Title

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Authors

Li, Qianyan

Engebrecht, JoAnne

Publication Date

2021

DOI

10.3389/fcell.2021.668309

Peer reviewed

frontiers in Cell and Developmental Biology

REVIEW
published: 23 April 2021
doi: 10.3389/fcell.2021.668309

BRCA1 and BRCA2 Tumor Suppressor Function in Meiosis

*Qianyan Li and JoAnne Engebrecht**

Department of Molecular and Cellular Biology, and Biochemistry, Molecular, Cellular and Developmental Biology Graduate Group, University of California, Davis, Davis, CA, United States

Meiosis is a specialized cell cycle that results in the production of haploid gametes for sexual reproduction. During meiosis, homologous chromosomes are connected by chiasmata, the physical manifestation of crossovers. Crossovers are formed by the repair of intentionally induced double strand breaks by homologous recombination and facilitate chromosome alignment on the meiotic spindle and proper chromosome segregation. While it is well established that the tumor suppressors BRCA1 and BRCA2 function in DNA repair and homologous recombination in somatic cells, the functions of BRCA1 and BRCA2 in meiosis have received less attention. Recent studies in both mice and the nematode *Caenorhabditis elegans* have provided insight into the roles of these tumor suppressors in a number of meiotic processes, revealing both conserved and organism-specific functions. BRCA1 forms an E3 ubiquitin ligase as a heterodimer with BARD1 and appears to have regulatory roles in a number of key meiotic processes. BRCA2 is a very large protein that plays an intimate role in homologous recombination. As women with no indication of cancer but carrying BRCA mutations show decreased ovarian reserve and accumulated oocyte DNA damage, studies in these systems may provide insight into why BRCA mutations impact reproductive success in addition to their established roles in cancer.

Keywords: BARD1, BRCA1, BRCA2, DSBs, meiosis, MSCI, recombination

OPEN ACCESS

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***Correspondence:**
JoAnne Engebrecht
jengebrecht@ucdavis.edu

Specialty section:
This article was submitted to Cell Growth and Division, a section of the journal Frontiers in Cell and Developmental Biology

Received: *16 February 2021*
Accepted: *19 March 2021*
Published: *23 April 2021*

Citation:
Li Q and Engebrecht J (2021) BRCA1 and BRCA2 Tumor Suppressor Function in Meiosis. Front. Cell Dev. Biol. 9:668309. doi: 10.3389/fcell.2021.668309

INTRODUCTION

Homologous recombination (HR) is a high-fidelity pathway that mediates error-free repair of DNA double strand breaks (DSBs) and is essential for maintaining genome integrity. In somatic cells, DSBs can arise when DNA replication is impeded or following exposure to irradiation or other genotoxic stress. Cells deficient for HR show genomic instability including chromosome rearrangements, characteristic of most cancers (Negrini et al., 2010). In contrast to somatic cells, where DSBs pose a risk to genome integrity, during meiosis, hundreds of DSBs are purposely introduced by the topoisomerase-like protein SPO11 in early meiotic prophase and these meiotic DSBs must be accurately repaired for the production of euploid gametes (Lam and Keeney, 2014). As meiosis proceeds, meiotic DSBs are processed by DNA end resection to reveal 3′ overhangs (Garcia et al., 2011). The RAD51 recombinase as well as the meiosis-specific paralog DMC1 assemble on the resulting single strand DNA to form nucleoprotein filaments that mediate strand invasion and homology search for accurate repair (Shinohara and Shinohara, 2004). Meiotic DSB repair occurs concomitantly with the assembly of the synaptonemal complex (SC), the meiosis-specific multi-protein structure that forms between homologous chromosomes. In many

organisms, SC assembly is driven by HR (Zickler and Kleckner, 2015). In the context of full length SC at the pachytene stage of meiotic prophase, a subset of recombination intermediates is processed into inter-homolog crossovers, which are essential for accurate separation of homologous chromosomes at meiosis I (Neale and Keeney, 2006; Baudat and de Massy, 2007). A large number of proteins are critical for HR, including the tumor suppressors BRCA1 and BRCA2, whose functions have been well characterized in somatic cells in the context of DNA damage and carcinogenesis. However, the roles of BRCA1 and BRCA2 during meiotic recombination have received less attention. Although the processing of DSBs by HR is similar in somatic cells and meiosis, meiotic recombination is unique in that SPO11 remains attached to the DNA end following DSB formation. Additionally, meiotic recombination occurs in the context of the SC and both sister and non-sister chromatids can serve as templates for repair. Thus, BRCA1 and BRCA2 function may be modified in meiosis to ensure accurate repair of meiotic DSBs. Studies in model organisms have provided insights into the roles of BRCA1 and BRCA2 in meiosis. This review will summarize the conserved and organism-specific meiotic functions of BRCA1 and BRCA2, focusing on recent studies in mice and *C. elegans*.

BRCA1 IN COMPLEX WITH BARD1 IS AN E3 UBIQUITIN LIGASE CRITICAL FOR GENOME INTEGRITY

Breast cancer susceptibility gene 1 (BRCA1) is a tumor suppressor gene, germline mutations of which are linked to familial breast and ovarian cancers (Hall et al., 1990; Futreal et al., 1994; Godwin et al., 1994; Miki et al., 1994). More than two decades of research has implicated BRCA1 function in multiple cellular pathways, including transcriptional regulation, DNA damage signaling, cell cycle checkpoints, centrosome regulation and in the repair of DNA DSBs through HR (Moynahan et al., 1999; Xu et al., 1999; Deng, 2002, 2006; Yarden et al., 2002; Caestecker and Van de Walle, 2013; Hill et al., 2014; Hatchi et al., 2015). Of critical importance, its role in promoting HR is directly linked to maintenance of genome integrity (Roy et al., 2011; Prakash et al., 2015).

In humans, the 1,863 amino acid BRCA1 protein has an N-terminal RING (Really Interesting New Gene) domain that coordinates two zinc cations in a cross-braced arrangement, a largely unstructured central region encoded by exon11, followed by a coiled coil domain and two C-terminal BRCT repeats (**Figure 1**). RING domains create a platform for binding to E2 ubiquitin conjugating enzymes and facilitate the transfer of ubiquitin from the E2 to substrates, thereby specifying E3 ubiquitin ligase activity (Deshaies and Joazeiro, 2009). The BRCT repeats are phosphopeptide interaction modules for binding to phosphorylated proteins (Manke et al., 2003; Rodriguez et al., 2003; Yu et al., 2003). BRCA1 forms a heterodimer with its obligate binding partner BARD1 (BRCA1-Associated RING Domain protein 1) through their N-terminal regions and the heterodimer exhibits efficient ubiquitin transfer activity (Wu et al., 1996; Meza et al., 1999; Brzovic et al., 2001; Hashizume et al., 2001; Baer and Ludwig, 2002). The BARD1 protein is 777 amino acids in length and similar to BRCA1, contains a RING domain at its N-terminus and two BRCT repeats at its C-terminus (**Figure 1**). In addition, four ankyrin repeats involved in chromatin recognition of newly replicated sister chromatids are present in the middle of the protein (FoxIII, Le Trong et al., 2008; Nakamura et al., 2019). Most studies indicate that BARD1 is indispensable for BRCA1 function and depletion of BARD1 leads to highly similar phenotypes as observed for BRCA1 mutants. Mutations in BARD1 have been identified in patients with breast, ovarian and other cancer types, although at a lower frequency than BRCA1 mutations (Thai et al., 1998; Ghimenti et al., 2002). Further, as with BRCA1, loss of BARD1 results in embryonic lethality in mice as well as defects in HR leading to chromosomal instability (McCarthy et al., 2003).

