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Recognition of DNA double strand breaks by the BRCA1 tumor suppressor network

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Abstract

DNA double-strand breaks (DSBs) occur in response to both endogenous and exogenous genotoxic stress. Inappropriate repair of DSBs can lead to either loss of viability or to chromosomal alterations that increase the likelihood of cancer development. In strong support of this assertion, many cancer predisposition syndromes stem from germline mutations in genes involved in DNA DSB repair. Among the most prominent of such tumor suppressor genes are the Breast Cancer 1 and Breast Cancer 2 genes (BRCA1 and BRCA2), which are mutated in familial forms of breast and ovarian cancer. Recent findings implicate BRCA1 as a central component of several distinct macromolecular protein complexes, each dedicated to distinct elements of DNA DSB repair and tumor suppression. Emerging evidence has shed light on some of the molecular recognition processes that are responsible for targeting BRCA1 and its associated partners to DNA and chromatin directly flanking DSBs. These events are required for BRCA1-dependent DNA repair and tumor suppression. Thus, a detailed temporal and spatial knowledge of how breaks are recognized and repaired has profound implications for understanding processes related to the genesis of malignancy and to its treatment.

DNA double-strand break repair and cancer

The eukaryotic genome continually cycles between diploid (G1) and tetraploid (G2) states. These different DNA contents, known as ploidy, have a dramatic influence on the processes available to repair DNA aberrations caused by both endogenous and exogenous genotoxic agents. The most onerous of such lesions are represented by DNA double strand breaks (DSBs). Commonly used anti-cancer agents such as ionizing radiation (IR) and topoisomerase inhibitors exert their cytotoxicity via increased DSB formation (Sung et al. 2006). DSBs can also arise as a result of physiologic events that occur while replicating DNA (replication forks) encounters a lesion that causes replication fork stalling and collapse (Osborn et al. 2002).

There are two predominant mechanisms for DSB repair—a process that utilizes homologous sequences on the sister chromatid as a template for repair known as homologous recombination (HR), and a repair mechanism termed nonhomologous end-joining (NHEJ) that can ligate either contiguous or non-contiguous sequences in the genome (Fig. 1a). Cell cycle stage is the primary determinant of DSB repair mechanism. By definition, HR by sister chromatid recombination (SCR) requires the presence of an intact sister chromatid and thus replication of the homologous DNA regions of a DNA DSB must exist in order for SCR to occur in S and G2 cell cycle phases. Indeed, many DNA repair proteins that are directly involved in HR, fail to concentrate at DSBs in G1 (Lisby et al. 2004). Thus G1 cells repair DSBs by NHEJ, while both NHEJ and HR can occur in S and G2 phases.

Asynchronous cell populations repair DSBs in a biphasic manner with the majority of repair occurring within 30 min and the remaining breaks being repaired over hours (Longo et al. 1997). Much of the early repair occurs via NHEJ (Nevaldine et al. 1997). BRCA1 and BRCA2 have a greater influence on the kinetics of the slow phase of DNA DSB repair that likely represents DSBs repaired by HR, or alternatively, more difficult to repair DSBs that require additional processing (Scully et al. 1999).

Given the cell cycle dependence of DNA repair mechanisms, it is thus not surprising that cyclin dependent kinase (CDK) activity appears to be a key driving force in the initiation of events necessary for HR. Studies in yeast reveal that CDK1 is required for 5' to 3' DSB resection required to generate single stranded DNA (ssDNA) for nucleation of Rad51 presynaptic filaments (Ira et al. 2004). Consistent with a model whereby HR and NHEJ are in dynamic competition (Myung et al. 2001; Pierce et al. 2001; Frank-Vaillant and Marcand 2002; Weinstock and Jasin 2006), HR is reduced and DSBs are instead preferentially repaired by NHEJ upon inhibition of CDK1 (Ira et al. 2004). Mammalian cells also process DSBs for HR only in S and G2 cell cycle phases as both RPA and Rad51 recruitment to laser-generated DSBs only occurs in S and G2 cell cycle phases (Bekker-Jensen et al. 2006). The molecular determinants of DNA repair mechanism choice at a DSB in S and G2 phases are largely unknown.

NHEJ is an error-prone process that enzymatically joins DNA ends. This repair process is performed by a protein complex consisting of the DNA binding Ku70/Ku80 heterodimer, the phosphoinositide kinase-related kinase (PIKK) family member DNA-PK, and the XRCC4/Ligase IV DNA ligase (Lieber et al. 2003). A particularly dangerous situation is when NHEJ is active in a cell containing more than 1 DSB. In this scenario NHEJ can repair breaks by joining non-contiguous chromosomal regions, resulting in a chromosomal translocation (Weinstock et al. 2006). As opposed to NHEJ, accurate HR does not contribute to translocation events stemming from the presence of multiple DSBs induced by endonucleases (Weinstock et al. 2006). A perhaps even more complicated array of reactions is necessary for HR repair of a DSB. Deficits in genes involved in any of these steps predisposes to cancer. In fact, the most common forms of hereditary breast and ovarian cancer occur in response to inactivating mutations to the breast cancer susceptibility genes, BRCA1 and BRCA2. These gene products are required for efficient HR repair of a DSB (Moynahan et al. 1999; Snouwaert et al. 1999; Moynahan et al. 2001). BRCA1 and BRCA2 mutated cells also show similar types of spontaneous chromosomal abnormalities including chromosome and chromatid breaks as well as triand quadriradial chromosomes (Venkitaraman 2002).

