Zhou Q, Kojic M, Holloman WK. Dss1 release activates DNA binding potential in Brh2. *Biochemistry*. 2012; 51:9137–9146. [PubMed: 23094644]
15. Carreira A, Hilario J, Amitani I, Baskin RJ, Shivji MK, Venkitaraman AR, Kowalczykowski SC. The BRC repeats of BRCA2 modulate the DNA-binding selectivity of RAD51. *Cell*. 2009; 136:1032–1043. [PubMed: 19303847]
16. Pellegrini L, Yu DS, Lo T, Anand S, Lee M, Blundell TL, Venkitaraman AR. Insights into DNA recombination from the structure of a RAD51-BRCA2 complex. *Nature*. 2002; 420:287–293. [PubMed: 12442171]
17. Cole DJ, Rajendra E, Roberts-Thomson M, Hardwick B, McKenzie GJ, Payne MC, Venkitaraman AR, Skylaris CK. Interrogation of the protein-protein interactions between human BRCA2 BRC repeats and RAD51 reveals atomistic determinants of affinity. *PLoS Comput Biol*. 2011; 7:e1002096. [PubMed: 21789034]
18. Chatterjee G, Jimenez-Sainz J, Presti T, Nguyen T, Jensen RB. Distinct binding of BRCA2 BRC repeats to RAD51 generates differential DNA damage sensitivity. *Nucleic Acids Res*. 2016; 44:5256–5270. [PubMed: 27084934]
19. Zhou Q, Kojic M, Cao Z, Lisby M, Mazloum NA, Holloman WK. Dss1 interaction with Brh2 as a regulatory mechanism for recombinational repair. *Mol Cell Biol*. 2007; 27:2512–2526. [PubMed: 17261595]
20. Kojic M, Yang H, Kostrub CF, Pavletich NP, Holloman WK. The BRCA2-interacting protein DSS1 is vital for DNA repair, recombination, and genome stability in *Ustilago maydis*. *Mol Cell*. 2003; 12:1043–1049. [PubMed: 14580353]
21. Song B, Sung P. Functional interactions among yeast Rad51 recombinase, Rad52 mediator, and replication protein A in DNA strand exchange. *J Biol Chem*. 2000; 275:15895–15904. [PubMed: 10748203]
22. Jeyasekharan AD, Liu Y, Hattori H, Pisupati V, Jonsdottir AB, Rajendra E, Lee M, Sundaramoorthy E, Schlachter S, Kaminski CF, Ofir-Rosenfeld Y, Sato K, Savill J, Ayoub N, Venkitaraman AR. A cancer-associated BRCA2 mutation reveals masked nuclear export signals controlling localization. *Nat Struct Mol Biol*. 2013; 20:1191–1198. [PubMed: 24013206]
23. Kojic M, Zhou Q, Fan J, Holloman WK. Mutational analysis of Brh2 reveals requirements for compensating mediator functions. *Mol Microbiol*. 2011; 79:180–191. [PubMed: 21166902]
24. Reuter M, Zelensky A, Smal I, Meijering E, van Cappellen WA, de Gruiter HM, van Belle GJ, van Royen ME, Houtsmuller AB, Essers J, Kanaar R, Wyman C. BRCA2 diffuses as oligomeric clusters with RAD51 and changes mobility after DNA damage in live cells. *J Cell Biol*. 2014; 207:599–613. [PubMed: 25488918]
25. Zaitseva EM, Zaitsev EN, Kowalczykowski SC. The DNA binding properties of *Saccharomyces cerevisiae* Rad51 protein. *J Biol Chem*. 1999; 274:2907–2915. [PubMed: 9915828]
26. Liu J, Doty T, Gibson B, Heyer WD. Human BRCA2 protein promotes RAD51 filament formation on RPA-covered single-stranded DNA. *Nat Struct Mol Biol*. 2010; 17:1260–1262. [PubMed: 20729859]
27. Zhao W, Vaithiyalingam S, San Filippo J, Maranon DG, Jimenez-Sainz J, Fontenay GV, Kwon Y, Leung SG, Lu L, Jensen RB, Chazin WJ, Wiese C, Sung P. Promotion of BRCA2-dependent homologous recombination by DSS1 via RPA targeting and DNA mimicry. *Mol Cell*. 2015; 59:176–187. [PubMed: 26145171]

Figure 1.