The mechanisms by which BRCA1-BARD1 promotes HR during DSB repair involve multiple steps. First, BRCA1 promotes DNA end resection by antagonizing 53BP1, a DNA damage response protein that promotes error-prone non-homologous end joining (NHEJ) (Bunting et al., 2010; Daley and Sung, 2014). Two, BRCA1 regulates the MRE11-RAD50-NBS1-CtIP complex essential for DNA end processing (Cruz-Garcia et al., 2014; Aparicio et al., 2016). There is also evidence that BRCA1 removes a chromatin barrier for DNA resection through ubiquitylation of histone H2A (Densham et al., 2016). In addition to promoting resection, BRCA1-BARD1 binds to DNA and interacts with RAD51 directly, enhancing RAD51 recombinase activity by promoting homologous strand invasion and synaptic complex formation (Zhao et al., 2017). However, whether BRCA1 functions by similar mechanisms to promote HR during meiosis for the repair of SPO11-induced DSBs has remained elusive.

BRCA1 FUNCTION IN MOUSE MEIOSIS

Mice homozygous for *Brca1* null alleles are embryonic lethal, excluding the possibility to assess BRCA1 function during meiosis (Gowen et al., 1996; Hakem et al., 1996; Liu et al., 1996; Ludwig et al., 1997). To circumvent this limitation, meiosis has been analyzed in mice carrying a hypomorphic mutation that deletes the large exon11 in the heterozygous *Trp53* (encoding p53) mutant background ($Brca1^{\Delta 11/\Delta 11}$ $Trp53^{+/-}$) (Xu et al., 2003; **Figure 1**). These mice develop and survive to adulthood; lethality likely bypassed by the reduced expression of *Trp53* (Cressman et al., 1999).

BRCA1 Is Essential for Meiotic Sex Chromosome Inactivation During Spermatogenesis

Although *Trp53* heterozygosity rescues the embryonic lethality of $Brca1^{\Delta 11/\Delta 11}$ mice, males are infertile as a result of pachytene arrest and apoptotic removal of germ cells (Xu et al., 2003). This observation revealed an essential role of BRCA1 in meiotic sex chromosome inactivation (MSCI). MSCI is a repressive mechanism that occurs during meiotic prophase I and involves elaboration of heterochromatin and transcriptional silencing of non-homologous regions of sex chromosomes (Turner, 2007).

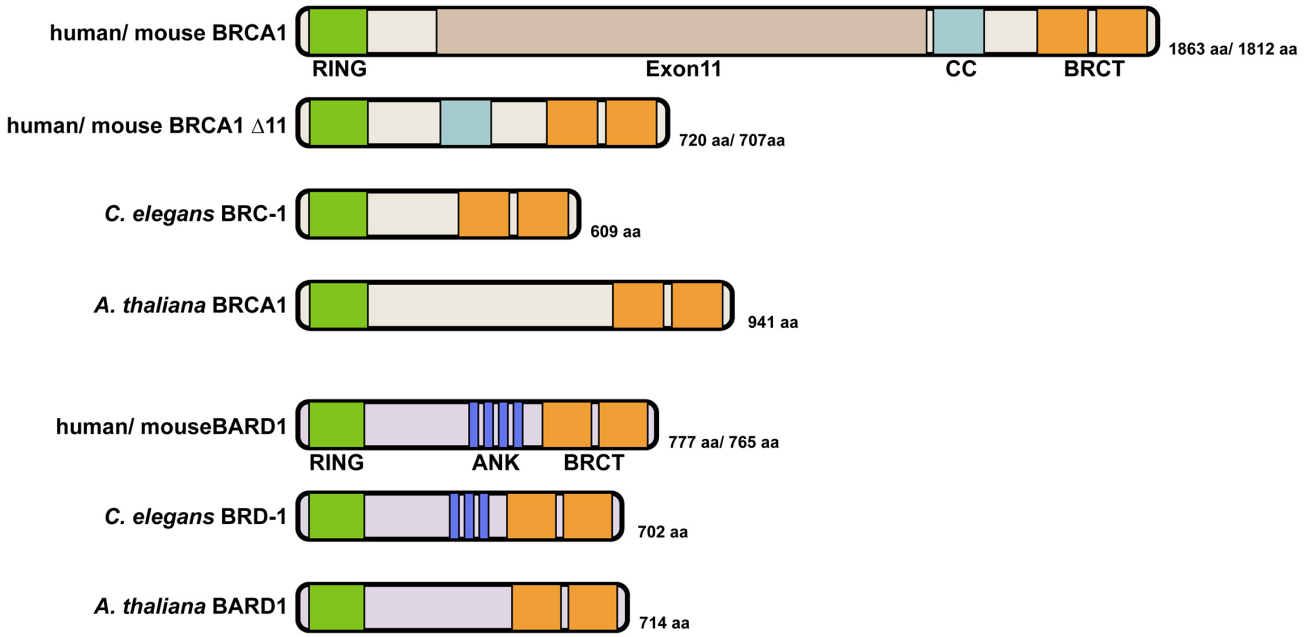

FIGURE 1 | Domain structure of BRCA1 and BARD1 proteins. Human BRCA1 contains an N-terminal RING domain, an unstructured central region encoded by the large exon11 followed by a coiled coil (CC) domain and two BRCA1 C-terminal (BRCT) repeats. Both human and mouse express an alternatively spliced variant BRCA1Δ11 that contains the N-terminal RING domain and C-terminal BRCT repeats but lacks the unstructured central region (Thakur et al., 1997; Huber et al., 2001). This truncated protein is expressed in the hypomorphic *Brca1*$^{\Delta 11/\Delta 11}$ mouse. *C. elegans* BRC-1 is structurally similar to the BRCA1Δ11 splicing variant with the presence of an N-terminal RING domain and two BRCT repeats at its C terminus. *A. thaliana* encodes a similarly structured BRCA1 ortholog that has a N-terminal RING and two C-terminal BRCT repeats. Human BARD1 and *C. elegans* BRD-1 are similar in size and domain structure, containing an N-terminal RING domain, ankyrin repeats in the middle and two C-terminal BRCT repeats. *A. thaliana* BARD-1 has a similar domain structure but appears to lack ankyrin repeats, which were not predicted by sequence alignment. BRCA1 interacts with BARD1 through their RING domains to form a heterodimer with E3 ubiquitin ligase activity.

MSCI is required for efficient meiotic progression in males as failure to repress the X and Y chromosomes results in elevated germline apoptosis (**Figure 2**).

In wild-type spermatocytes, BRCA1 localizes to asynapsed chromosome axes, including the mostly unsynapsed X and Y sex chromosomes (Scully et al., 1997). BRCA1 recruits the checkpoint kinase ataxia telangiectasia and Rad3-related protein (ATR) to the hemizygous regions of sex chromosomes; ATR phosphorylates a histone variant, H2AX, to form γH2AX, leading to sex chromosome compaction and transcriptional silencing (Fernandez-Capetillo et al., 2003; Turner et al., 2004). In the absence of full length BRCA1, ATR and γH2AX localization is disrupted, formation of XY sex body fails, and transcriptional silencing is abolished, leading to ectopic gene transcription from the hemizygous regions of the sex chromosomes (Xu et al., 2003; Turner et al., 2004; Broering et al., 2014). The inability to execute successful MSCI in the *Brca1*$^{\Delta 11/\Delta 11}$ mutant has been attributed to a direct role of BRCA1 in establishing heterochromatin on the X and Y chromosomes and XY body morphogenesis, rather than an indirect consequence of defective meiotic recombinational repair in the absence of full-length BRCA1 (Broering et al., 2014).