Homologous recombination proceeds by the general scheme outlined in Fig. 1a. DSB 5' to 3' end resection produces a stretch of single-stranded DNA (ssDNA) that is coated by the single-stranded DNA binding protein RPA. This allows recruitment of the ATRIP-ATR heterodimer needed to phosphorylate and activate the CHK1 kinase (Zou and Elledge 2003) as well as other repair factors required for S and G2 checkpoint activation (Zou et al. 2003). These regions of ssDNA become the substrate for subsequent Rad51-dependent strand invasion reactions into homologous regions of the sister chromatid. Rad51 catalyzes the strand exchange reaction allowing invasion into the homologous region on the sister chromatid to create Holliday Junction (HJ) recombination intermediates.

HR is tightly controlled by delivery systems for the Rad51 recombinase. Two factors necessary for efficient Rad51 delivery are the BRCA1 and BRCA2 tumor suppressors. BRCA2 likely plays a direct role in delivering Rad51. The strand invasion reaction has been elegantly modeled in vitro to demonstrate that BRCA2 delivers Rad51 to displace RPA coated by ssDNA (Yang et al. 2005). The Rad51 presynaptic filament develops at the 3' overhanging ssDNA when BRCA2 nucleates Rad51 polymers specifically at the double-stranded–single-stranded DNA

junctions to displace RPA, creating a Rad51 nucleofilament (Yang et al. 2005). Additional support for an essential role of these tumor suppressors in HR comes from recent reports that Rad51 is overexpressed in BRCA1 mutated tumors and can rescue the IR sensitivity of BRCA1 deficient cells (Martin et al. 2007).

Following HJ formation, DNA polymerase-mediated copying of the sister chromatid template and resolution of the HJ intermediates by RecQ helicases is necessary for completion of this reaction (Ira et al. 2003; Wu and Hickson 2003). Failure at almost any step of this complex process has been implicated in cancer predisposition (Scully and Livingston 2000; Jasin 2002).

Molecular anatomy of a DSB

DNA DSBs rapidly induce a series of cooperative and parallel signaling events at the site of DNA damage to activate DNA repair and checkpoint responses. Numerous and varied post-translational modifications and DNA processing events facilitate recognition of DNA DSBs by an elaborate system of macromolecular DNA repair complexes.

The exact temporal order of events for DSB repair is unknown; however, studies in yeast and in mammalian systems suggest a number of interdependent events ensue rapidly at breaks (Celeste et al. 2003; Lisby et al. 2004; Bekker-Jensen et al. 2006; Greenberg et al. 2006; Sobhian et al. 2007). ATP-dependent chromatin relaxation occurs within minutes at the site of DNA damage (Kruhlak et al. 2006). Nucleosome structure is disrupted at DNA directly flanking the DSB by chromatin remodeling complexes, (Shroff et al. 2004; Bradshaw et al. 2005; Bekker-Jensen et al. 2006; Berkovich et al. 2007). Chromatin is extensively modified next to the non-nucleosomal DNA at DSBs via processive phosphorylation of a C-terminal serine residue on the histone variant H2AX. Approximately 10% of the histone H2A species within a cell is derived from H2AX, which is distinguished by the presence of consensus PIKK phosphorylation sites within its C-terminal tail. The phosphorylated form of H2AX is referred to as γ H2AX. Seminal studies by Bonner and colleagues demonstrated that H2AX flanking a DSB is extensively modified by DNA damage inducible, wortmannin-sensitive phosphorylation (Rogakou et al. 1998; Rogakou et al. 1999). Estimates support a model whereby megabases of chromatin flanking a single DSB are modified by H2AX phosphorylation (Rogakou et al. 1999); however, more direct measures of γ H2AX boundaries are required to precisely determine the extent of γ H2AX involvement along chromatin adjacent to DSBs.

Mechanisms responsible for spreading of H2AX phosphate along chromatin have recently become clearer. In mammalian cells, H2AX is phosphorylated by redundant PIKK signaling from ATM, ATR and DNA-PK (Ward and Chen 2001; Furuta et al. 2003; Stiff et al. 2004). Extensive phosphorylation by PIKKs proceeds to modify H2AX that is present at contiguous stretches of chromatin. This allows binding of H2AX by the mediator of DNA damage checkpoint protein 1 (MDC1) through the MDC1 BRCT (BRCA1 C-terminal repeat) domain (Stewart et al. 2003; Stucki et al. 2005). While MDC1 is predicted to be downstream of H2AX phosphorylation with respect to DSB recruitment, the processes are cooperative rather than linear. MDC1 directs ATM to chromatin regions flanking DSBs, resulting in a feed-forward phosphorylation of H2AX (Lou et al. 2006). The binary γ H2AX-MDC1 interaction appears to mediate nearly all of the chromatin associated foci formation following DNA DSBs (Bassing et al. 2002; Celeste et al. 2002; Celeste et al. 2003).

