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The BRCA Tumor Suppressor Network in Chromosome Damage Repair by Homologous Recombination

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Abstract

Mutations in the *BRCA1* and *BRCA2* genes predispose afflicted individuals to breast, ovarian, and other cancers. The BRCA-encoded products form complexes with other tumor suppressor proteins and with the recombinase enzyme RAD51 to mediate chromosome damage repair by homologous recombination, and also to protect stressed DNA replication forks against spurious nucleolytic attrition. Understanding how the BRCA tumor suppressor network executes its biological functions would provide the foundation for developing targeted cancer therapeutics, but progress in this area has been greatly hampered by the challenge of obtaining purified BRCA complexes for mechanistic studies. In this article, we review how recent effort begins to overcome this technical challenge, leading to functional and structural insights into the biochemical attributes of these complexes and the multi-faceted roles that they fulfill in genome maintenance. We also highlight the major mechanistic questions that remain.

Keywords

Homologous recombination; DNA damage repair; replication fork repair; replication fork protection; genome maintenance; BRCA1; BARD1; BRCA2; tumor suppression

1. INTRODUCTION

The integrity of our genome is challenged continuously by environmental agents, such as high-energy radiations, and by reactive metabolites, e.g. aldehydes and free radicals, that

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damage DNA (1–3). Accordingly, a complex network of DNA damage checkpoints and repair pathways has evolved to minimize the harm that may be caused by these genotoxic agents. As such, a defect in any of these detoxification mechanisms leads to genome destabilization and disease, cancer in particular (2, 4). Studies on chromosome damage repair have taken center stage in cancer biology in recent years, and knowledge garnered from these endeavors is being translated into cancer therapeutics (5).

DNA damage checkpoints:

signal transduction cascades that result in cell cycle arrest and activation of DNA damage repair.

Below, we review chromosome damage repair that is mediated by homologous recombination (HR) genes of the *RAD52* epistasis group (see Section 3.1 for description). This genome maintenance pathway helps eliminate DNA double-strand breaks (DSBs) and interstrand crosslinks (ICLs), and also repairs damaged DNA replication forks and protects stressed forks against nucleolytic digestion. We will specifically focus on the functional and structural insights derived from recent studies on the tumor suppressors BRCA1-BARD1 and BRCA2 that are integral components of the HR machinery.

2. BIOLOGICAL ROLES OF HOMOLOGOUS RECOMBINATION

2.1 Function as a DNA Double-Strand Break Repair Tool

HR and non-homologous DNA end-joining (NHEJ) are the major, mechanistically distinct DSB repair pathways in eukaryotic cells. HR primarily operates in the S and G2 phases of the cell cycle when the sister chromatid is available to template repair, whereas NHEJ functions throughout the cell cycle (6–8). HR also mediates the pairing of homologous chromosomes in meiosis to help ensure their proper disjunction in the first meiotic division (9).

Meiosis: encompasses two cell divisions to generate haploid gametes. Pairing of meiotic chromosomes is triggered by DSBs and mediated by HR.

For HR to occur, a DNA break end must first undergo nucleolytic resection of the 5′-terminated strand to generate 3′-tailed single-stranded (ss) DNA to serve as the template for assembling the HR machinery (see Figure 1 and later sections). Under specific circumstances, e.g. when HR is inactivated by a genetic mutation, the ssDNA tails derived from DSB processing may be utilized by two other repair pathways, namely, single-strand annealing (SSA) and alternative DNA end joining (alt-EJ), to yield deletion type products. Details on the choice and regulation of DSB repair pathways can be found in several recent reviews (6–8, 10).

2.2 Involvement in Replication Fork Repair and Protection

DNA replication fork stalling induced by stress, such as upon nucleotide depletion or encountering a DNA lesion, triggers the DNA replication checkpoint for fork stabilization (11). One major means to circumvent fork stalling is the activation of a nearby, normally

dormant replication origin (11). Alternatively, DNA lesions may be bypassed by one of several specialized DNA polymerases in a mechanism called translesion synthesis (12). In addition, the stalled fork can be converted by an ATP-dependent nucleic acid motor protein into a "chicken-foot" structure, in which the free DNA end is engaged by HR to mediate fork restart (11). Importantly, recent studies have provided evidence that a subset of HR factors, including the recombinase RAD51 and the tumor suppressors BRCA1 and BRCA2, help protect stressed replication forks from being degraded by nucleases such as MRE11 and DNA2 (13–17).

Translesion synthesis: employs a specialized DNA polymerase to synthesize DNA across a lesion in the template strand.

Chicken foot structure: also called a regressed fork, has four double-stranded arms, of which one harbors a free end.

2.3 Role in Interstrand DNA Crosslink Removal

An ICL arises when a covalent bond is formed between bases in the complementary strands of duplex DNA. This lesion impedes transcription and DNA replication, and the mechanism for its removal is exceedingly complex and normally coupled to DNA replication (18–21). The prevailing model for DNA replication-coupled ICL repair posits that lesion removal is triggered by two replication forks converging from opposite sides of the crosslink (19, 20). Then, lesion "unhooking" via endonucleolytic strand scissions 5′ and 3′ to the ICL generates two DNA molecules, with one harboring a DSB and the other a DNA gap with the unhooked crosslink appended as a mono-adduct (19, 20). Upon gap filling by DNA polymerase ζ (a translesion synthesis polymerase) and ligation of the resulting DNA nick, a duplex template is created to enable elimination of the DSB via HR (18, 20, 21). Accordingly, DSBs accumulate in HR-defective cells upon treatment with agents that induce ICLs (18, 20, 21). Ultimately, the unhooked DNA crosslink is removed by nucleotide excision repair (22). Aside from components of the HR pathway, the genes responsible for Fanconi Anemia (FA) are also critically important for replication-dependent ICL repair (18, 20, 21). We refer interested readers to recent reviews and research highlights on this topic (18–21, 23, 24).

Nucleotide excision repair: removes bulky DNA lesions that distort the conformation of the DNA double helix.

The FANCONI ANEMIA DNA DAMAGE RESPONSE PATHWAY: Fanconi anemia (FA) is an autosomal recessive disorder characterized by bone marrow failure, developmental abnormalities, and a strong cancer predisposition in children and young adults. Cells from FA patients are hypersensitive to DNA damaging agents, in particular those that induce ICLs, and to reactive aldehydes that stem from cellular metabolism (18, 20, 21, 25). FA is genetically complex, comprising at least 22 complementation groups. Importantly, biallelic mutations in a number of HR genes can result in FA. This provides compelling evidence that HR-mediated DNA repair constitutes an integral component of the FA DNA damage response pathway (18, 20, 21, 25).

3. GENETICS OF HOMOLOGUS RECOMBINATION

3.1 Genes of the *RAD52* Epistasis Group

The budding yeast *S. cerevisiae* has been a valuable model for dissecting the genetic complexity and mechanistic intricacy of HR. In *S. cerevisiae*, the majority of DSBs are resolved via HR, and the initial attempts at isolating mutants based on their hypersensitivity to X-rays found eight genetic loci designated *RAD50* to *RAD57* (26, 27). Later efforts utilizing genetic screens and bioinformatics led to the isolation of other HR genes (28, 29). For instance, *SHU1 and SHU2* were identified as suppressors of the slow growth phenotype and DNA damage sensitivity of cells lacking DNA topoisomerase 3 (30). Collectively, these HR genes constitute the *RAD52* epistasis group, and the majority of them possess an ortholog in metazoans ((31, 32); Table 1). Generally speaking, mutants of the *RAD52* group are hypersensitive to agents that induce DSBs and replication stress, and are also defective in meiotic chromosome segregation (31). As will be discussed in the next section, metazoans rely on the tumor suppressors BRCA1, BARD1, BRCA2, and PALB2 for HR execution (4). Notably, *S. cerevisiae* does not harbor a structural homolog of any of these tumor suppressors.