Association of Dss1 with Brh2 depends on CRE. (A) Schematic showing MBP-tagged Brh2 variants with domains: BRC (black box), NBD (gray rectangle), CTD with HD (hatched), OB1 (gray oval), OB2 with an intervening tower (gray oval/black rectangle), and CRE (black box). Brh2 polypeptides as shown schematically were co-expressed with Dss1 in *E. coli*. (B) Sequential coprecipitation of MBP-Brh2/His-Dss1 complexes. Cell-free extracts were prepared and the soluble fractions mixed with Ni²⁺-NTA agarose and then eluted with 0.3 M imidazole. The eluate was mixed with amylose resin, and specific complexes eluted with 10 mM maltose. The protein composition was analyzed by SDS gel electrophoresis. (C) Parallel precipitations of MBP-Brh2 fragments not associated with His-Dss1. Cell-free extracts were prepared and the soluble fractions mixed with Ni²⁺-NTA agarose or amylose resin and then eluted with 0.3 M imidazole or 10 mM maltose. Lane numbers in the gels correspond to the MBP-Brh2 variants in the schematic. MBP-Brh2 variants are indicated according to the predicted size by the asterisks; His-Dss1 is indicated by the arrow.

Figure 2.

Interaction of CRE with Rad51. In panels A–F, association reactions were performed with MBP-tagged CRE 72mer polypeptide ($0.7 \mu\text{M}$) and the indicated levels of Rad51 and Dss1. After incubation for 30 min, amylose resin was added. Specific complexes were eluted with 10 mM maltose as described in Experimental Procedures. The protein composition of the unbound and specifically eluted fractions was analyzed by SDS gel electrophoresis. (A) Effect of temperature. After being mixed with input Rad51 ($3.5 \mu\text{M}$), samples were held at the indicated temperatures and then mixed with amylose resin as described above. (B) Protein amounts were quantified by comparison of band intensities with CRE and Rad51 standards in the panel. (C) Increasing levels of Rad51. The MBP-tagged CRE polypeptide and increasing levels of Rad51 were incubated in reaction mixtures at 37°C . (D) Protein amounts were quantified. (E) Effect of Dss1. Reaction mixtures containing the MBP-tagged CRE polypeptide ($0.7 \mu\text{M}$), Rad51 ($1.1 \mu\text{M}$), and increasing levels of Dss1 as shown were incubated at 0°C for 30 min and then processed with amylose resin. (F) Protein amounts were quantified. Levels are depicted as means $\pm$ the standard deviation of at least three experiments. In panels G–I, association reactions were performed with MBP-tagged Brh2^{CT} ($0.7 \mu\text{M}$) with input Rad51 ($3.5 \mu\text{M}$). (G) Schematic illustrating the sequential procedure for capturing MBP-Brh2^{CT}/His-Dss1 complexes and Rad51 with Ni^{2+} -NTA beads and amylose resin. (H) After mixing, MBP-Brh2^{CT}/His-Dss1 and Rad51 samples were held at 0 or 32°C and processed by sequential precipitations. (I) Protein amounts were quantified. Protein components in reactions 1–5 are tabulated in the panel below. Rad51 by itself (reaction 5) did not appear to associate with either Ni^{2+} -NTA beads or amylose resin. Levels are depicted as means $\pm$ the standard deviation of three experiments.

Figure 3.

Interaction of Rad51 with Brh2 as a function of Dss1 association. (A) Schematic showing the method for capturing Brh2 and associated Rad51 on amylose resin after passage through Ni^{2+} -NTA beads to separate Dss1-free Brh2 from Brh2/Dss1 complexes. Brh2 and Dss1 mentioned below contained MBP and His tags, respectively. (B) Reaction mixtures (50 μL) contained 840 nM Brh2 prepared as a complex with His-Dss1, 3.4 μM Rad51, and the indicated levels of additional Dss1: lane 1, no added Dss1; lane 2, 840 nM Dss1; lane 3, 1.78 μM Dss1; lane 4, 3.36 μM Dss1. Lane 5 contained only Rad51 as a control for nonspecific binding. After incubation, Ni^{2+} -NTA beads were added to capture free Dss1 and Brh2/Dss1 complexes. The unbound fraction was mixed with amylose resin to capture Brh2 and associated Rad51. After specific elution of protein from amylose by addition of 10 mM maltose as described in Experimental Procedures, the beads were boiled in SDS sample buffer to strip off any protein bound nonspecifically. The inset below panel B shows standards that were used for quantifying the recovered protein. (C) Rad51:Brh2 ratios were quantified as a function of additional Dss1 that was added to the starting reaction mix containing Brh2/Dss1 complexes. The top curve shows Rad51 associated with Brh2 after amylose capture. The bottom curve shows Rad51 associated with Brh2 after Ni^{2+} -NTA capture. (D) Reaction mixtures were prepared and processed as described for panel B but with 840 nM Brh2^{W1052A}. Brh2^{W1052A} protein did not form a stable complex with Dss1 and was not retained by Ni^{2+} -NTA beads. Rad51 associated with Brh2^{W1052A} on amylose resin was quantified in the top portion of panel C. (E) Reaction mixtures were prepared and