Despite this dramatic effect on DSB morphology as assessed by imaging studies of DNA repair protein ionizing radiation induced foci (IRIF), γ H2AX or MDC1 deficiency does not have as significant an influence on DNA DSB repair as deficiency of many of the repair factors that it assembles into IRIF (Celeste et al. 2002; Evers and Jonkers 2006; Lou et al. 2006). While

BRCA1 and the M/R/N (Mre11/Rad50/NBS1) complex are all γ H2AX-MDC1 dependent for foci formation (Celeste et al. 2002; Furuta et al. 2003), they are not completely epistatic with respect to MDC1- γ H2AX and DSB repair. BRCA1 or M/R/N null mice die early during embryonic development, whereas MDC1 or γ H2AX $-/-$ mice are viable (Celeste et al. 2002; Lou et al. 2006). Moreover, the repair deficiencies in M/R/N (Luo et al. 1999; Kang et al. 2002) or BRCA1 (Gowen et al. 1996; Hakem et al. 1996; Liu et al. 1996) null mice are more severe than mice lacking either MDC1 or γ H2AX. In addition, while H2AX deficiency impairs HR (Unal et al. 2004; Xie et al. 2004), BRCA1 depletion further reduces HR in this setting (Xie et al. 2004), indicating that BRCA1 and H2AX have nonlinear roles in DNA repair. Finally, BRCA1 and the M/R/N are recruited to laser-induced DSBs in the absence of H2AX or MDC1 (Celeste et al. 2003); however, this MDC1- γ H2AX independent recruitment is limited to the non-nucleosomal regions directly flanking DSBs (Lukas et al. 2004).

These observations point to adjunct or parallel roles for chromatin-associated BRCA1 and M/R/N during DSB repair and suggest association with the non-nucleosomal regions flanking the breaks instead mediates their major contributions to DNA repair. This makes some intuitive sense, as the repair chemistry actually occurs in these regions. Extensive imaging of breaks reveals the particular subdomain (chromatin vs DNA proximal to the break) of the DSB region recognized by BRCA1 and many of its associated repair proteins (Bekker-Jensen et al. 2006; Sartori et al. 2007). Within the resolution limits of fluorescence microscopy, many repair factors are exclusively limited to either the chromatin regions or the nonnucleosomal DNA regions flanking laser-induced DNA DSBs (Fig. 1b). For example, MDC1 and ATM display chromatin association, delineated by colocalization with γ H2AX and not RPA at laser-generated DSBs. ATR and Rad51 display primarily non-nucleosomal DNA localization, and BRCA1 and the M/R/N concentrate to both chromatin regions and to non-nucleosomal regions flanking breaks (Bekker-Jensen et al. 2006).

Strict placement of repair proteins to one particular region of a DSB, however, does not adequately explain all observed checkpoint and repair signaling dependencies. For example, elegant studies have revealed that CHK2 is phosphorylated at a DSB by the ATM kinase and then migrates throughout the nucleus to phosphorylate its substrates (Lukas et al. 2003). CHK2 phosphorylation, however, is independent of H2AX or MDC1 (Fernandez-Capetillo et al. 2002; Goldberg et al. 2003). Thus, retention of ATM by γ H2AX-MDC1 on chromatin is not required for all ATM signaling events at DSBs. Perhaps ATM recruitment to DNA regions proximal to the break via interaction with the C terminus of NBS1 is sufficient for ATM-dependent signaling events to CHK2 (Falck et al. 2005), while ATM chromatin association is responsible for a different subset of phosphorylation events among the vast network of ATM substrates (Matsuoka et al. 2007). The recent advent of chromatin IP (CHIP) methods to determine DSB association has the potential to shed light on some of these unanswered questions by determining the precise nucleotide position of repair factor association at a DSB (Potts et al. 2006; Rodrigue et al. 2006; Berkovich et al. 2007).

The BRCA1 tumor suppressor network

Breast cancer is the most common malignancy and the second leading cause of cancer-related mortality among women in the US. The majority of breast cancer is termed sporadic. Its occurrence is thought to be unrelated to genetic inheritance, as these cancers do not cluster in familial patterns. Approximately 10% of breast cancers do show a familial pattern of distribution and roughly half of these are derived from germline mutations in BRCA1 and BRCA2 (Nathanson et al. 2001). Rare syndromes, and unidentified genetic causes contribute to a small percentage of inherited breast cancer cases and more than half of all familial breast cancer is derived from unknown genetic causes, commonly referred to as *breast cancer susceptibility X (BRCA) genes* (Nathanson et al. 2001). The identification of the *BRCA1* and

BRCA2 genes (Futreal et al. 1994; Miki et al. 1994; Wooster et al. 1994) has enabled researchers a means to investigate the molecular basis of inherited breast cancers. Such endeavors have revealed a network of genes relevant to *BRCA1* and *BRCA2* genome integrity function in breast cancer susceptibility (Walsh and King 2007).