Epistasis group: encompasses genes functioning in the same pathway, such that double mutants exhibit no more severe a phenotype than single mutants.

THE PALB2 GENE AND PROTEIN: PALB2 (Partner and Localizer of BRCA2) protein is stably associated with BRCA2 and required for the localization of BRCA2 to DNA damage (33). Through its interaction with BRCA1, PALB2 nucleates the assembly of the BRCA Mediator Complex (see Section 5.6b). Pathogenic mutations in PALB2 predispose afflicted individuals to breast, pancreatic, prostate, and other cancers (34, 35). Importantly, point mutations that disrupt the interaction of PALB2 with either BRCA1 or BRCA2 engender DNA damage sensitivity, HR defects, and cancer susceptibility (34, 35). PALB2 binds DNA and physically interacts with RAD51, and it also enhances the recombinase activity of RAD51 via promotion of synaptic complex formation (36, 37).

3.2 Dependence of HR on Tumor Suppressors in Metazoans

BRCA1, BARD1, BRCA2, and PALB2 are needed for the suppression of a wide variety of tumors, and they do so by enhancing the DSB repair capacity of cells. Efforts by many research groups have shown that these tumor suppressors form distinct complexes among themselves and with other HR proteins. These protein complexes play key roles in different stages of HR, DSB and replication fork repair, the protection of stressed replication forks, and ICL removal ((4); see Section 2). Given the important roles that HR fulfills during DNA replication, it is no surprise that deletion of these tumor suppressors engenders embryonic lethality at an early stage of development (38–43). Interestingly, biallelic mutations in *BRCA1*, *BRCA2*, and *PALB2* can cause FA (44–46) and, hence, these genes are also known as *FANCS*, *FANCD1* and *FANCN*, respectively (21). No FA-specific mutations have been described for BARD1 yet. It should be noted that cancer causative mutations have been identified in other HR genes such as those that encode the RAD51 paralogs (47) and FANCJ (48), a DNA helicase that is mutated in a subset of FA patients.

THE RAD51 PARALOGS IN HR AND CANCER—RAD51 paralogs are conserved HR factors that exhibit sequence similarity with and are structurally related to the recombinase RAD51 (4). Six such paralogs, namely, RAD51B, RAD51C, RAD51D, XRCC2, XRCC3 and SWS1AP1 (Table 1), have been described in humans, and they form complexes among themselves and with other factors (4, 49). However, the RAD51 paralogs are devoid of recombinase activity. Rather, they regulate RAD51 presynaptic filament assembly and appear to enhance the catalytic potential of the presynaptic filament (50, 51). Existing evidence suggests that the RAD51 paralogs function together with BRCA2 in presynaptic filament assembly (51, 52). Mutations in the RAD51 paralogs have been linked to breast and ovarian cancers and to Fanconi anemia (47).

4. CRITICAL STAGES IN HOMOLOGOUS RECOMBINATION

4.1 DNA End Resection

As touched upon earlier (see Section 2.1), the repair of DSBs or replication forks by HR is initiated via the resection of the 5′-terminated DNA strand at the break site, which generates a 3′-tailed ssDNA substrate for the assembly of the HR machinery that harbors the RAD51 recombinase and its ancillary factors (53). Studies, first done in *S. cerevisiae* and later in metazoans, have shown that DNA end resection occurs in two distinct stages (53). At DNA ends that are occluded by the NHEJ factor Ku, Sae2 (CtIP in humans; Table 1) activates the Mre11-Rad50-Xrs2 complex (MRE11-RAD50-NBS1 in humans) to incise the 5′-terminated DNA strand endonucleolytically (Figure 1). Subsequent long-range resection is mediated by two apparently redundant mechanisms that are dependent on either the 5′→ 3′ exonuclease Exo1 or the ensemble comprising the DNA helicase/endonuclease Dna2. Both of these long-range resection nucleases are dependent on other protein factors for optimal activity (54, 55). Specifically, Dna2 functions within the context of an obligate ensemble with RPA, the RECQ family helicase Sgs1 (BLM in metazoans), and other cofactors (Table 1), while the activity of Exo1 is enhanced by RPA and, in the case of the human enzyme, by BLM as well (53, 55, 56). Interestingly, WRN, another RECQ family helicase, can substitute for BLM in DNA2-dependent resection (57, 58). It should be noted that nucleolytic resection of the "chicken foot" structure stemming from the regression of stressed replication forks appears to proceed via mechanisms different than what occurs at DSBs (59, 60).

Notably, yeast Rad9 and its mammalian counterpart 53BP1 (p53-binding protein 1) suppress DNA end resection in the G1 phase of the cell cycle. The restriction on resection imposed by Rad9/53BP1 effectively renders NHEJ the default DSB pathway in G1 cells. The inhibitory effect of these proteins is relieved in the S cell cycle phase (6, 61). In metazoans, relaxation of the 53BP1-dependent resection block requires BRCA1 (61, 62). A great deal of effort has been expended to identify protein factors that act with 53BP1. The current model posits that the recruitment of 53BP1 occurs via its interaction with modified histones H2A-K15ub and H4-K20Me_2, which then nucleates the formation of a higher order ensemble that encompasses RIF1, the CST complex and Shieldin ((6, 63–67); Figure 1).

4.2 Presynaptic Filament Assembly

4.2a Recombinase presynaptic filament.—Rad51 belongs to the RecA/Rad51 family of recombinases that catalyze the pairing of homologous ssDNA and dsDNA and the exchange of DNA strands between the synapsed DNA molecules (68–70). Homologous DNA pairing and strand exchange occur within the confines of a right-handed, filamentous recombinase polymer assembled on the 3´-tailed ssDNA derived from DNA end resection ((53, 69, 70); Figure 1). Within the HR community, the Rad51-ssDNA nucleoprotein filament is referred to as the "presynaptic filament" or "presynaptic complex" (69, 70). The presynaptic filament of Rad51 has been examined by negative stain electron microscopy (71, 72) and, more recently, by cryo-electron microscopy (cryo-EM; (73, 74)). As revealed in earlier work (71, 72, 75) and highlighted in a near-atomic cryo-EM structure (73, 74), the presynaptic filament of human RAD51 comprises ~6 protein protomers and ~18 bases of DNA per turn with a 100 Å pitch ((74); Figure 2, A and B). Importantly, the ssDNA is extended to ~1.5 times the length of B-form DNA, a conserved feature of presynaptic filaments of RecA/Rad51 family members (74, 75). The majority of RAD51 protomer-protomer interactions in a functional presynaptic filament occur upon ATP being sandwiched between two adjacent protomers. Within the filament, RAD51 protomers primarily interact with the phosphate backbone of the DNA, with key amino acid residues in the L1 and L2 DNA binding loops of three contiguous protomers organizing the DNA ligand in base triplets (Figure 2C). In this configuration, the base triplets point towards the axial direction of the presynaptic filament and are poised to undergo pairing and strand exchange with a homologous duplex target ((74); Figure 2C).

4.2b Mediators of presynaptic filament assembly and maintenance.—Owing its abundance and high affinity for ssDNA, RPA promptly occupies the 3´-tailed ssDNA derived from DNA end resection (69). Even though RPA protects the DNA tail from nucleolytic attack and removes secondary structure therein, it presents a strong impediment to the initial nucleation of RAD51 (76). Facilitators of RPA-Rad51 exchange have been found in different organisms, including *S. cerevisiae* Rad52 and the tumor suppressor BRCA2 in metazoans (69, 76). These HR "mediators" interact with RAD51 and bind ssDNA. Interestingly, while Rad52 associates with RPA, the small, acidic protein DSS1 provides the RPA-interacting interface in its stoichiometric complex with BRCA2 ((77); see Section 6.5).