The related process of meiotic silencing of unsynapsed chromatin (MSUC) also requires BRCA1 and operates in both male and female germ cells (Mahadevaiah et al., 2008; Kouznetsova et al., 2009). As with MSCI, MSUC leads to accumulation of repressive chromatin and transcriptional silencing on any asynapsed chromosomal regions. MSUC promotes the elimination of gametes with chromosome asynapsis and is initiated by the recruitment of BRCA1 to unsynapsed chromosomes through the interaction with the SC axial component SYCP3. Interestingly, oocytes have a limited capacity to silence unsynapsed chromosomes and this appears to be a consequence of the amount of BRCA1 available to accumulate on unsynapsed chromosomes. Thus, the role of BRCA1 in transcriptional silencing contributes to ensuring the production of euploid gametes.

Potential BRCA1 Role in Meiotic Recombination

In addition to MSCI failure, spermatocytes from *Brca1*$^{\Delta 11/\Delta 11}$ Trp53$^{+/-}$ mice exhibited a prolonged autosomal γH2AX signal with greatly reduced numbers of RAD51 (but not DMC1) and MLH1 foci, suggesting that BRCA1 plays a role in meiotic DSBs repair and crossover formation (Xu et al., 2003). In contrast, a separate study utilizing Cre/LoxP mediated conditional germline-specific deletion of *Brca1* exon11 in the presence of both wild-type *Trp53* alleles showed that RAD51 foci were not reduced, although decreased numbers of MSH4 foci and delayed appearance of MLH1 foci were observed. These authors concluded that while BRCA1 is not essential for meiotic DSB repair, BRCA1 might be involved in the regulation of the

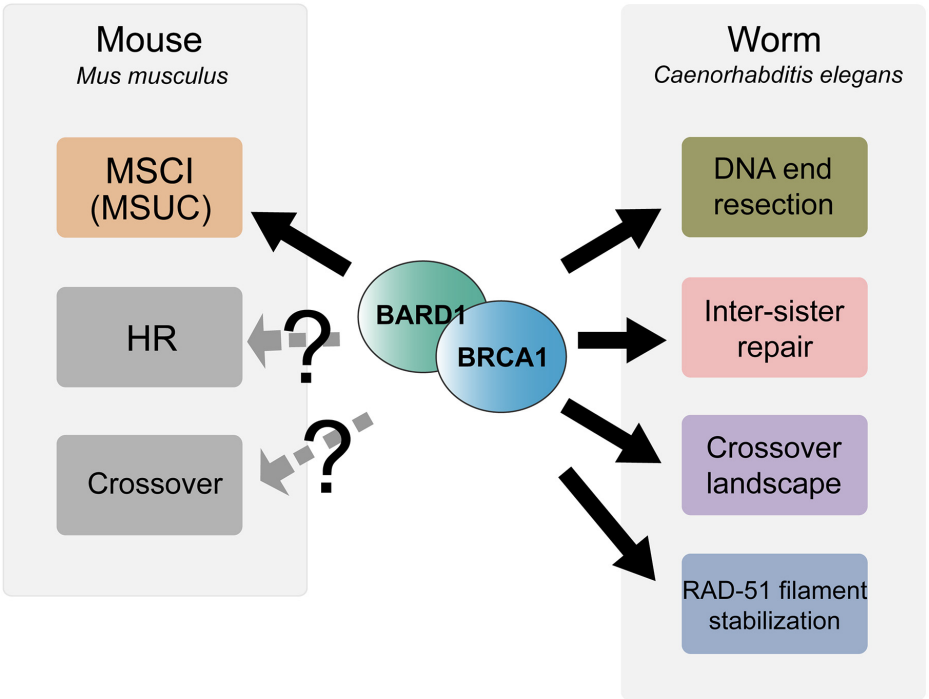

FIGURE 2 | Summary of meiotic functions of BRCA1-BARD1 in mouse and *C. elegans*. BRCA1-BARD1 is critical for meiotic sex chromosome inactivation (MSCI) and meiotic silencing of unsynapsed chromatin (MUSC) during mouse meiosis while it remains an open question as to whether it functions in meiotic recombination and crossover regulation. In contrast to mouse, *C. elegans* BRC-1-BRD-1 is dispensable for MSCI but functions in DNA end resection, inter-sister recombinational repair, RAD-51 filament stabilization and regulation of the crossover landscape.

timing of crossover formation (Broering et al., 2014). In a recent study using END-seq on mouse spermatocytes that allows direct examination of meiotic DSB processing at the single nucleotide level, hypomorphic *Brca1*$^{\Delta 11/\Delta 11}$ Trp53$^{+/-}$ mice did not exhibit a reduction in resection track length, suggesting that BRCA1 does not promote DNA resection in meiotic DSB repair as in somatic cells (Paiano et al., 2020). Together these results suggest that the critical meiotic role for BRCA1 is in transcriptional silencing; however, it is possible that BRCA1 function in meiotic recombination is obscured by the use of the hypomorphic *Brca1*$^{\Delta 11/\Delta 11}$ mutant (**Figure 2**).

Analysis of female meiosis in the hypomorphic *Brca1*$^{\Delta 11/\Delta 11}$ mutation revealed no observable phenotypes. Female *Brca1* mutants are fertile and the number of MLH1 foci are comparable to that observed in wild-type oogenesis, suggesting that the region deleted in *Brca1*$^{\Delta 11/\Delta 11}$ is not required for meiotic recombination during female meiosis (Xu et al., 2003; Broering et al., 2014). Therefore, the observed sex-specific phenotypes in the hypomorphic *Brca1*$^{\Delta 11/\Delta 11}$ mutant are likely a consequence of the presence of unsynapsed sex chromosomes in males. It is also important to note that the region encoded by exon11 is thought to be unstructured with no resemblance to known domain structures (Li and Greenberg, 2012). Future studies focusing on the RING domain, which confers E3 ubiquitin ligase activity, and the BRCT repeats, are necessary to reveal whether these domains play important roles in the repair of meiotic DSBs in both male and female meiosis. Finally, to the best of our knowledge a functional role of BARD1 in mice gametogenesis has not been examined.