Heterozygous *BRCA1* and *BRCA2* mutation carriers display high penetrance cancer phenotypes, with up to an 85% lifetime risk of breast cancer (King et al. 2003). They also have a strong predisposition to ovarian epithelial cancer, with *BRCA1* mutations conferring a greater risk than *BRCA2* (King et al. 2003). These genetic loci display classical tumor suppressor alterations characterized by loss of heterozygosity at the wild-type (wt) allele and retention of mutant copies. Family history enables identification of at-risk individuals that can be screened for germline *BRCA1* and two mutations. Screening has clear clinical value, as mutation carriers that have undergone prophylactic salpingo-oophorectomy and mastectomy display an 80–90% reduction in the incidence of breast and ovarian cancer in 8-year periods following surgery (Rebbeck et al. 1999; Rebbeck et al. 2002).

The initial clues to *BRCA1* function came from seminal studies by Scully and Livingston. Discreet, intra-nuclear *BRCA1* foci were observed to colocalize with the Rad51 recombinase in response to DNA damaging agents (Scully et al. 1997a; Scully et al. 1997b). Contemporaneous discovery of *BRCA2* interaction with Rad51 (Sharan et al. 1997) led to the hypothesis that *BRCA1* and *BRCA2* play integral roles in specific types of DNA repair activities in response to endogenous and exogenous genotoxic stresses (Scully and Livingston 2000; Elledge and Amon 2002). Consistent with this DNA repair hypothesis of inherited breast cancer suppression, *BRCA2* associates with *BRCA1* in a protein complex that colocalizes with Rad51 at DNA damage sites (Chen et al. 1998). *BRCA1* and *BRCA2* cells display similar types of spontaneous chromosomal abnormalities and sensitivity to DNA damaging agents that require HR for repair (Venkitaraman 2002). Moreover, *BRCA1* is extensively phosphorylated after DNA damage by the checkpoint kinases and suppressors of inherited breast cancer, ataxia telangiectasia mutated (ATM) (Cortez et al. 1999; Tibbetts et al. 2000), and CHK2 (Lee et al. 2000).

Clinical *BRCA1* mutations invariably disrupt multiple cellular checkpoint and repair responses to genotoxic stress (Xu et al. 1999; Xu et al. 2001a), buttressing the hypothesis that *BRCA1* DNA repair activities are germane to its roles in tumor suppression. In particular, *BRCA1* is required for the IR-induced S phase and G2 checkpoints and for HR repair of DSBs (Moynahan et al. 1999; Moynahan et al. 2001). The molecular basis underlying these repair activities has been largely undefined. Given the evidence that a network of DNA repair proteins functions in concert with *BRCA1* to maintain genome stability, it will be important to identify new members in the *BRCA1* network that might also contribute to suppression of breast and ovarian cancer.

BRCA1 is a large protein of 1,863 amino acids. It has several distinct domains that are essential for its DNA repair functions (Fig. 2a), and each of these domains is affected by cancer causing mutations to the *BRCA1* gene. It has an amino terminal RING domain with E3 ubiquitin ligase activity that is activated on chromatin by DNA damage (Polanowska et al. 2006). Clinical *BRCA1* RING domain mutations disrupt this E3 activity suggesting its importance for tumor suppression (Brzovic et al. 2001a; Brzovic et al. 2001b). DNA damage inducible *BRCA1* E3 ligase activity requires Mre11 and PIKK-dependent association with the E2 enzyme UbcH5c (Polanowska et al. 2006). *BRCA1* E3 ubiquitin ligase activity is required to ubiquitinate the BRCT interacting partner CtIP (Yu et al. 2006).

DNA damage induces *BRCA1*-dependent, CtIP chromatin association and IRIF formation, suggesting the ubiquitinated form of CtIP is targeted to DNA damage sites, rather than to the

proteasome for degradation (Yu et al. 2006). Should BRCA1-dependent CtIP ubiquitination be a paradigm for how BRCA1 E3 activity influences the DNA damage response, it suggests that BRCA1 functions in an analogous manner to the ATM kinase to modify proteins at the site of DSBs for appropriate DNA repair and checkpoint functions (Lukas et al. 2003; Kitagawa et al. 2004).

Approximately 60% of the BRCA1 protein is derived from exon 11 of the BRCA1 locus. The BRCA1 region encoded by exon 11 is extensively phosphorylated in response to IR by the checkpoint kinases and breast cancer suppressors CHK2 and ATM (Cortez et al. 1999; Lee et al. 2000; Tibbetts et al. 2000). Mutations disrupting exon 11 results in the production of a hypomorphic BRCA1 protein derived from an in-frame splicing event between exons 10 and 12. Mice possessing exon 11 deletions can survive to adulthood in some strains or in combination with p53 heterozygosity (Cressman et al. 1999a). While BRCA1 Δ 11 is recruited to DNA damage sites, it fails to deliver Rad51 to DSBs (Huber et al. 2001) and cells expressing Δ 11 have HR deficiency (Moynahan et al. 1999; Snouwaert et al. 1999). Exon 11 mutations predispose to breast and ovarian cancer in humans, and Δ 11 mice develop spontaneous mammary gland cancers as well as lymphomas and squamous cell carcinomas (Cressman et al. 1999b; Ludwig et al. 2001; Xu et al. 2001b).