The yeast Rad55-Rad57 and human RAD51B-RAD51C complexes enhance presynaptic filament assembly on RPA-coated ssDNA. As such, these complexes of Rad51 paralogs are HR mediators in their own right (78, 79). Moreover, Rad55-Rad57 has been shown to attenuate the ability of the Srs2 helicase, one of several anti-recombinases that have been identified, to dismantle the Rad51 presynaptic filament (8, 80, 81). Interestingly, the yeast Rad51 paralogs associate and synergize with Rad52 to facilitate Rad51 presynaptic filament assembly (51). Whether the metazoan RAD51 paralogs (76, 82) similarly interact and function with BRCA2 remains to be determined, but it should be noted that some of the human RAD51 paralogs show an epistatic relationship with BRCA2 (52).

Anti-recombinases: ATP-dependent motor proteins that either dismantle the presynaptic filament or HR intermediates, or inhibit repair DNA synthesis.

4.3 Homologous DNA Pairing and Strand Exchange

4.3a Assembly of the synaptic complex: The presynaptic filament possesses a site, termed the secondary site, that engages duplex DNA to enable the search for sequence homology in the latter (83). Work done with *E. coli* RecA has provided evidence that DNA homology search occurs by way of random collisions between the presynaptic filament and the duplex DNA (83). Upon the location of homology in the duplex partner, the presynaptic filament forms a triple-stranded structure that entails limited base pairing between the ssDNA and dsDNA molecules. This nascent DNA joint is called the "paranemic joint", and the three-stranded nucleoprotein ensemble is referred to as the "synaptic complex" ((69, 83, 84); Figure 3A). Even though the paranemic joint is transient in nature, its formation allows for iterative three-dimensional search through sequence space to facilitate downstream events ((85); see Section 4.3b). Biochemical and biophysical methods have been developed to study paranemic joint formation (36, 86, 87). As revealed in single-molecule imaging of homologous DNA pairing by RecA/Rad51 family recombinases, a contiguous homology tract of eight nucleotides is sufficient for paranemic joint formation (85). HR factors that enhance the assembly of the synaptic complex have been identified (36, 69, 86, 88). Below (see Section 5.5), we discuss recent findings that identify the BRCA1-BARD1 complex as such a stimulatory factor of RAD51 (89).

4.3b Formation of the post-synaptic complex: In DSB repair, upon synaptic complex formation (see Section 4.3a), strand exchange between the 3´-tailed ssDNA and the homologous dsDNA partner initiates from the free end of the former. This gives rise to the "plectonemic joint" in which the two DNA strands are topologically intertwined (Figure 3A). The nucleoprotein ensemble harboring the plectonemic joint has been termed the "post-synaptic" complex, with the corresponding DNA structure that harbors a displaced ssDNA region being referred to as the displacement loop, or D-loop (69, 83, 84). In congruence with the organization of ssDNA as base triplets in the presynaptic filament, DNA strand exchange occurs in 3-nucleotide steps (85, 90). Analysis by cryo-EM has revealed the hierarchy of protein-DNA interactions that help stabilize the plectonemic joint within the context of base pair triplets ((74); Figure 2D–F).

4.4 Repair DNA Synthesis and Resolution of HR Intermediates

Following D-loop formation, DNA synthesis commences from the terminus of the invading strand to copy sequence information from the donor DNA molecule (Figure 1). In both yeast and metazoans, DNA polymerase (Pol) δ catalyzes the repair synthesis reaction (91). The extended D-loop structure can be resolved by one of several mechanistically distinct pathways, namely, canonical DSB repair (DSBR), synthesis-dependent DNA strand annealing (SDSA), and double Holliday Junction (dHJ) dissolution. Of these pathways, only DSBR is capable of generating recombinants that harbor an exchange of chromosome arms, or crossover (92, 93). As was first revealed in genetic studies done in *S. cerevisiae*, during mitotic DSB repair, there is a strong tendency for cells to employ SDSA or dHJ dissolution to form non-crossover recombinants. Shunting the D-loop away from the DSBR pathway

helps prevent chromosome arm translocations. The mechanistic details and regulation of HR pathways are highly complex, and we refer interested readers to recent reviews on this topic (8, 32, 93).

5. MULTIFACETED ROLE OF BRCA1-BARD1 IN GENOME MAINTENANCE

5.1 BRCA1: Discovery and Protein Domain Structure

The *BRCA1* (Breast Cancer 1) gene was identified based on genetic linkage to familial breast cancer susceptibility (94, 95). Importantly, BRCA1 mutations cause familial ovarian cancer and sporadic cancer in different organs as well (96, 97). Deletion of *Brca1* in mice engenders embryonic lethality (38–40). The human protein comprises 1,863 amino acid residues and harbors a RING (Really Interesting New Gene) domain at its N-terminus and two copies of the BRCT (BRCA1 Carboxy-Terminal) repeats at its C-terminus (Figure 3B). Through its RING domain, BRCA1 combines with BARD1 to form a heterodimer that possesses E3 ubiquitin ligase activity (98, 99). The tandem BRCT repeats confer the ability to associate with DNA damage response factors such as CtIP and FANCJ (100). Immediately preceding the BRCT repeats is a coiled coil domain that mediates complex formation with PALB2 (33, 101, 102). The central region of BRCA1 that is encoded by exon 11 accounts for >60% of the protein and is disordered based on secondary structure prediction (100). This large central region harbors sequences that confer capabilities for binding DNA (89, 103, 104) and interaction with various DNA damage response and repair factors, including RAD51 (89, 100, 105).

5.2 BARD1: Discovery, Protein Domain Structure, and Tumor Suppression Activity

BARD1 was identified as a BRCA1 interactor in a yeast two-hybrid screen (98). BARD1 comprises 777 amino acid residues and, like BRCA1, possesses an N-terminal RING domain and tandem BRCT repeats at its C-terminus (Figure 3C). The RING domain mediates heterodimer formation with BRCA1 (98, 106), and the BRCT repeats interact with proteins distinct from those recognized by the equivalent domain in BRCA1 (107). There are four ankyrin motifs that are involved in protein-protein interactions (107). Like BRCA1, BARD1 binds DNA and physically interacts with RAD51 (89). *BARD1* mutations have been identified in breast, ovarian, and uterine cancers (107, 108). Importantly, BARD1 variants are also associated with neuroblastoma and other cancer types (107). In mouse studies, the ablation of *Bard1* engenders embryonic lethality (42), while its conditional deletion in mammary epithelial cells induces breast cancer (107, 109).

5.3 Involvement of BRCA1 and BARD1 in Chromosome Damage Repair by HR

Following the discovery of BRCA1, intense efforts by different research groups provided concrete evidence for its involvement in chromosome damage repair. In one study, BRCA1 was found to colocalize with RAD51 in S phase cells and in human meiotic spermatocytes, and to also coimmunoprecipitate with RAD51 from cell extracts (105). Other endeavors showed that BRCA1-deficient cells are hypersensitive to DNA damaging agents and display chromosomal instability (110–112). Importantly, with the use of a HR reporter, an impairment of HR-mediated DSB repair in *Brca1* deficient mouse embryonic stem cells was revealed (112, 113). Likewise, genetic ablation of BARD1 engenders a defect in

chromosome damage repair, genome instability, and an inability to execute HR (42, 89, 114).

5.4. Role of BRCA1-BARD1 in DNA End Resection and RAD51 Recruitment

DNA damage-induced RAD51 focus formation becomes impaired when BRCA1 or BARD1 is absent, thus implicating the BRCA1-BARD1 complex in DNA end resection and RAD51 recruitment (89, 115, 116). As mentioned earlier, cells in the G1 cell cycle phase are poorly equipped to resect DSBs. The G1 restriction on end resection occurs at several levels, namely, **(i)** general shielding of the DNA lesion by an ensemble of proteins being anchored by 53BP1; **(ii)** occlusion of DNA ends by Ku and associated factors; and **(iii)** absence of cyclin-dependent kinase (CDK) activity needed for the recruitment or activation of key resection factors, such as CtIP (53, 61, 63–67, 117). In particular, BRCA1-BARD1 is believed to help counter the inhibitory effect of the 53BP1-associated ensemble when cells enter S phase. In BRCA1 mutant cells, ablation of 53BP1, RIF1, or any of the subunits of Shieldin or the CST complex (Table 1) partially suppresses the DNA end resection deficiency of these cells and their hypersensitivity to DNA damaging agents (61–67, 118, 119). Importantly, acting as E3 ligase, BRCA1-BARD1 conjugates ubiquitin to three lysine residues (K125/127/129) in histone H2A, a modification that leads to the recruitment of the chromatin remodeler SMARCAD1 to mobilize chromatin-bound 53BP1 and to facilitate DNA end resection (106, 116, 120).