BRCA1 FUNCTION IN *C. ELEGANS* MEIOSIS

The *C. elegans* Germ Line as a Model for Studying Meiosis and BRCA1-BARD1 Function

Caenorhabditis elegans has emerged as an excellent model for investigating meiosis: many genes required for meiotic recombination are conserved in this metazoan and the animals possess prominent gonads that exhibit a spatial temporal organization of germ cells undergoing meiotic prophase I (**Figure 3A**). At the distal tip, germline stem cells divide to produce cells that will advance down the gonad and enter meiosis. In transition zone (corresponding to leptotene/zygotene), homologous chromosomes are paired together, facilitated by Zn-finger ZIM-1/2/3 and HIM-8 proteins that bind to special sequences present on each homolog pair. Beginning at this stage, SPO-11 induces meiotic DSBs, which are processed and bound by RAD-51 for homologous recombinational repair. In pachytene, the SC is fully assembled between the homologs and within this context strictly one crossover forms between each chromosome pair in late pachytene. Upon crossover formation, the SC

disassembles and homologs undergo remodeling and compaction to reveal six bivalents at diakinesis stage, representing the six pairs of homologs connected by chiasmata (**Figure 3B**; Lui and Colaiacovo, 2013; Hillers et al., 2017). Although the overall process is very similar to other systems, it is important to note that there are differences unique to *C. elegans* meiosis. These include the absence of DMC1 in this organism, thus RAD-51 is the sole recombinase acting during both mitotic and meiotic recombination (Brown and Bishop, 2014). Interestingly, *C. elegans* RAD-51 contains three amino acids conserved in the DMC1 lineage that stabilize mismatch-containing heteroduplex DNA, critical for meiotic recombinase function (Steinfeld et al., 2019). Another unique feature of *C. elegans* meiosis is that chromosome synapsis does not depend on meiotic recombination initiation (Dernburg et al., 1998). Nevertheless, the availability of molecular markers combined with genetic and genomic approaches has made the *C. elegans* germ line a powerful system that provides a unique opportunity to dissect gene function at any particular sub-stage of meiotic prophase. Most importantly, proteins with conserved domain structure and sequence similarity to BRCA1 and BARD1, referred to as BRC-1 and BRD-1, are encoded in the *C. elegans* genome. *brc-1* and *brd-1* null mutants exhibit elevated IR sensitivity and a higher incidence of males among self-progeny (a readout of X chromosome non-disjunction) compared to wild type, but are mostly fertile, allowing analysis of meiotic outcomes in the absence of functional BRCA1 and BARD1 (Boulton et al., 2004; Li et al., 2018). Similar to *C. elegans*, *Arabidopsis AtBRCA1* and *AtBARD1* mutants are also fertile, suggesting that the essentiality of mammalian BRCA1-BARD1 is not broadly conserved (Reidt et al., 2006).

C. elegans brc-1 encodes a 609 amino acid protein with highly conserved N-terminal RING domain and C terminal BRCT repeats, similar to the human protein. Structurally, *C. elegans* BRC-1 is analogous to the BRCA1Δ11 splicing variant (**Figure 1**). AtBRCA1 with 941 amino acids is also considerably smaller than the human protein. The *C. elegans* BRD-1 and AtBARD1 proteins are similar in both size and domain architecture to the human protein, although AtBARD1 does not have recognizable ankyrin repeats (**Figure 1**). Interestingly, *C. elegans* BRC-1-BRD-1 exhibits dynamic localization throughout meiotic prophase. Discrete foci of BRC-1-BRD-1 that partially colocalize with RAD-51 are present in both proliferative/mitotic region and early meiotic prophase, from leptotene to early pachytene (Li et al., 2018, 2020). As meiotic prophase progresses, BRC-1-BRD-1 localizes with the SC between the maternal and paternal chromosomes (Polanowska et al., 2006; Janisiw et al., 2018; Li et al., 2018). This localization is in contrast to BRCA1 localization in mammalian meiocytes, where BRCA1 is found on the axes of asynapsed chromosomes (Turner et al., 2004). In late pachytene upon crossover maturation, BRC-1-BRD-1 concentrates on one subdomain of the chromosome pair termed the "short arm", suggesting an intimate connection of BRC-1-BRD-1 to crossover sites and potential involvement in crossover regulation.

BRC-1-BRD-1 Is Not Essential for Meiotic Sex Chromosome Inactivation but Promotes HR in Spermatogenesis

C. elegans BRC-1-BRD-1 is absent from the single asynapsed X chromosome in male germ cells, and consistent with

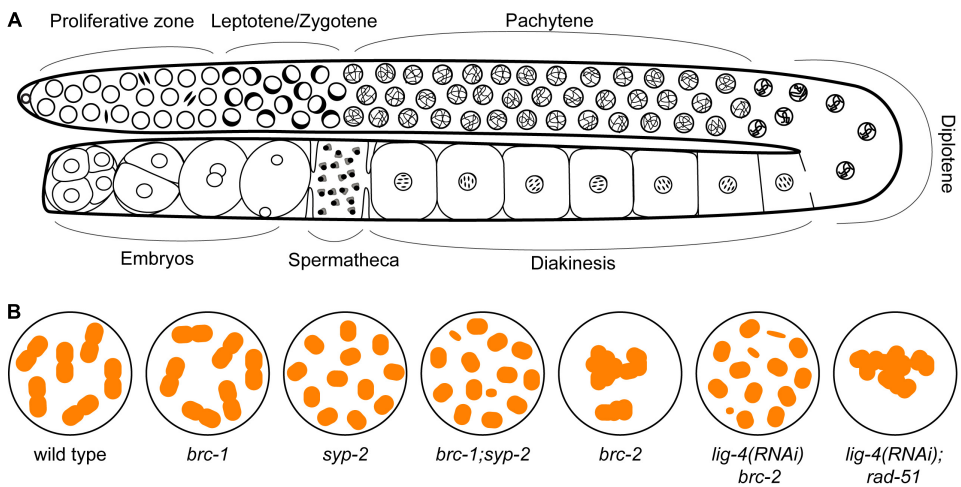

FIGURE 3 | The *C. elegans* germ line presents a spatial temporal organization of events during meiotic prophase I. **(A)** At the distal proliferative zone, germline stem cells mitotically divide to produce cells that will advance down the gonad and enter meiosis. Chromosome pairing and DSBs induction by SPO-11 occur in leptotene/zygotene (transition zone), which is characterized by the presence of clustered chromatin on one side of the nuclei. DSBs are processed and bound by RAD-51 for homologous recombinational repair, which are visible as RAD-51 foci by immunostaining starting in leptotene through pachytene. The synaptonemal complex (SC) is fully assembled between the homologs in pachytene and strictly one crossover forms between each chromosome pair in late pachytene. Upon crossover formation, the SC disassembles and homologs undergo remodeling and compaction to reveal six bivalents at diakinesis stage. **(B)** Cartoon of chromosome structure observed in diakinesis nuclei in WT (6 bivalents), *brc-1* (6 bivalents), *syp-2* (12 univalents), *brc1; syp-2* (> 12 univalents/DNA fragments) (Adamo et al., 2008), *brc-2* (aggregation), *lig-4* (RNAi) *brc-2* (12 univalents with some DNA fragments) and *lig-4* (RNAi); *rad-51* (aggregation) (Martin et al., 2005) mutants.

this observation, BRC-1-BRD-1 is not required for MSCI during spermatogenesis. In *brc-1* and *brd-1* null mutants, deposition of the repressive chromatin mark H3K9me2 and the absence of Pol2-S2P (actively transcribing RNA polymerase II) signal on the X chromosome are indistinguishable from wild-type animals, suggesting that MSCI is successful in these mutants. As such, the null mutants do not exhibit pachytene arrest and germ cells complete meiotic prophase in preparation for the meiotic divisions (Li et al., 2020).