At the far C terminus of BRCA1 is the BRCT repeat. BRCT domains recognize their cognate interacting partners by binding to phosphoserine residues. The BRCA1 BRCT domain has a recognition element for sequences S (PO₄) PXF (Manke et al. 2003; Yu et al. 2003). This sequence is embedded in its three known direct interaction partners—the BACH1 helicase (Cantor et al. 2001), recently found to be mutated in Fanconi Anemia Syndrome and in familial breast cancer, CtIP (Wong et al. 1998; Yu et al. 1998), and Abraxas (Kim et al. 2007b; Liu et al. 2007; Wang et al. 2007). The BRCT phosphopeptide-binding domain is frequently present in DNA repair proteins and is the most common region of clinical missense mutations within BRCA1. Cancer causing BRCT domain mutations invariably disrupt BRCT tertiary structure (Williams et al. 2001) and ability to interact with phosphorylated forms (Manke et al. 2003; Yu et al. 2003) of each of BACH1, CtIP, and Abraxas.

The BRCA1-associated ring domain protein 1 (BARD1) is the only known stoichiometric binding partner of BRCA1 (Wu et al. 1996). BARD1 has a similar domain structure to BRCA1 and the direct, binary interaction with BARD1 increases BRCA1 in vitro E3 ligase activity (Xia et al. 2003). Null phenotypes for BRCA1 and BARD1 in mice and frogs are indistinguishable and epistatic with respect to progression through embryogenesis, suggesting a single, common biological function for the BRCA1-BARD1 heterodimer (Joukov et al. 2001; McCarthy et al. 2003). While BARD1 mutations have been reported (Thai et al. 1998), it is surprising that it appears that BARD1 mutations do not significantly contribute to inherited breast cancer, as is the case for BRCA1 interacting partners BRCA2 and BACH1 (Walsh and King 2007).

BRCA1 has been reported to interact with many DNA repair factors that when mutated, each lead to breast cancer (Wang et al. 2000). This spawned a model of a single megacomplex required to repair DNA damage, implying a linear pathway to tumor suppression (i.e., mutation of BRCA1 interacting partners would produce similar phenotypes in vivo with respect to cancer development). But several observations suggested a more complex picture. BRCA1 and two tumors are histopathologically different with BRCA2 tumor commonly being estrogen receptor (ER) positive and BRCA1 tumors typically being of the basaloid class, or ER, progesterone receptor (PR), and Her 2 negative (Sorlie et al. 2003). Expression patterns at the mRNA level for BRCA1 and BRCA2 breast and ovarian cancers can also be used to segregate each to different subclasses of malignancy (Jazaeri et al. 2002; Sorlie et al. 2003).

This nonlinearity of in vivo phenotypes is more indicative of a network model for BRCA1 function in tumor suppression, perhaps analogous to oncogenic Ras signaling in cellular transformation (White et al. 1995). Oncogenic Ras constitutively activates multiple signaling pathways and each of these contributes in a subtotal manner to Ras signaling and oncogenicity. The partially overlapping responses of BRCA1 and associated partners in the DNA damage response may explain the different clinical manifestations of mutations to genes encoding BRCA1 interacting partners. Indeed, BRCA1-BARD1 heterodimers exist in several distinct complexes before genotoxic stress and each migrates to the site of DNA damage where it forms new protein associations (supercomplexes) following checkpoint kinase signaling (Fig. 2b) (Greenberg et al. 2006). Each of these complexes is responsible for distinct elements of the BRCA1-dependent DNA damage response. Thus, clinical mutations of genes encoding BRCA1 interacting partners might only partially recapitulate true BRCA1 nullizygosity (Greenberg et al. 2006), providing an explanation for the different susceptibility syndromes of BRCA2 carriers (Goggins et al. 1996; Consortium 1999) and lower penetrance for PalB2 (Erkko et al. 2007; Rahman et al. 2007; Xia et al. 2007), and BACH1 (Seal et al. 2006) mutations.

Initial studies predicted the existence of at least three different DNA damage inducible BRCA1-BARD1 super-complexes defined by new protein interactions with the checkpoint and repair proteins topoisomerase II B binding protein 1 (TopBP1) and members of the M/R/N complex (Greenberg et al. 2006). TopBP1 primarily associated with the BRCA1 complex defined by the BRCT interacting protein BACH1, while the M/R/N complex was largely associated with the BRCA1 complex defined by an interaction at the BRCT domain with CtIP. This BRCA1-CtIP-M/R/N complex has recently been implicated in DSB end resection in an orthologous manner to M/R/X-Sae2 complexes in yeast (Sartori et al. 2007; Chen et al. 2008). The third BRCA1-BARD1 complex is defined by interaction with BRCA2 and Rad51. This complex contains the Rad51 recombinase protein, but not the M/R/N or TopBP1 inducible interactions. The BRCA1-BRCA2 complex likely contains an additional member required for function in HR, the recently discovered tumor suppressor protein PalB2 (Xia et al. 2006) (Greenberg laboratory, unpublished results).

More recently, a fourth protein complex defined by new BRCA1 BRCT interactions was discovered (Kim et al. 2007a; Sobhian et al. 2007; Wang et al. 2007). This new complex demonstrates phosphorylation-dependent interactions at the BRCA1 BRCT domain with a novel protein termed Abraxas. Abraxas mediates interaction between BRCA1 and the ubiquitin binding protein Rap80 (Kim et al. 2007b; Liu et al. 2007; Wang et al. 2007). This BRCA1-BARD1 complex also contains the BRCC36 deubiquitinating (DUB) enzyme (Sobhian et al. 2007). Each of these members is required for the IR-induced G2 checkpoint and repair of IR-induced DNA damage (Kim et al. 2007a; Sobhian et al. 2007; Wang et al. 2007). This complex is mutually exclusive to the complexes containing CtIP or BACH1 and also appears to lack interaction with the BRCA1 complex containing BRCA2.