5.5 Function of BRCA1-BARD1 in Synaptic Complex Assembly

An insect cell-based platform has been developed to assemble and purify wild type and mutant variants of the BRCA1-BARD1 complex (89). Biochemical testing confirmed previous findings that BRCA1 binds DNA (103, 104) and physically interacts with RAD51 (105). Interestingly, BARD1 was found to possess DNA binding and RAD51 interaction attributes as well (89). In DNA binding, BRCA1-BARD1 has the highest affinity for the D-loop structure, followed by the replication fork structure, dsDNA, and then ssDNA (89). Importantly, BRCA1-BARD1 was shown to enhance the ability of RAD51 to form D-loops, although not to promote RPA-RAD51 exchange on ssDNA (89). Biochemical and single-molecule imaging analyses provided evidence that stimulation of D-loop formation stems from an ability of BRCA1-BARD1 to promote synaptic complex assembly ((89); Figure 3A). Control experiments revealed that BRCA1-BARD1 does not associate with or affect the activity of yeast Rad51 or *E. coli* RecA. Several BARD1 mutations that weaken or ablate RAD51 interaction were constructed and shown to compromise the ability of BRCA1-BARD1 to promote D-loop and synaptic complex formation, in a manner that mirrors the degree of RAD51 interaction deficiency imparted by the mutations (89). Cell-based analysis of one of the BARD1 mutants provided evidence that the BARD1-RAD51 interaction is indispensable for chromosome damage repair and the execution of HR (89).

5.6 Knowledge Gaps Regarding BRCA1-BARD1 in Genome Repair

Much remains to be learned regarding how BRCA1-BARD1 helps mediate crucial steps in chromosome damage repair (see Section 5.4). Below, we outline some of the prevailing

issues key to achieving holistic understanding of the multifaceted role of BRCA1-BARD1 in genome maintenance.

5.6a Role in DNA end resection.—How BRCA1-BARD1 helps relieve the restriction imposed by the 53BP1-RIF1-Shieldin-CST ensemble on DNA end resection in S phase cells remains largely undefined. While published work has implicated BRCA1-BARD1 in the recruitment of the chromatin remodeler SMARCAD1 to facilitate resection, it will be important to determine whether BRCA1-BARD1 also recruits other factors, e.g. additional chromatin remodelers, or conjugates ubiquitin to components of the 53BP1-associated ensemble to further enhance access of the resection machinery to DNA damage. Even though BRCA1-BARD1 forms a complex with the DNA end resection factor CtIP (121), cells expressing a CtIP mutant defective in this interaction show only a mild resection deficiency (122). It is possible that BRCA1-BARD1 directly influences the activity of the nuclease entities, namely, the MRN complex, EXO1, BLM-DNA2 or WRN-DNA2, that catalyze the resection process. This premise awaits future investigations.

5.6b Function in presynaptic filament assembly.—Existing evidence suggests that BRCA1-BARD1 aids in the assembly of the presynaptic filament (33, 63, 66, 101, 102). Since purified BRCA1-BARD1 is devoid of HR mediator activity (89), it likely functions within the context of the pentameric BRCA Mediator Complex that harbors PALB2 and BRCA2-DSS1 ((4); Figure 1). In this regard, one would expect the DNA binding and RAD51 interaction attributes of BRCA1-BARD1, BRCA2, and PALB2 (36, 103–105, 123) to provide a reservoir of RAD51 protomers and to increase the dwell time of the BRCA Mediator Complex on ssDNA to maximize the likelihood and efficiency of RPA replacement (Figure 1). This model awaits experimental validation. A recent study suggests that Shieldin interferes with the loading of RAD51 onto resected DNA ends (63). The basis for this effect of Shieldin on RAD51 presynaptic filament assembly and how it may be overcome by BRCA1-BARD1 remain to be delineated.

5.6c Relationship with other RAD51 co-factors.—Like BRCA1-BARD1 (89), PALB2, RAD54, and the RAD51AP1-UAF1 complex each can enhance Rad51-mediated D-loop formation (8, 36, 37, 88). While RAD54 uses the free energy from ATP hydrolysis to generate negative supercoiling in the duplex target to facilitate DNA strand invasion, PALB2 and RAD51AP1-UAF1 enhance synaptic complex formation (8, 36, 37, 88). Given the association of PALB2 with BRCA1-BARD1 (Figure 1), it will be important to test whether they work together in synaptic complex assembly, and if BRCA2-DSS1 influences the synaptic activity of BRCA1-BARD1-PALB2. Moreover, it will be of considerable interest to examine whether BRCA1-BARD1-PALB2 functions in parallel or as a single entity with RAD54 or RAD51AP1-UAF1 in DNA strand invasion.

5.6d Role in R-loop resolution.—R-loops are RNA-DNA hybrids that harbor a displaced ssDNA region. This structure arises mostly during transcription and serves important biological roles, e.g. in gene promoter regulation and transcription termination (124). However, R-loops that form upon perturbation of transcription or transcription-coupled mRNA splicing can interfere with DNA replication and lead to replication fork

collapse. Cells possess at least three distinct mechanisms to avoid such transcription-replication conflicts, namely **(i)** avoidance of R-loop formation via topoisomerase I-mediated removal of negative DNA supercoiling; **(ii)** digestion of the RNA moiety in R-loops by RNase H; and **(iii)** dissociation of R-loops by putative RNA-DNA helicases, such as Senataxin (SETX) (124, 125). BRCA1 interacts with SETX and has been postulated to regulate the R-loop dissociative activity of the latter (124, 125). It will be important to test whether BRCA1 partners with BARD1 in the recognition of R-loops and in the enhancement of SETX to resolve these pathological structures.

5.6e Function of the ubiquitin E3 ligase activity.—The E3 ubiquitin ligase activity of BRCA1-BARD1 is required for the protection of cells from the harmful effects of a variety of genotoxic agents but dispensable for the response to DNA replication stress or to treatment with cisplatin that induces ICLs (116). Thus far, the BRCA1-BARD1-mediated ubiquitination of histone H2A has been linked to the DNA end resection step of HR ((106, 116); see Section 5.4). It remains to be determined whether H2A ubiquitination is also germane for subsequent HR stages and whether there are other targets of the BRCA1-BARD1 E3 ligase activity relevant for the execution of HR and chromosome damage repair.

6. ROLE OF BRCA2 IN PRESYNAPTIC FILAMENT ASSEMBLY

6.1 Discovery of BRCA2 and Domain Structure

BRCA2 was identified based on genetic linkage analysis of non-*BRCA1* families afflicted with breast cancer at a young age (126, 127). Subsequently, *BRCA2* mutations were shown to engender a high risk to familial ovarian cancer and other cancer types (97). BRCA2 protein is exceptionally large, comprising 3,418 amino acid residues. The N-terminus of BRCA2 is involved in complex formation with PALB2 ((33); Figure 1). There are eight BRC (Breast Cancer) repeats (with each harboring ~30 residues) that interact with RAD51 individually (128, 129), and an additional, unrelated C-terminal RAD51-binding (CTRB) domain (41). BRCA2 possesses two DNA binding domains, one of which comprises three oligonucleotide/oligosaccharide binding (OB-1, 2, and 3) folds and is called the DBD (123), and the second, N-terminally proximal DNA binding module has been named the NTD (130, 131). A portion of OB1 and a neighboring helical domain constitute the interface for association with the small acidic protein DSS1 ((123, 132); Figure 4A; see Section 6.5).