processed as described for panel B but with 800 nM Brh2 that had been stripped of Dss1: lane 1, no added Dss1; lane 2, 560 nM Dss1; lane 3, 1.1 μ M Dss1; lane 4, 2.24 μ M Dss1. Lane 5 contained only Rad51 as a control for nonspecific binding. The inset below panel E shows Brh2 and Rad51 standards that were used for quantifying the recovered protein. (F) Rad51:Brh2 ratios were quantified as a function of Dss1 that was added to the starting reaction mix. Levels are depicted as means $\pm$ the standard deviation of three determinations. (G) Reaction mixtures (50 μ L) contained where indicated 3.4 μ M Rad51, 2 mM ATP, 2 mM MgCl₂, 3.4 μ M Dss1, and 150 nM single strand 60mer. After incubation, Ni²⁺-NTA beads were added to capture Dss1 and Dss1/Rad51 complexes. (H) Rad51:Dss1 ratios were quantified.

Figure 4.

Mapping additional Rad51 contact sites. (A) Schematic showing the location of FxTP motifs and two Brh2 fragments tested for physical interaction with Rad51. (B) Reaction mixtures (50 μ L) contained MBP-tagged Brh2 fragments (0.7 μ M) and 3.5 μ M Rad51. After incubation for 30 min, amylose resin was added. Specific complexes were eluted with 10 mM maltose as described in Experimental Procedures. The protein composition of the unbound and specifically eluted fractions was analyzed by SDS gel electrophoresis. (C) Protein amounts were quantified by comparison of band intensities with Brh2 fragments and Rad51 standards in the panel.

Figure 5. Rad51 inhibits binding of Brh2 to DNA. (A) Schematic showing the method for capturing Brh2 and Rad51 on biotinylated DNA-bound streptavidin-coated magnetic particles. (B) Reaction mixtures contained 840 nM Brh2 complexed with Dss1 in Tris-Mg²⁺ buffer without ATP. Rad51 was added as follows: lane 1, none; lane 2, 210 nM; lane 3, 420 nM; lane 4, 840 nM; lane 5, 1.68 μ M; lane 6, 3.36 μ M; lane 7, 3.36 μ M but no biotinylated DNA. After reaction, particles were collected and the composition of bound components was determined. (C) Levels of Brh2 bound are depicted as means $\pm$ the standard deviation of three determinations. (D) Binding of Brh2 to DNA determined by a gel shift assay. Reaction mixtures contained 3.3 nM IRD800-labeled 60mer, Brh2 or NT or CT variant at 330 mM, and increasing levels of Rad51 at the ratios shown. (E) Quantification. (F) Binding of Rad51 to DNA determined by a gel shift assay. Reaction mixtures contained 2 mM Mg²⁺ or Ca²⁺ with or without 2 mM ATP as shown and increasing levels of Rad51. (G) Quantification.

Figure 6.

Brh2 mediator feedback loop. The scenario envisioned features a 1:1:1 primed complex of Brh2 (gray torus with indicated DNA and Rad51 interaction regions) and Dss1 (black pickle) associated with a single Rad51 protomer (gray oval) in a locked conformation (step 1). As the complex approaches exposed ssDNA, Dss1 is released, triggering opening of the Brh2 torus, attraction of a short oligomer of Rad51 to the protein surface, and binding of NBD and CTD domains to DNA (steps 2 and 3). In turn, the Rad51 oligomer forces Brh2 off the DNA while transferring itself on (step 4). Free Dss1 associates with DNA-bound Rad51.