Recognition of DNA DSBs by BRCA1 protein complexes

Imaging studies using a far-wave UV laser to create defined subnuclear volumes of DNA DSBs reveal that BRCA1 migrates to both chromatin regions flanking the DSBs and to the ssDNA regions directly at the DSB (Bekker-Jensen et al. 2006). In addition, BRCA1 can recognize DNA DSBs occurring in G1 of the cell cycle, while BRCA2-Rad51 DSB association is restricted to S and G2 cell cycle phases (Bekker-Jensen et al. 2006). It is possible that this G1 DSB association for BRCA1 is responsible for its reported involvement in promoting certain types of NHEJ (Guirouilh-Barbat et al. 2004; van Heemst et al. 2004; Zhuang et al. 2006). BRCA1 chromatin association at DSBs is dependent on epigenetic modifications initiated by γ H2AX-MDC1. In the absence of these proteins, BRCA1 still migrates to laser-induced DSBs,

but now its DSB association is restricted to the DNA regions at the center of the stripe, rather than to the chromatin regions flanking the breaks (Bekker-Jensen et al. 2006).

Clinical mutations at the C-terminal BRCA1 BRCT domain disrupt DSB localization. BRCA1 fails to efficiently access DSBs in HCC1937 breast cancer cells. These cells express detectable levels of a single BRCA1 allele containing a frameshift mutation at codon 1755 that deletes the second BRCT domain. BRCA1 (1–1755) fails to localize to DNA DSBs in the form of intra-nuclear foci and is very weakly detectable at laser-induced DSBs (Greenberg et al. 2006). Such BRCT mutations disrupt BRCA1 interaction with its known BRCT interacting partners, implying that one or more BRCT ligands is utilized to target BRCA1 to DSBs.

Reconstitution with wt BRCA1 in HCC1937 cells restores DSB localization and DNA damage response activities (Scully et al. 1999; Xu et al. 2001a; Xu et al. 2002). Thus, HCC 1937 cells reconstituted with wt vs clinical BRCA1 mutants provide a convenient genetic system to examine relationships between BRCA1 and its associated partners with respect to DSB recruitment. BARD1, BRCA2, and Rad51 all failed to efficiently access DSBs when associated with clinical BRCA1 BRCT mutants (Greenberg et al. 2006). The converse is not true as BRCA1 efficiently migrates to laser-induced DSBs in BRCA2-mutated cells (Greenberg et al. 2006). BRCA2 similarly depends on its binding partner Palb2 for chromatin association and DSB localization (Xia et al. 2006), and Palb2 is readily detectable in BRCA1–BARD1 complexes (Greenberg lab, unpublished results).

These observations imply that BARD1–BRCA1 heterodimers control HR in conjunction with Palb2 to deliver BRCA2–Rad51 complexes to DSBs, although formal proof of this hypothesis is currently lacking. BRCA2 could then facilitate Rad51-mediated presynaptic filament formation on ssDNA and strand invasion into the homologous region of the sister chromatid (Yang et al. 2005). As BRCA2 depends on both BRCA1 and Palb2 for DSB localization, it is plausible that either BRCA1 or Palb2 provides the DSB recognition element for this complex. An additional possibility is that each of these proteins merely stabilizes BRCA2 on chromatin flanking DNA damage, allowing BRCA2 to migrate to non-nucleosomal regions where it can bind to single-strand–double-strand DNA junctions (Yang et al. 2002).

In contrast to many of its constitutive interactions, DNA damage-inducible interactions with TopBP1 and the M/R/N were BRCA1-independent for DSB recruitment. Both the M/R/N and TopBP1 accessed DSBs indistinguishably in cells containing wt or mutated BRCA1 (Greenberg et al. 2006). Similarly, BRCA1 did not depend on either TopBP1 or the M/R/N for DSB recruitment. These results suggest that BRCA1 forms supercomplexes at the site of DNA damage by parallel and independent recognition of DSB elements with its DNA damage inducible interacting partners. In agreement with this assertion, BRCA1 BRCT mutants that inefficiently access DSBs also largely fail to interact with TopBP1 (Greenberg et al. 2006).

The sought-after BRCT interacting partner that provides the chromatin-associated, γ H2AX/MDC1-dependent DSB recognition element for BRCA1 was recently discovered as the ubiquitin-binding protein Rap80 (Fig. 3). Rap80 binds to the BRCA1 BRCT domain, but fails to associate with clinical BRCT mutants that are deficient in DSB localization. Moreover, Rap80 localization to DSBs is independent of BRCA1 interaction. Rap80 has tandem ubiquitin interaction motifs (UIM) that are essential for its DSB localization. UIM domains are alpha helical structures that can recognize ubiquitin (Harper and Schulman 2006). Conjugated ubiquitin is rapidly generated at DSBs (Morris and Solomon 2004), although it was initially unclear if ubiquitin chain accumulation at breaks was relevant to BRCA1 recruitment. Both UIM domains are essential for Rap80 DSB localization and for targeting BRCA1 to DSBs, suggesting ubiquitin is the DSB targeting signal for BRCA1–Rap80 complexes. In strong

support of this assertion, Rap80 UIM mutants fail to bind ubiquitin and to recognize IR-induced DSBs (Kim et al. 2007a; Sobhian et al. 2007; Wang et al. 2007).