6.2 BRCA2 in Chromosome Damage Repair and Replication Fork Maintenance

Evidence that BRCA2 plays a role in chromosome damage repair and is essential for life came first from mouse studies (41). Specifically, mouse embryos deleted for exon 27 of the *Brca2* gene were found to be hypersensitive to ionizing radiation and to arrest in early development. Moreover, yeast two-hybrid analysis revealed an interaction between the CTRB of Brca2 with the N-terminal region of Rad51 (41). Subsequently, the BRCA2 deficient CAPAN-1 cell line, derived from a pancreatic cancer patient, and a mouse cell line that harbors alleles of *Brca2* deleted for the CTRB were shown to be deficient in HR-mediated gene targeting and also in the repair of a site-specific DSB by HR (133). More recently, BRCA2 was implicated in preventing the accumulation of pathogenic R-loops and also in the protection of stressed DNA replication forks against nucleolytic attack (15, 134).

The replication fork protection activity of BRCA2 is thought to stem from its ability to nucleate the formation and stabilization of RAD51 filaments on the "chicken foot" structure derived from the enzymatic regression of a stressed fork (14, 135). Within this context, RAD51 likely shields regressed forks from various nucleases (14, 15, 135).

6.3 HR Mediator Activity of BRCA2 and its *Ustilago maydis* Ortholog Brh2

BRCA2 mutant cells are proficient in DNA end resection but fail to assemble RAD51 foci upon the occurrence of DNA damage (136), indicating that BRCA2 functions in the assembly of the RAD51 presynaptic filament. In this regard, biochemical testing of Brh2, the BRCA2 ortholog in *U. maydis* (a fungus that causes corn smut) that possesses a single BRC repeat, in complex with Dss1 furnished the first evidence for a HR mediator activity able to promote Rad51 presynaptic filament assembly on RPA-bound ssDNA (137). Notably, Brh2-Dss1 is devoid of HR mediator activity with *E. coli* RecA, being reflective of the fact that the BRC repeat in Brh2 has no affinity for RecA (137). In concordance with the above findings, fusing the BRC3 and BRC4 repeats and the DBD of human BRCA2 generates a polypeptide, BRC1/2-DBD (Figure 4B), that can physically interact with human RAD51 but not with yeast Rad51 or RecA. Importantly, the BRCA2 polypeptide could deliver human RAD51, but neither of the heterologous recombinases, to ssDNA pre-coated with RPA to form the presynaptic filament (138). Interestingly, BRC3/4-DBD is just as adept at mediating RAD51 presynaptic filament assembly on ssDNA decorated with the *E. coli* ssDNA binding protein SSB (138). Unlike RAD51, which binds ssDNA and dsDNA equally well (139), BRC3/4-DBD has higher affinity for ssDNA (138). Accordingly, BRC3/4-DBD specifically targets RAD51 to ssDNA when an excess of dsDNA is present (138). Thus, studies on Brh2-Dss1 and the BRCA2 polypeptide BRC3/4-DBD revealed two key attributes expected of a HR mediator, namely, preferential RAD51 targeting to ssDNA and seeding the assembly of the presynaptic filament on ssDNA pre-occupied by a ssDNA binding protein (137, 138).

The eventual purification of full-length human BRCA2 (77, 140–143) afforded the opportunity to test its HR mediator activity biochemically (77, 140–142) and by electron microscopy (143). Results from these endeavors have shown that the full-length protein is more adept than the BRC3/4-DBD polypeptide in RAD51 presynaptic filament assembly (77, 140). Like BRC3/4-DBD, BRCA2 is specific for its cognate recombinase and is equally efficacious with ssDNA bound by *E. coli* SSB (77, 140). In congruence with the latter finding, no physical interaction of BRCA2 with RPA has been detected (77, 140). As we will discuss in Section 6.5, DSS1 confers to the BRCA2-DSS1 complex specificity for RPA. As was first shown for BRC3/4-DBD, full length BRCA2 helps target RAD51 to ssDNA when dsDNA is present (140, 142). Analysis by electron microscopy of negatively stained BRCA2 has yielded an 18.5 Å reconstruction revealing a homodimeric structure (143).

6.4 Distinct Functions of the BRC Repeats

A systematic biochemical analysis has placed the BRC repeats in human BRCA2 into two distinct categories (139, 144). The first repeat class, comprising BRC1, 2, 3, and 4, is characterized by its high affinity for RAD51 that is not DNA bound. In contrast, BRC5, 6, 7, and 8, members of the second class, possess little affinity for free RAD51 but associate with

the presynaptic filament avidly. Both types of BRC repeats stabilize the presynaptic filament, but they do so via a different mechanism. Specifically, while members of the first class work by attenuating ATP hydrolysis by RAD51, repeats from the second class do not affect this RAD51 attribute. Moreover, repeats of the first class suppress binding of RAD51 to dsDNA and enhance the ability of the presynaptic filament to catalyze DNA strand exchange. Thus, multiple BRC repeats with distinctive properties synergize to ensure the timely assembly and maintenance of the presynaptic filament, and also to enhance the catalytic potential of the filament. However, it should be noted that conjugation of selected BRC repeats to the DBD and/or CTRB yields polypeptides that are capable of complementing BRCA2 deficiency in cells (130, 145, 146), indicating that the eight BRC repeats function in an additive fashion in chromosome damage repair.

6.5 RPA-targeting and DNA Mimicry by DSS1

DSS1 comprises 70 amino acid residues and is highly acidic. Aside from its role in proteasome biogenesis and RNA metabolism (147), DSS1 also forms a stoichiometric complex with BRCA2 that is indispensable for chromosome damage repair (148, 149). Specifically, depletion of DSS1 engenders phenotypic changes reminiscent of BRCA2 deficiency, including hypersensitivity to agents that damage DNA or interfere with DNA replication and an impairment of RAD51 focus formation upon the occurrence of DNA damage (148, 149). Even though BRCA2 becomes destabilized when DSS1 is absent (77, 149), overexpression of BRCA2 fails to complement DSS1 deficiency (77), indicating that the latter fulfills at least one other role in HR distinct from BRCA2 stabilization. Importantly, the cancer-associated BRCA2-D2723H mutation, which affects HR and chromosome damage repair adversely (150), has been found to attenuate the interaction of BRCA2 with DSS1 and to cause mis-localization of BRCA2 to the cytoplasm due to the exposure of a nuclear export signal in the latter (151). As summarized below, a recent study documents how DSS1 influences BRCA2-dependent presynaptic filament assembly (77).

The BRCA2-DSS1 complex has been assembled in insect cells and purified for biochemical testing (77). In a side-by-side comparison, BRCA2-DSS1 was shown to be significantly more active than BRCA2 in the mediation of presynaptic filament assembly on ssDNA coated with human RPA. Importantly, while BRCA2 alone is equally adept at nucleating presynaptic filament assembly on ssDNA coated with *E. coli* SSB, the enhanced efficacy of BRCA2-DSS1 is specific for RPA-bound ssDNA. By affinity pulldown, DSS1 was found to physically interact with the N domain and all three DNA binding OB folds (OB-A, B, and C) of RPA70, the largest of the three RPA subunits. NMR analysis revealed that DSS1 makes contacts with DNA binding residues within the basic cleft of the OB folds, thus providing evidence for a DNA mimetic function in DSS1. Indeed, DSS1 was found to inhibit ssDNA binding by RPA but have no such effect on *E. coli* SSB. The X-ray crystal structure of the Brca2-Dss1 complex solved earlier (123) not only identifies the interfaces involved in protein complex formation, but also a solvent-exposed, disordered loop in Dss1 that is lined with acidic residues ((123); Figure 4C). To test the relevance of this solvent-exposed acidic loop in RPA interaction, a compound DSS1 point mutant that changes eight of the loop residues to alanine was constructed. The $DSS1^{8A}$ mutant, while being proficient in BRCA2 interaction, is greatly compromised for the ability to associate with RPA or to attenuate

ssDNA binding by RPA. Importantly, the BRCA2-DSS1^{8A} mutant complex is no more efficacious than BRCA2 in the promotion of presynaptic filament assembly. Accordingly, cells expressing the DSS1^{8A} mutant are hypersensitive to DNA damaging agents, defective in DNA damage-induced RAD51 focus formation, and impaired for HR. Thus, DSS1 targets BRCA2-RAD51 to RPA-bound ssDNA and also functions as a DNA mimetic to facilitate RPA-RAD51 exchange ((77); Figure 4D).