Analysis of RAD-51 immunostaining in the *brc-1* and *brd-1* null male germ lines showed reduced levels of RAD-51 foci in early meiotic prophase and this reduction was suppressed by inhibiting the NHEJ pathway. Moreover, quantification of GFP:RPA-1 foci, indicative of single stranded DNA, showed a significant reduction in overall foci number and intensity in the absence of BRC-1-BRD-1, suggesting that BRC-1-BRD-1 favors HR at the expense of NHEJ through promoting resection of DSBs during male meiosis (Li et al., 2020; **Figure 2**). This role is similar to what is proposed for BRCA1 function in promoting HR in somatic cells.

BRC-1-BRD-1 Promotes Inter-Sister Recombination and Stabilizes the RAD-51 Filament Under Checkpoint Activation in Oogenesis

In contrast to male meiosis, *brc-1* and *brd-1* null mutants exhibited an increased number of RAD-51 foci at late pachytene in oogenic germ lines, with no obvious difference in RAD-51 kinetics in early meiotic prophase as compared to wild-type animals (Adamo et al., 2008; Janisiw et al., 2018; Li et al., 2018). The elevated RAD-51 foci observed in late pachytene suggests that the repair of a subset of DSBs is delayed in the absence of BRC-1-BRD-1. The high fertility and presence of six bivalents, representing the six homologs connected by chiasmata, at diakinesis in *brc-1* and *brd-1* mutants (**Figure 3B**) suggest that BRC-1-BRD-1 is not essential for crossover formation. To test the hypothesis that BRC-1 promotes repair of DSBs by the inter-sister recombination pathway, Adamo and coworkers disrupted SC assembly and thereby inter-homolog crossovers by mutation of *syp-2* (one of six components in the central region of the SC) in the *brc-1* mutant. *syp-2* mutants have twelve intact univalents at diakinesis (**Figure 3B**), suggesting efficient repair of DSBs by the inter-sister pathway. On the other hand, in the *brc-1; syp-2* double mutant more than twelve DAPI staining bodies were often observed (**Figure 3B**), indicating the presence of chromosome fragmentation and failure in inter-sister repair. These results are consistent with BRC-1 playing an important role in inter-sister repair during oogenesis (Adamo et al., 2008). A recent study extended these findings by showing that mutation of *brc-1* enhanced the phenotype of phosphorylation defective mutants in *syp-1* (another component of the central region of the SC), presumably through impairment of inter-sister recombination (Garcia-Muse et al., 2019; **Figure 2**). Importantly, BRC-1-dependent inter-sister repair prevents erroneous recombination (recombination between heterologous sequences) in meiosis, suggesting one mechanism by which BRC-1 prevents genome instability (Leon-Ortiz et al., 2018).

In addition to promoting inter-sister repair, BRC-1 is required to stabilize the RAD-51 filament from premature disassembly in late pachytene under meiotic checkpoint activation conditions. In *zim-1/2/3* or *syp-1* mutants, which lack crossovers on a subset or all chromosomes, respectively, and activate meiotic checkpoints, extensive RAD-51 foci are present throughout meiotic prophase (Yu et al., 2016). Removing BRC-1 in these mutant backgrounds results in a region in late pachytene with significantly reduced RAD-51 levels, with high levels of RAD-51 both prior to and after this region. Both the number of RAD-51 foci as well as the fluorescence intensity of residual foci was greatly diminished in this region and thus this pattern has been referred to as a RAD-51 "dark zone". Taking advantage of the spatial temporal organization of the germ line, time course analysis of *spo-11; brc-1; syp-1* mutants exposed to irradiation (IR) was performed. The *spo-11* mutant was used so that breaks could be induced uniformly in the germ line at a single point in time by IR and as nuclei moved through the germ line no new breaks were formed. This analysis revealed that RAD-51 installed on processed DSBs in nuclei residing in early prophase at the time of DSB induction was dismantled once the nuclei reached late pachytene, suggesting that BRC-1 promotes the stability of the RAD-51 filament under these conditions (Li et al., 2018). The mechanism underlying BRC-1-dependent RAD-51 stabilization is currently unknown and could be either through direct interaction with RAD-51 to reduce its ATP hydrolysis and/or regulation of helicases which dismantle the RAD-51 filament. Interestingly, the requirement for BRC-1 to stabilize RAD-51 filaments under checkpoint activation conditions is oogenesis-specific, as a RAD-51 dark zone was not observed in the male germ line (Li et al., 2020; **Figure 2**).

Recent studies examining the mutational signatures of *brc-1* and *brd-1* mutants propagated over multiple generations revealed elevated levels of small deletions, deletions-insertions, single nucleotide variants and tandem repeats (Kamp et al., 2020; Volkova et al., 2020). Analysis of *brc-1* and *brd-1* mutants in combination with mutations in different repair pathways provided evidence that theta-mediated end joining (TMEJ), but not NHEJ, was responsible for the mutational profiles observed. TMEJ anneals short regions of microhomology and catalyzes template-dependent DNA synthesis to repair the broken DNA molecule. These results suggest that in the absence of BRC-1-BRD-1, TMEJ repairs inefficiently resected DSBs. It will be important to distinguish whether the mutations are a consequence of repair of meiotic DSBs, or repair of breaks generated during replication prior to meiotic entry or during embryogenesis, to understand the complete spectrum of BRC-1-BRD-1 function in both the soma and in meiosis. Nonetheless, the mutational profile of *C. elegans brc-1* and *brd-1* mutants is very similar to that found in BRCA1-deficient tumor cells, suggesting that TMEJ repair in the absence of BRCA1 contributes to carcinogenesis (Kamp et al., 2020; Volkova et al., 2020).

BRC-1-BRD-1 Regulates Crossover Patterning

Given that there are many more DSBs than crossovers, a subset of processed DSBs is chosen to be resolved as crossovers in a process referred to as crossover designation (Gray and Cohen, 2016). To investigate whether BRC-1 plays a role in crossover designation and/or resolution, genetic linkage analysis on meiotic products of *brc-1* mutants was performed and revealed an altered crossover landscape. Although the genetic map length was not significantly different between wild type and *brc-1* mutants, there was a shift in crossover distribution from chromosome arms, which are most often observed in wild-type animals, to more central regions on chromosomes (Li et al., 2018, 2020). Altered crossover distribution to the chromosome center has been observed in many other *C. elegans* mutants defective for various aspects of meiotic recombination (Zetka and Rose, 1995; Wagner et al., 2010; Meneely et al., 2012; Saito et al., 2012, 2013; Chung et al., 2015; Hong et al., 2016; Jagut et al., 2016). While the underlying mechanisms are currently unknown, it has been suggested that this could result from an altered chromatin landscape (Saito and Colaiacovo, 2017). Thus, BRCA1 may regulate chromatin structure in *C. elegans* meiosis, as it does in mouse meiosis (Broering et al., 2014; Densham et al., 2016), although the specific types of chromatin modification regulated by BRCA1 may not be identical in *C. elegans* and mouse.