Proteins can be modified by polymers of ubiquitin formed by isopeptide linkages between any of seven different lysine residues and the C-terminal ubiquitin glycine residue. In vivo, all seven different linkages are used with distinct consequences (Peng et al. 2003). Proteins covalently linked to K48-ubiquitin (K48-Ub) chains are targeted for degradation by the proteasome, whereas substrates of K63-linked ubiquitin (K63-Ub) are utilized for signaling events and not proteolytic destruction (Pickart and Fushman 2004). Rap80 demonstrates specificity for the non-K48 linked ubiquitin polymers (Sobhian et al. 2007). The Rap80 UIM domains bind K63-Ub polymers with a preference for ubiquitin chains of four ubiquitin molecules or more. They do not efficiently recognize K48-Ub chains under similar conditions.

The Rap80 UIM domains also recognize proteins tagged in vivo with K6-linked ubiquitin (Sobhian et al. 2007). It will thus be interesting, from a molecular recognition standpoint, to determine the structural basis for Rap80 ubiquitin binding specificity. This in vitro binding specificity for Rap80 appears relevant to DSB recognition in vivo. BRCA1 colocalizes at DSBs with wt, conjugated Ub, and with K6 and K63-Ub. K48-Ub chains were not detected at IRIF. This does not signify that K48-linked ubiquitination events are absent at DNA damage sites; rather that they are simply unlikely to form discernable structures for DSB recognition events (Morris and Solomon 2004; Sobhian et al. 2007).

Depletion of Rap80 greatly reduces BRCA1 IR-induced foci formation in a manner reminiscent of MDC1 and γ H2AX null cells (Kim et al. 2007a; Sobhian et al. 2007; Wang et al. 2007), suggesting Rap80 may be the missing link between epigenetic events involving H2AX phosphorylation on chromatin and recruitment of BRCA1 to higher order structures at breaks. Consistent with this notion, Rap80 is almost completely abolished at DSBs by MDC1 depletion (Sobhian et al. 2007). BRCA1 is recruited to DSBs at similar frequencies in MDC1 and Rap80 depleted cells however its intensity at DSBs is diminished (Celeste et al. 2003; Bekker-Jensen et al. 2006; Sobhian et al. 2007). In MDC1 null cells, BRCA1 is restricted to non-nucleosomal DNA subdomains directly flanking the breaks. Thus, not all BRCA1 complexes are MDC1 or Rap80 dependent for DSB recruitment. Perhaps different regions of BRCA1 provide unique DSB recognition elements for the BACH1, CtIP, and BRCA2 containing complexes.

Independent lines of investigation have provided additional genetic evidence to support that Rap80 targets BRCA1 to K63-Ub at DSBs (Zhao et al. 2007). The Ubc13 E2 enzyme is responsible for K63-Ub synthesis and has an established role in postreplicative repair in conjugation with the Rad5 E3 enzyme in lower eukaryotes. Rad5-Ubc13 modifies a specific PCNA lysine residue with K63-Ub to enhance error-free mechanisms of repair at stalled replication forks (Hoege et al. 2002; Stelter and Ulrich 2003). Vertebrates appear to have commandeered this pathway for repair of DSBs by HR. Ubc13 deficiency reduces HR and inhibits conjugated ubiquitin formation at DSBs and BRCA1 IRIF (Zhao et al. 2007). Several laboratories have recently shown that Ubc13 functions in concert with the Ring Finger 8 (RNF8) E3 ligase to ubiquitinate substrates at DSBs (Huen et al. 2007; Kolas et al. 2007; Mailand et al. 2007).

Both RNF8 and Ubc13 are required for Rap80-mediated BRCA1 DSB localization and are also needed for the G2 checkpoint and prevention of supersensitivity to IR (Fig. 3). As K63-Ub is likely to be a recognition element for only one specific BRCA1 complex containing Rap80, it will be interesting to determine if other BRCA1 complexes are also Ubc13 dependent for DSB localization.

A second important finding is that BRCA1 failed to demonstrate DNA damage-induced E3 ligase activity in cells depleted of Ubc13. These results suggest that BRCA1 E3 activity is

stimulated by recruitment to K63-Ub structures at DSBs. If this hypothesis is correct, Rap80 should also be required for DNA damage-induced BRCA1 E3 activity. Furthermore, Rap80 provides a DSB recognition capacity for the BRCC36 DUB (Sobhian et al. 2007). BRCC36 is capable of K63-Ub hydrolysis, the same type of Ub linkage recognized by Rap80 and synthesized by RNF8-Ubc13. It is tempting to speculate that this may provide a negative feedback system to terminate DNA damage recognition signals at DSBs (Fig. 3). An alternative scenario is that Rap80 directs ubiquitin-remodeling events at DSBs that are required for appropriate DSB repair, by targeting both BRCC36 mediated K63-Ub hydrolysis and BRCA1 E3 ligase activities to DSB sites. Thus, concomitant K63-DUB activity and K6-Ub ligase activity would alter the landscape of ubiquitin on chromatin flanking breaks to influence DSB repair.