6.6 Questions Regarding the Genome Maintenance Role of BRCA2-DSS1

As mentioned earlier (see Section 5.6b and Figure 1), it remains to be determined how BRCA2-DSS1 synergizes with BRCA1-BARD1 and PALB2 within the context of the BRCA Mediator Complex in the assembly and maintenance of the presynaptic filament. Likewise, given that BRCA1 and BRCA2 function together to protect stressed replication forks from nucleolytic attack (15, 16), it will be important to verify that the other subunits of the BRCA Mediator Complex work in the same pathway of replication fork protection, and that DSS1 acts in the same manner as in presynaptic filament assembly.

BRCA2 possesses two DNA binding domains, the DBD and NTD (123, 130, 131). In one study, the NTD was found to have a higher affinity for DNA than the DBD (131), but the opposite conclusion was drawn in a separate investigation (130). Future work will resolve the discrepancy regarding DNA binding affinity of the NTD and DBD (130, 131), and will determine whether these domains fulfill different roles or function additively in presynaptic filament assembly and in replication fork protection.

Finally, like BRCA1 (125), BRCA2 suppresses the accumulation of pathogenic R-loops (134). It will be important to ascertain the role of DSS1 in this process and whether BRCA2 acts in conjunction with BRCA1-BARD1 and PALB2 in the recruitment of SETX and other R-loop processing enzymes, or if separate pathways of R-loop resolution exist that are dependent on subsets of these tumor suppressor proteins.

7. EPILOGUE

Deleterious mutations in HR genes have been documented for many cancer types and this common trait has been termed “BRCAness” (5). Notably, a recent pan-cancer analysis of the Cancer Genome Atlas data set revealed that over 5% of all cancers have bi-allelic pathogenic alterations in genes that mediate or regulate HR, and that the HR pathway is genetically compromised in 25% and 10% of all ovarian and breast cancers, respectively (152). Those cancer patients with an abnormality in HR may respond favorably to HR-directed therapies that entail the use of inhibitors of PARP, of cell cycle regulators, or of DNA damage checkpoints (5, 153, 154). With the availability of purified BRCA complexes (77, 89, 140–142), the field is now poised to delineate the mechanistic underpinnings of BRCA-mediated chromosome damage repair and replication forks protection. Such endeavors can be expected to provide the knowledge base to help drive the development of targeted cancer therapeutics, predict patient response to therapy, and explain acquired drug resistance.

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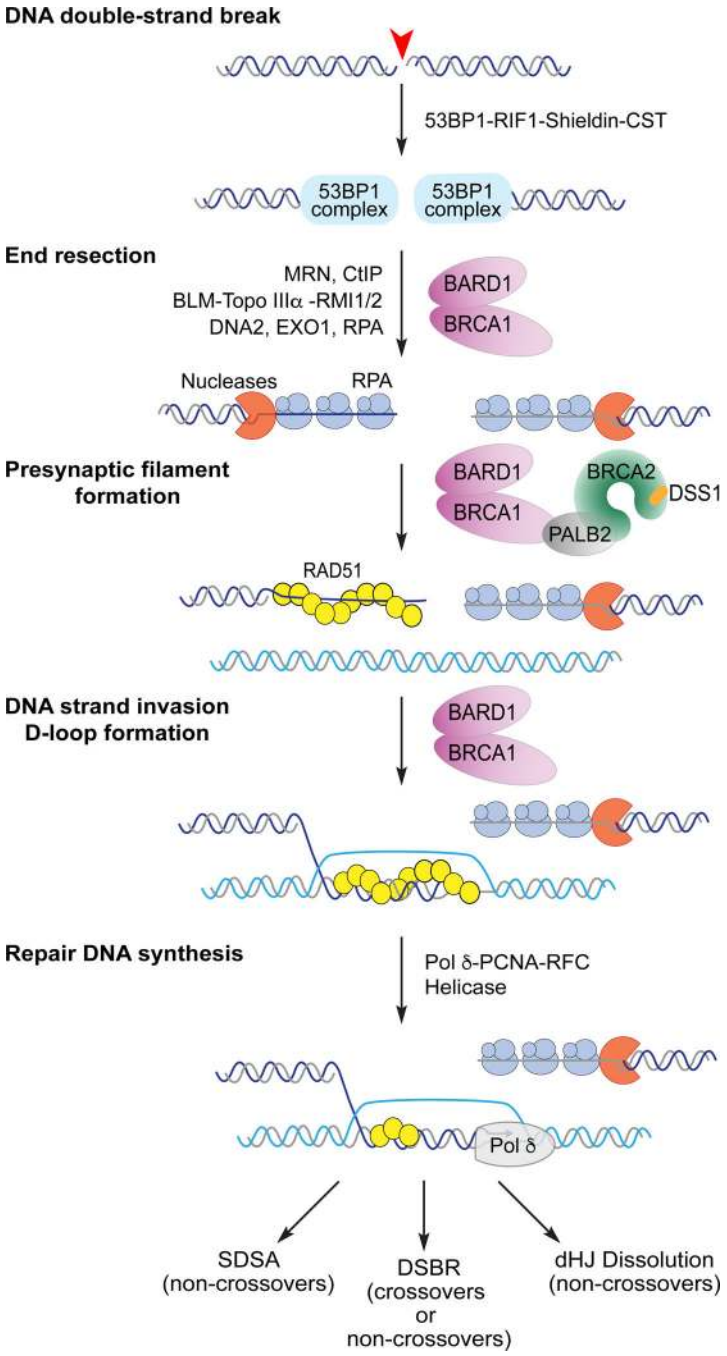

Figure 1.

DNA double-strand break repair by homologous recombination.

Emphasis is placed on the roles of BRCA1-BARD1 in DNA end resection and strand invasion and on the BRCA mediator complex (DSS1-BRCA2-PALB2-BRCA1-BARD1) in presynaptic filament formation. Note that many other factors that function in different stages of the recombination reaction are not shown (see Table 1 for the list of uncited factors). The three pathways of D-loop resolution are briefly discussed in Section 4.4 and in detail in recent reviews (8, 93). The four subunits of Shieldin are REV7 (MAD2L2), SHLD1

(C20orf196), SHLD2 (FAM35A), and SHLD3 (CTC534A2.2). The three subunits of the CST complex are CTC1, SNT1, and TEN1.