Surprisingly, in the *zim-1* mutant where two chromosomes fail to pair and synapse, BRC-1-BRD-1 promoted the formation of extra COSA-1 marked crossover designation events on the remaining chromosome pairs during oogenesis. COSA-1 (CrossOver Site Associated protein 1) is generally accepted to mark canonical crossovers in *C. elegans* meiosis (Yokoo et al., 2012); therefore, the number of COSA-1 foci has been used as a cytological readout of the number of genetic crossovers. The reduced COSA-1 foci in the *brc-1; zim-1* double mutant, however, was not accompanied by a smaller genetic map distance, measured by SNP marker-based linkage analysis. These results suggest that not all crossovers are marked by COSA-1 in the *brc-1; zim-1*double mutant. Further, while the map length was similar in the absence of BRC-1, CO patterning was altered such that there were elevated levels of single crossovers (SCOs) with a concomitant reduction in double crossovers (DCOs). As a crossover can form between any two non-sister chromatids within paired homologs, two, three or four-strand DCOs are possible outcomes of elevated crossover formation. However, only DCOs between the same two chromatids can be detected as DCOs in SNP marker-based analysis, because only one sister chromatid is inherited in the product of meiosis. DCOs involving three or four chromatids will be detected as SCOs. Therefore, the aforementioned observation is consistent with a model whereby inactivation of BRC-1 in the *zim-1* mutant results in a shift from two-strand DCOs that are marked by COSA-1 and observed in the DCO class, to three- and four-strand DCOs that lack the COSA-1 marker and are detected as SCOs (Li et al., 2018). In contrast to oogenesis, BRC-1 inhibits the formation of extra COSA-1 marked crossover precursors in spermatogenesis. Elevated levels of COSA-1 foci were observed in the *brc-1; zim-1* double mutant as compared to *zim-1*. Additionally, the genetic map distance was enlarged in the *brc-1*; *zim-1* double mutant, suggesting that BRC-1 inhibits the formation of extra canonical crossovers in spermatogenesis (Li et al., 2020). Together, these results suggest that BRC-1 plays a role in CO patterning, perhaps through regulating both canonical and non-canonical CO pathways under conditions of meiotic dysfunction (**Figure 2**).

Why does *brc-1* and *brd-1* mutation exhibit sex-specific phenotypes? One hypothesis is that BRC-1-BRD-1 interacts with unique partners to form different complexes during male and female meiosis. This would be analogous to what has been established for BRCA1 function in somatic cells, where it forms three different complexes with distinct functions under different physiological conditions (Li and Greenberg, 2012). Alternatively, or in addition, the sex-specific phenotypes could be a consequence of BRC-1-BRD-1 being differentially regulated by post-translational modifications in the diverging environments of male and female meiosis. Future studies on BRC-1-BRD-1 interacting proteins and the regulation of complex(es) will provide insight into the functions of BRC-1-BRD-1 during spermatogenesis and oogenesis. These studies may also shed light on the sex-specific regulation of the BRCA1-BARD1 complex in mammals.

BRCA2 FUNCTIONS AS AN ESSENTIAL MEDIATOR FOR HR

Breast cancer susceptibility gene 2 (BRCA2) is an essential mediator of HR (Jensen et al., 2010; Liu et al., 2010; Kowalczykowski, 2015). Similar to *BRCA1,* germline mutations in *BRCA2* predispose patients to breast and ovarian cancer and genome instability (Wooster et al., 1995; Yu et al., 2000; Venkitaraman, 2002; King et al., 2003). Biochemical, cell biological and genetic studies have supported a role of BRCA2 in recruiting the RAD51 recombinase to resected single strand DNA at DSBs and promoting nucleoprotein filament assembly to mediate homology search and strand exchange (Sharan et al., 1997; Wong et al., 1997; Abbott et al., 1998; Chen et al., 1999; Tutt et al., 1999; Yuan et al., 1999; Moynahan et al., 2001; Xu et al., 2001; Jensen et al., 2010; Liu et al., 2010; Thorslund et al., 2010).

Human BRCA2 encodes an exceptionally large protein consisting of 3,418 amino acids with multiple functional domains: an N-terminal domain that facilitates binding with Partner And Localizer of BRCA2 (PALB2), eight BRC repeats that define the RAD51 binding motif, a DSS1 and DNA binding domain (DBD, composed of one helix-rich domain (HD), three oligonucleotide/oligosaccharide binding (OB) folds and a tower domain), and a C terminal RAD51 binding domain (CTRB) (**Figure 4**; Yang et al., 2002; Esashi et al., 2005; Xia et al., 2006; Carreira et al., 2009; Shivji et al., 2009). Given its essential role in HR, it is not surprising that BRCA2 is conserved in fungi, plants and metazoans. While overall similar, BRCA2 orthologs possess different numbers of BRC repeats and OB folds, which are signature domains of BRCA2, and vary considerably in size (Gudmundsdottir and Ashworth, 2004; **Figure 4**). For example, Brh2, the BRCA2 ortholog in the fungus *Ustilago maydis,* contains a single BRC repeat and

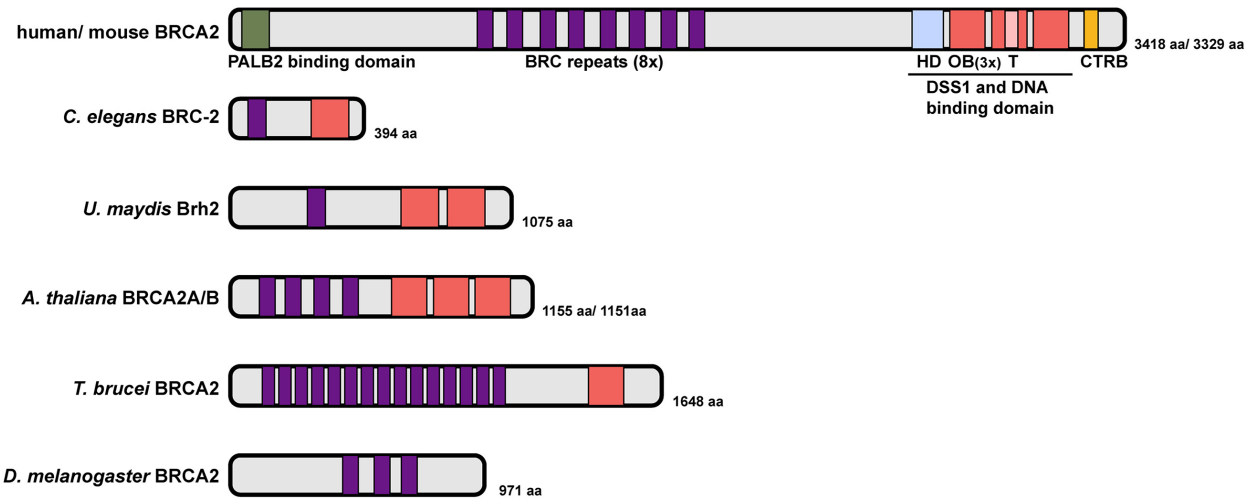

FIGURE 4 | Domain structure of BRCA2 proteins. Human BRCA2 encodes an exceptionally large protein with an N-terminal PALB2 binding domain, eight BRC repeats, a DSS1 and DNA binding domain (DBD) composed of one helix-rich domain (HD), three oligonucleotide/oligosaccharide binding (OB) folds and a tower domain, and a C terminal RAD51 binding domain (CTRB). *C. elegans* BRC-2 represents a simplified version with a single BRC repeat and OB fold. The number of BRC repeats and OB fold domains vary greatly in different organisms (*U. maydis* Brh2, *A. thaliana* BRCA2A/B, *T. brucei* BRCA2 and *D. melanogaster* BRCA2). Sequence alignment did not identify a putative OB fold/DNA binding domain in *Drosophila* BRCA2 (Yang et al., 2002).

two OB folds (Kojic et al., 2002, 2005). *Drosophila melanogaster* BRCA2 contains three BRC repeats but no recognizable OB fold (Klovstad et al., 2008). Two almost identical BRCA2 orthologs were identified in *Arabidopsis thaliana*, each containing four BRC repeats (Siaud et al., 2004). In contrast, the parasite *Trypanosoma brucei* possess a single BRCA2 ortholog with 15 BRC repeats (Hartley and McCulloch, 2008). The BRC repeat is highly conserved among species; despite the different number of repeats, BRC domains in all BRCA2 orthologs examined so far have been shown to bind RAD51 directly and to promote RAD51 nucleoprotein filament formation on ssDNA, which is essential for homology search and strand exchange during HR. In addition, BRCA2 interaction with the highly conserved DSS1 protein also contributes to HR through promoting RAD51-recruitment activity and stability of BRCA2 (Li et al., 2006; Liu et al., 2010; Siaud et al., 2011). The CTRB domain, while conferring RAD51 binding and stabilizing RAD51 filaments on ssDNA, is not essential for HR (Davies and Pellegrini, 2007; Esashi et al., 2007; Prakash et al., 2015).

C. elegans BRCA2 (BRC-2) contains domain signatures similar to mammalian BRCA2 but is approximately 1/8 the size, with just 394 amino acids. BRC-2 contains a single BRC repeat that directly interacts with RAD51 and a single OB fold that preferentially binds to ssDNA (Martin et al., 2005; Petalcorin et al., 2006; **Figure 4**). The single BRC repeat is comprised of two RAD-51 interaction regions, one that preferentially binds to free RAD-51, and the other to the RAD-51-DNA nucleoprotein filament that exhibits inhibitory activity on RAD-51 ATPase hydrolysis. Together, these two RAD-51 interaction regions within the BRC repeat are proposed to coordinate the activity of BRC-2 for promoting RAD-51 nucleation on ssDNA and stabilizing existing RAD51 filament from disassembly through inhibiting ATP hydrolysis (Petalcorin et al., 2007). Recent single-molecule analysis has revealed that BRC-2 acts primarily as a RAD-51 nucleation factor on RPA-coated ssDNA (Belan et al., 2021).

BRCA2 Role in Meiotic Recombination

In addition to a role of promoting RAD51 mediated HR in somatic cells, studies on BRCA2 orthologs have revealed a requirement for BRCA2 during meiosis. In *Ustilago maydis,* mutation of Brh2 led to a failure in the formation of meiotic spore products (Kojic et al., 2002). Null mutants of BRCA2 ortholog in *Drosophila* led to sterility in both male and female flies (Klovstad et al., 2008; Weinberg-Shukron et al., 2018). A transgenic mouse line expressing low levels of human BRCA2 in the gonad showed reduced RAD51 and DMC1 foci formation and prophase arrest of spermatocytes, due to the inability to complete meiotic recombination (Sharan et al., 2004). Depletion of *A. thaliana* BRCA2 by RNAi showed meiotic defects similar to *rad51; dmc1* double mutants (Siaud et al., 2004) and *C. elegans brc-2* mutant produced completely inviable progeny (Martin et al., 2005), suggesting an indispensable role of BRCA2 during meiosis. Studies on human and *Arabidopsis* BRCA2 proteins demonstrated that BRCA2 directly binds to the meiosis-specific recombinase DMC1, which functions together with RAD51 to promote strand invasion and joint molecule formation during meiotic recombination (Dray et al., 2006; Thorslund et al., 2007; Jensen et al., 2010; Martinez et al., 2016). As with RAD51, the BRC repeats facilitate binding between BRCA2 and DMC1, although binding affinities for each individual BRC repeat differ between RAD51 and DMC1 (Martinez et al., 2016). Moreover, different mechanisms have been proposed for BRCA2 stimulation of RAD51 versus DMC1 recombinase activity. In the context of RAD51 mediated recombination, BRCA2 and its eight BRC repeats function by a combination of inhibiting RAD51 ATPase activity, promoting RAD51 filament formation

on ssDNA but not dsDNA, and enhancing strand exchange activity of RAD51. In contrast, stabilization of DMC1 filament on ssDNA was proposed to be the major mechanism by which BRCA2 functions with DMC1 (Martinez et al., 2016; **Figure 5**).

BRCA2 localization to DSBs in somatic cells depends on PALB2 (Xia et al., 2006). It has remained mysterious until recently, how BRCA2 is recruited to DSBs during meiosis. The Shibuya group identified a BRCA2 localizer in mice, which they named meiotic localizer of BRCA2 (MEILB2). MEILB2 is specifically expressed in germ cells and localizes to meiotic recombination sites on the chromosome axis. In the absence of MEILB2, the recruitment of DMC1 and RAD51 recombinase to meiotic DSBs is abolished, leading to sterility in male mice. Furthermore, MEILB2 directly binds to BRCA2 *in vitro* and is a physiological binding partner of BRCA2 *in vivo*. Removing MEILB2 impairs BRCA2 localization to resected ssDNA in spermatocytes, suggesting that MEILB2 recruits BRCA2 to sites undergoing meiotic recombination (Zhang et al., 2019). In contrast to males, female *Meilb2*$^{-/-}$ mice show only a ∼50% reduction in the localization of DMC1 and RAD51, and are sub-fertile, suggesting that redundant mechanisms exist to localize BRAC2 in oogenesis. One possibility is that PALB2 functions in concert with MEILB2 in female meiosis to localize BRCA2. Interestingly, PALB2 knockout mice show reduced male, but not female, fertility. This reduction in fertility is likely due to PALB2 interaction with BRCA1 (Simhadri et al., 2014). Future studies addressing the roles, redundancies and interconnections between PALB2, BRCA1 and BRCA2 will be important for understanding how meiotic DSBs are processed in male and female meiosis. Recently a third component of the BRCA2 complex, BRCA2 and MEILB2-associating protein 1 (BRME1), was identified. BRME1 forms a ternary complex with BRCA2 and MEILB2 and in the absence of BRME1, meiotic DSB repair, homologous chromosome synapsis and crossover formation were impaired in spermatogenesis (Takemoto et al., 2020; Zhang et al., 2020). MEILB2 is conserved among vertebrate taxa; whether binding partners promote meiotic regulation of BRCA2 in organisms such as worms and plants remain to be investigated.

Non-conserved Role of BRCA2 in *C. elegans* Meiosis

BRCA2's role in promoting RAD51/DMC1 nucleoprotein filament formation for homology search and strand exchange in meiotic recombination is conserved among all organisms where it has been examined. A RAD-51 independent, non-conserved role of BRC-2 was uncovered in *C. elegans* meiosis (Martin et al., 2005; Petalcorin et al., 2006). Without BRC-2, SPO-11 induced DSBs are resected, but RAD-51 is not recruited to the single stranded DNA, blocking strand invasion for error-free repair. As the presence of DSBs is extremely deleterious, alternative repair pathways are engaged to remove any remaining breaks before cells exit meiotic prophase I. In *rad-51* or *brc-2* single mutant, oogenic diakinesis nuclei exhibit aggregated DAPI staining chromosome structures, in contrast to the six