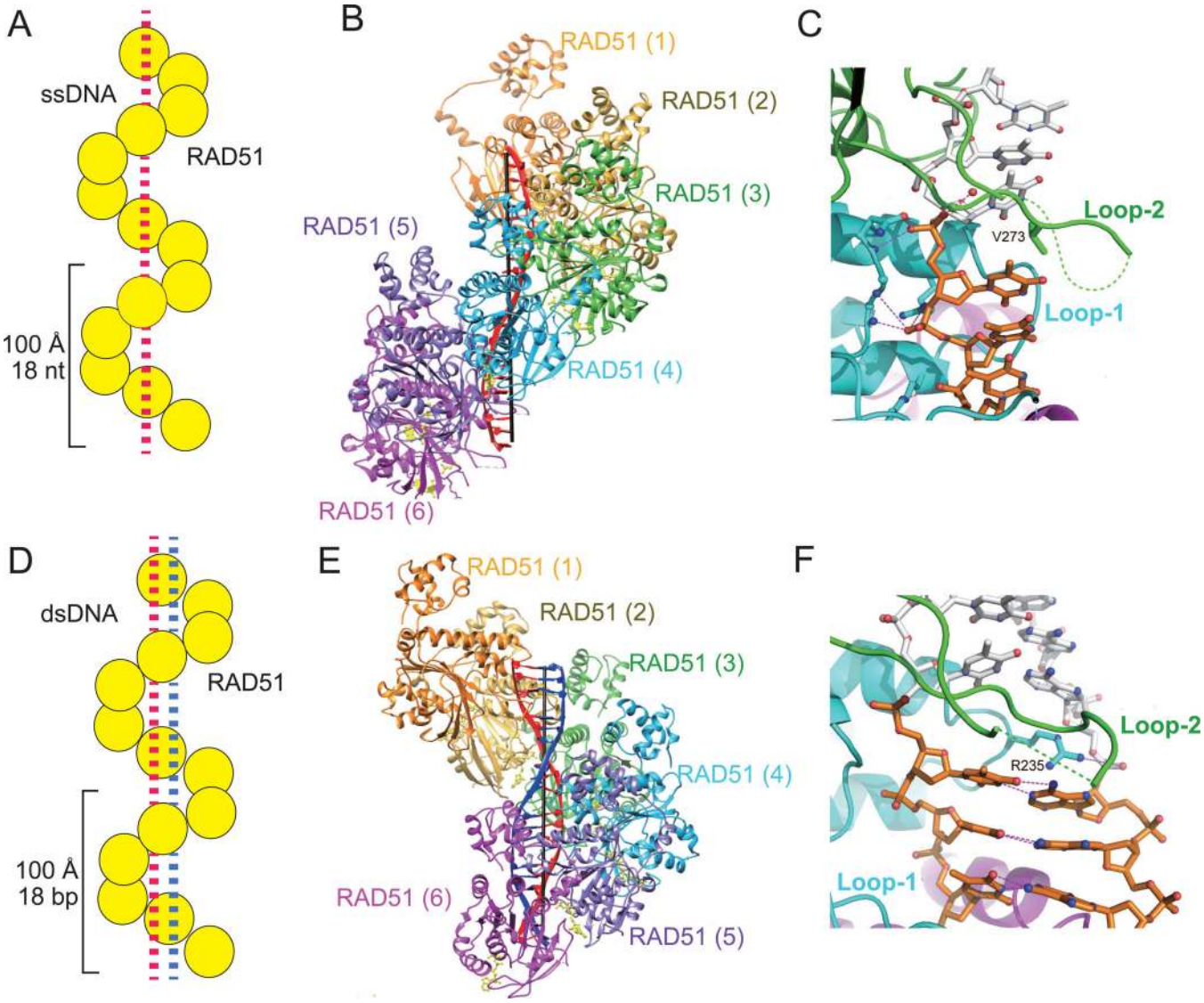

Figure 2.

Structure of the RAD51-ssDNA presynaptic filament and RAD51-dsDNA post-synaptic complex.

(A, D) Cartoon illustrations of the presynaptic filament (A) and post-synaptic complex (D); RAD51 is shown in yellow and DNA is depicted as dashed lines. (B, E) Atomic structure of the presynaptic filament (B) and post-synaptic complex (E), the invading ssDNA is shown in red and complementary strand in blue. (C, F) RAD51-DNA interactions in the presynaptic filament (C) and post-synaptic complex (F). Base triplets interacting with V273 in RAD51 DNA binding Loop 2 in the presynaptic filament (C), and base pair triplets with R235 in DNA binding Loop 1 in the post-synaptic complex (F) are highlighted. See reference 74 for details.

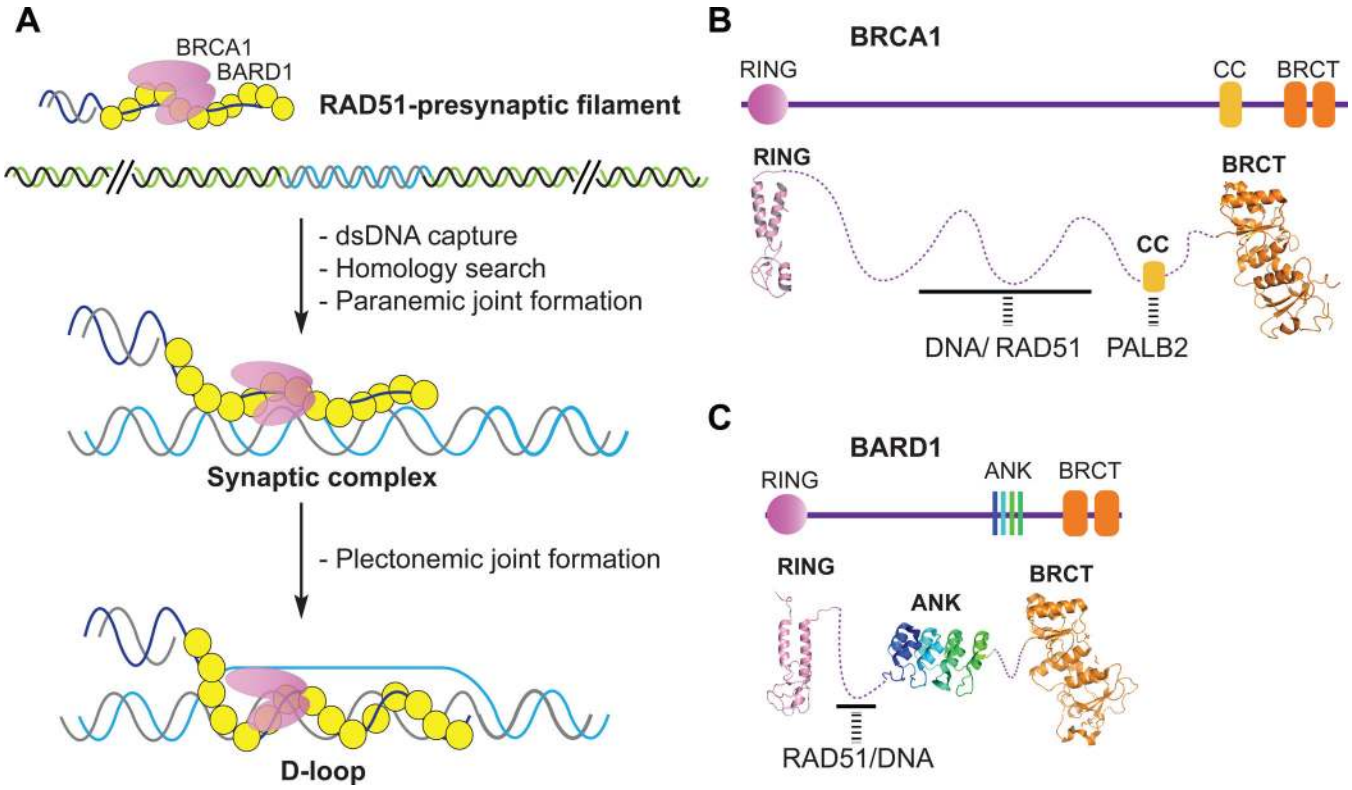

Figure 3.

BRCA1-BARD1 structure and function.

(A) Cartoon illustrating the role of BRCA1-BARD1 in the promotion of paranemic and plectonemic joint formation (89). BRCA1-BARD1 aids in the capture of the recombining duplex molecule to facilitate paranemic joint formation and the assembly of the triple-stranded synaptic complex. Subsequent plectonemic joint formation yields the D-loop intermediate. (B) Functional motifs and domains in BRCA1 (B) and BARD1 (C) including the RING (Really Interesting New Gene) domain (PDB: 1JM7), CC (Coiled Coil) domain, ANK (Ankyrin) motifs (PDB: 3C5R), and BRCT (BRCA1 Carboxy-Terminal) repeats (PDB: 1T2U for BRCTs of BRCA1; PDB: 2NTE for BRCTs of BARD1). The known atomic structures are linked by the dash lines depicting protein regions of which no structural information is available.

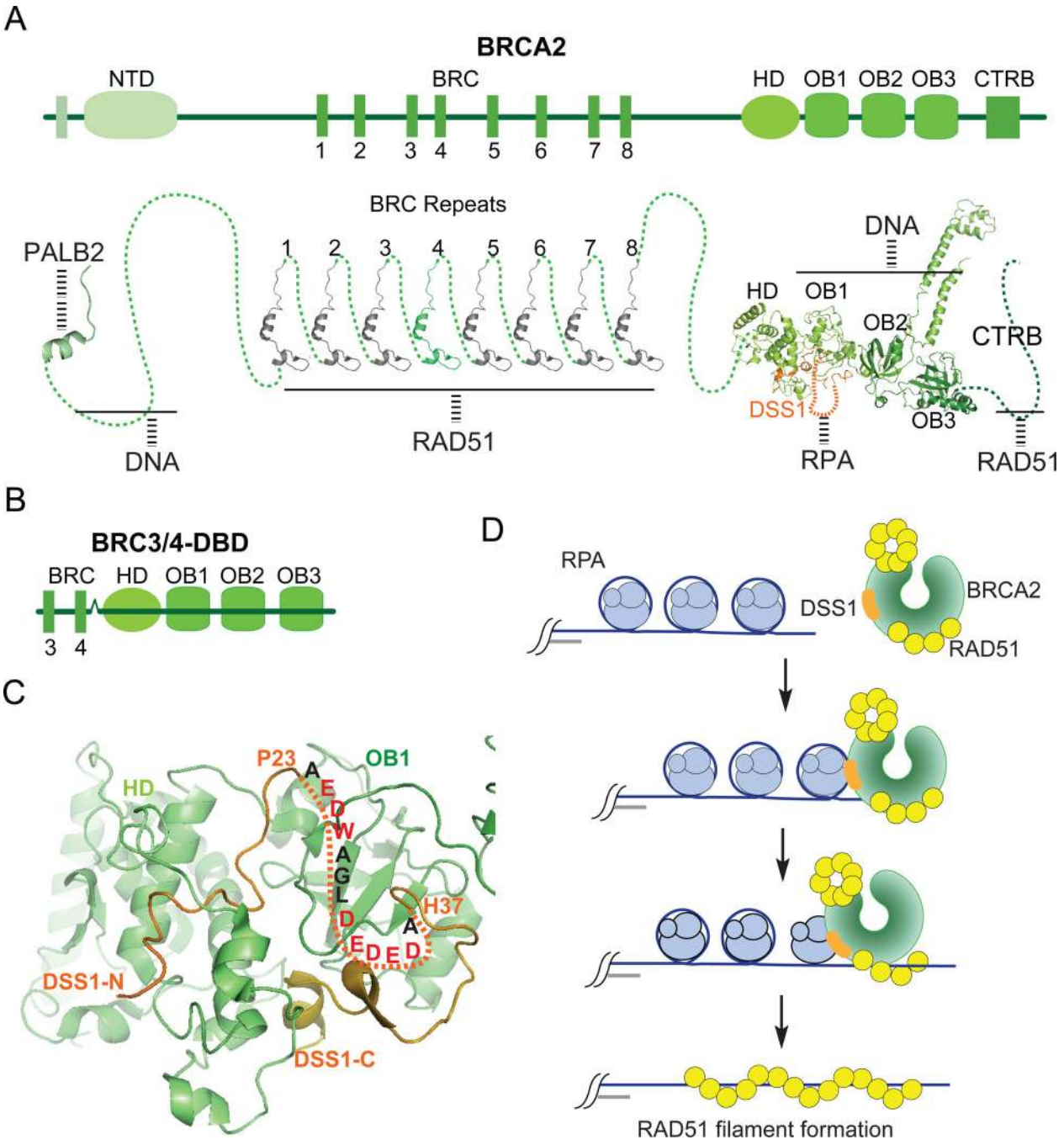

Figure 4.

BRCA2 structure and function.

(A) Functional motifs and domains in BRCA2. The BRC (Breast Cancer) repeats, HD (helical domain), NTD (N-terminal DNA Binding Domain), DBD (DNA Binding Domain) comprising three OB (oligonucleotide/oligosaccharide binding) folds, and CTRB (C-terminal RAD51-binding) domain are shown. The HD and a portion of OB1 constitute the interface for association with DSS1 that physically interacts with RPA via a solvent-exposed acidic loop (77). The known atomic structures (PDB: 3EU7,1N0W and 1MIU) are linked by

the dash lines depicting protein regions of which no structural information is available. (B) Schematic of the BRCA2-derived polypeptide composed of BRC3, BRC4 and the DBD. (C) Structure of the BRCA2-DSS1 complex ((123); PDB: 1IYJ)) with the BRCA2 parts in green. Residues in the solvent-exposed loop of DSS1 targeted for mutagenesis in the construction of a mutant defective in RPA interaction are in red (77). (D) Cartoon illustrating the HR mediator function of BRCA2-DSS1 in which BRCA2 provides the DNA binding and RAD51 interaction attributes, while DSS1 facilitates presynaptic filament assembly through its ability to interact with RPA and to act as a DNA mimetic to attenuate the affinity of RPA for ssDNA (77).

Table 1

Proteins and their functions in homologous recombination

Function	*H. sapiens*	*S. cerevisiae*
DNA end resection		
Initiation and short-range	MRE11-RAD50-NBS1 (MRN), CtIP BRCA1-BARD1	Mre11-Rad50-Xrs2 (MRX), Sae2
Long-range	EXO1	Exo1
	DNA2, BLM or WRN, TOPOIIIα-RMI1-RMI2	Dna2, Sgs1-Top3-Rmi1
	RPA	RPA
	SMARCAD1	Fun30
	Possibly BRCA1-BARD1	
Inhibitors of DNA resection	53BP1-RIF1-Shieldin (REV7-SHLD1-SHLD2-SHLD3)-CST(CTC1-STN1-TEN1)	Rad9
	PTIP, Artemis	
Presynaptic filament assembly and dynamics		
Recombinase	RAD51	Rad51
Mediators	BRCA2-DSS1, Possibly BRCA1-BARD1, PALB2	Rad52
Rad51 paralog complexes and partners	RAD51B-RAD51C-RAD51D-XRCC2, RAD51C-XRCC3, SWS1-SWSAP1	Rad55-Rad57, Psy3-Csm2-Shu1-Shu2
Recombinase stimulatory factors	RAD54, RAD54B SWI5-SFR1	Rad54, Rdh54 *Mei5-Sae3
	MMS22L-TONSL	
Anti-recombinases	FBH1	Srs2
	RECQ5	
Synaptic complex formation		
	BRCA1-BARD1	
	PALB2	
	RAD51AP1-UAF1	
	HOP2-MND1	*Hop2-Mnd1
DNA synthesis		
DNA polymerase complex	Pol δ, PCNA, RFC	Pol δ, PCNA, RFC
DNA helicase	To be identified	Pif1
DNA ligase	Ligase I	Ligase I

Protein factors, including DNA helicases and resolvases, that help determine HR pathway choice are not listed above. Readers are referred to recent reviews on this topic (8, 32, 93). Blanks in the *S. cerevisiae* column indicate that no ortholog of the human factors exits. *S. cerevisiae* factors denoted by the asterisk (*) are expressed only during meiosis and function specifically with the RecA/Rad51-related recombinase Dmc1 (8, 69).