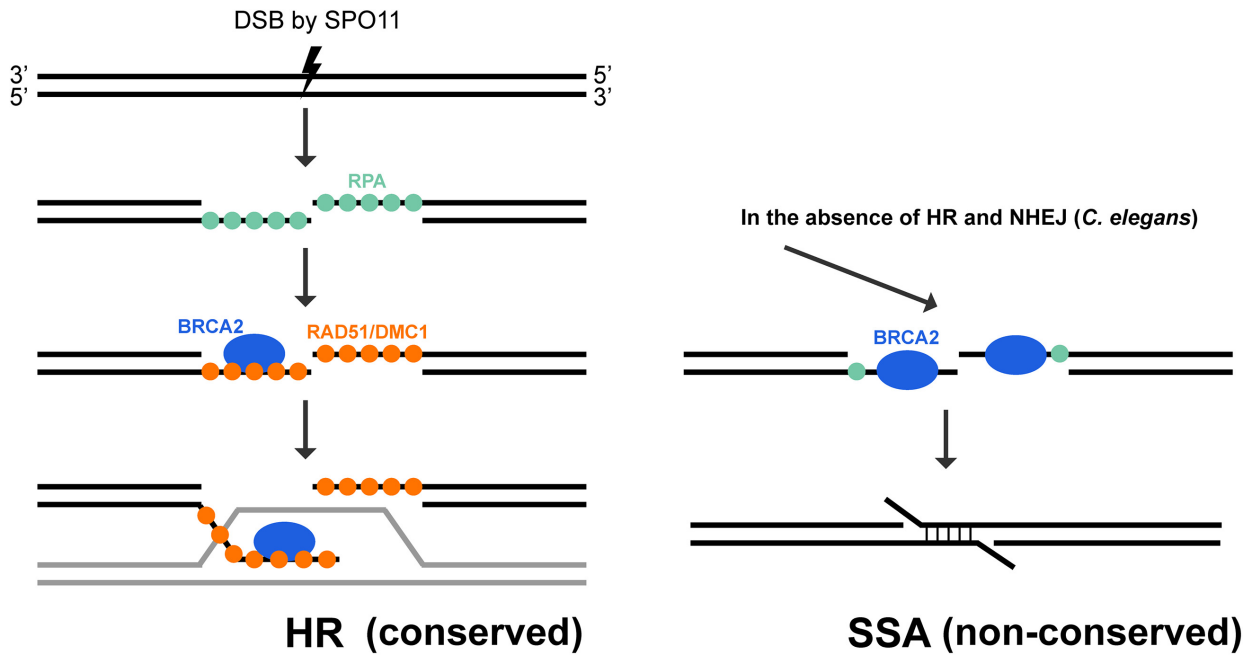

FIGURE 5 | Conserved and non-conserved roles of BRCA2 during meiosis. BRCA2 is an essential mediator of homologous recombination in meiosis. After SPO-11 induced DSB is resected, the 3′ ssDNA is coated with RPA. BRCA2 is critical for recruiting DMC1/RAD51 recombinases to displace RPA molecules on the ssDNA, promoting the formation and stabilization of nucleoprotein filaments to mediate homology search and strand exchange. This function of BRCA2 is highly conserved during meiosis among a large variety of organisms, including *C. elegans*. However, *C. elegans* BRC-2 also exhibits a non-conserved role in promoting single strand annealing when HR (*rad-51* mutant) and NHEJ (*lig-4* knock down) are not available for repair (Martin et al., 2005).

morphologically distinct bivalent structures in wild-type animals (**Figure 3B**). Inactivating NHEJ (*lig-4*) in the *brc-2* mutant resulted in mostly twelve DAPI bodies (**Figure 3B**), suggesting that the aggregation observed in *brc-2* is due to inappropriate repair of meiotic DSBs by NHEJ. However, when a functional BRC-2 was present, as in the case of the *lig-4; rad-51* double mutant, diakinesis nuclei contained clumped DAPI structures as seen in *brc-2* and *rad-51* single mutants (**Figure 3B**; Martin et al., 2005). This observation suggests that BRC-2 promotes an alternative repair pathway when both HR and NHEJ fail to be executed in meiocytes. A possible candidate for this repair pathway is single strand annealing (SSA). Indeed, *in vitro* experiments showed that purified *C. elegans* BRC-2 protein promoted annealing of single strand oligonucleotide coated with RPA (Petalcorin et al., 2006), an activity that mammalian BRCA2 does not possess (Jensen et al., 2010; **Figure 5**). It is likely that *C. elegans* BRC-2 has acquired this function to promote SSA during meiosis, as an ortholog of RAD52, which mediates SSA, is missing.

CONCLUSION

That organisms such as mice, *C. elegans*, and *A. thaliana* carrying mutations in their respective BRCA1 and BRCA2 orthologs exhibit meiotic phenotypes is consistent with BRCA1 and BRCA2 playing critical roles in meiosis. While important for meiotic recombination, BRCA1 and BRCA2 orthologs have acquired divergent functions throughout evolution. BRCA1 together with BARD1 functions as an E3 ubiquitin ligase that promotes ubiquitin transfer to a number of substrates and therefore plays regulatory roles in various processes. Not surprisingly, BRCA1 function during meiosis is quite diverse in different organisms (**Figure 2**). For example, BRCA1 is essential for MSCI in mice but is dispensable for MSCI in *C. elegans*, while *C. elegans* BRC-1 promotes DNA end resection, stabilizes the RAD-51 filament and regulates the crossover landscape. It remains an open question whether BRCA1-BARD1 functions in any of these aspects of meiotic recombination in mammals. Future studies taking advantage of conditional expression and genome editing tools should facilitate analyses on the role of E3 ligase activity, including identification of substrates, and the conserved BRCT domains. In contrast to BRCA1, BRCA2 plays a fundamental and conserved role in HR as a mediator to recruit RAD51 and DMC1 for nucleoprotein filament formation and strand invasion. However, *C. elegans* BRC-2 also uniquely promotes the alternative SSA pathway, perhaps as a consequence of a streamlined set of repair proteins (e.g., absence of DMC1 and RAD52) (**Figure 5**). While not identical, knowledge on meiotic roles of BRCA1 and BRCA2 from model organisms will continue to provide valuable insights into the mechanisms by which these two genes function during human meiosis. Clinical data has shown a correlation between the presence of BRCA1 and BRCA2 mutations in healthy carriers and ovarian aging, which is measured by elevated accumulation of DNA damage in oocytes and reduced primordial follicle reserve (Oktay et al., 2010; Lin et al., 2017; Lambertini et al., 2018). This indicates that the functions of BRCA1 and BRCA2 during human meiosis are likely to influence sperm and egg quality. Interestingly, some cancers inappropriately express meiotic genes and recent evidence suggests that this may lead to altered BRCA2 function (Hosoya et al., 2011; Zhang et al., 2020). HR was inhibited in somatic cells when the SC protein SYCP3 and the meiotic partners of BRCA2, MEILB2 and BRME1, were aberrantly expressed, presumably as a result of BRCA2 protein being sequestered when bound by the meiotic proteins. Future studies focusing on meiotic aspects of BRCA1 and BRCA2 may advance our knowledge in human reproduction as well as tumorigenesis to provide tools for improving fertility and health.

AUTHOR CONTRIBUTIONS

QL wrote the manuscript with content and editorial input from JE. Both authors contributed to the article and approved the submitted version.

FUNDING

Work in the Engebrecht lab is supported by National Institutes of Health GM103860.

ACKNOWLEDGMENTS

We are grateful to members of the Engebrecht lab for thoughtful discussions. We also acknowledge the meiosis research community and apologize to those researchers whose work we were unable to include.

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.