The BRCA1-BACH1-TopBP1 complex has been even less definitively assigned to a particular DSB region. This BRCA1 subcomplex contains members of the mismatch repair family MLH1 and MSH2 (Greenberg et al. 2006; Peng et al. 2007). Thus, it is composed of several DNA recognition elements in addition to BACH1 and TopBP1, suggesting it is involved in DNA recognition rather than chromatin-associated events at DSBs.

While recognition of K63-Ub vs DNA appears to explain, at least in part, BRCA1 chromatin vs DNA association at DSBs, there are many unresolved questions regarding the functional consequences of this differential localization. One could reasonably hypothesize that a specific BRCA1 complexes recognizes either non-nucleosomal DNA or chromatin regions. A second equally plausible model predicts the existence of a dynamic equilibrium between chromatin and DNA localization for a given BRCA1 complex. This would necessitate the same BRCA1 complex to be capable of recognizing both chromatin and DNA at breaks. In such a scenario, BRCA1 would track along a gradient of modified chromatin in cis to a DSB to more efficiently target the nonnucleosomal regions for recognition and delivery of repair factors.

In support of the first model, it appears that the BRCA1-CtIP (Sartori et al. 2007) and BRCA1-BRCA2-Rad51 (Bekker-Jensen et al. 2006) complexes localize to DNA regions at DSBs in S and G2 cell cycle phases, while recent evidence links the BRCA1-Rap80 complex to K63-Ub structures on γ H2AX-MDC1 containing chromatin (Kim et al. 2007a; Sobhian et al. 2007; Wang et al. 2007). These data, while compelling, are qualitative and do not precisely define the location of each complex at a DSB. Thus, in the absence of higher resolution data, they do not definitively rule out the possibility that a particular BRCA1 complex can traverse both chromatin and DNA regions of a DSB.

Sophisticated imaging or CHIP studies will be required to determine the mobility of each BRCA1 subcomplex within the vicinity of a DSB. A related question is to what extent each of these complexes plays cooperative vs completely distinct roles in DSB repair. The answers to these questions will have relevance to determining relationships between DSB localization, BRCA1-controlled repair activities, and tumor suppression.

Concluding remarks

While less than half of familial breast cancer is explained by BRCA1 and BRCA2 mutations, a significant portion of inherited cancers is derived from mutation of BRCA1 interacting partners, implicating a BRCA1 network of interactions responsible for tumor suppression (Table 1). Recently, three additional BRCA1-associated proteins BACH1, PalB2 (BRCA1- and BRCA2-associated) and TopBP1 were discovered to contain germline mutations in familial breast cancer pedigrees (Karppinen et al. 2006; Seal et al. 2006; Rahman et al. 2007). Perhaps additional rare alleles that contribute to breast cancer susceptibility remain to be discovered among the many BRCA1-BARD1 interacting partners. By understanding the basic

biology of this genome integrity network, it may be possible to more efficiently search for additional suppressors of breast and ovarian cancer.

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References

- Bassing CH, Chua KF, Sekiguchi J, Suh H, Whitlow SR, Fleming JC, Monroe BC, Ciccone DN, Yan C, Vlasakova K, Livingston DM, Ferguson DO, Scully R, Alt FW. Increased ionizing radiation sensitivity and genomic instability in the absence of histone H2AX. *Proc Natl Acad Sci U S A* 2002;99(12):8173–8178. [PubMed: 12034884]
- Bekker-Jensen S, Lukas C, Kitagawa R, Melander F, Kastan MB, Bartek J, Lukas J. Spatial organization of the mammalian genome surveillance machinery in response to DNA strand breaks. *J Cell Biol* 2006;173(2):195–206. [PubMed: 16618811]
- Berkovich E, Monnat RJ Jr, Kastan MB. Roles of ATM and NBS1 in chromatin structure modulation and DNA double-strand break repair. *Nat Cell Biol* 2007;9(6):683–690. [PubMed: 17486112]
- Bradshaw PS, Stavropoulos DJ, Meyn MS. Human telomeric protein TRF2 associates with genomic double-strand breaks as an early response to DNA damage. *Nat Genet* 2005;37(2):193–197. [PubMed: 15665826]
- Brzovic PS, Meza JE, King MC, Kleivit RE. BRCA1 RING domain cancer-predisposing mutations. Structural consequences and effects on protein–protein interactions. *J Biol Chem* 2001a;276(44):41399–41406. [PubMed: 11526114]
- Brzovic PS, Rajagopal P, Hoyt DW, King MC, Kleivit RE. Structure of a BRCA1–BARD1 heterodimeric RING–RING complex. *Nat Struct Biol* 2001b;8(10):833–837. [PubMed: 11573085]
- Cantor SB, Bell DW, Ganesan S, Kass EM, Drapkin R, Grossman S, Wahrer DCR, Sgroi DC, Lane WS, Haber DA, Livingston DM. BACH1, a novel helicase-like protein, interacts directly with BRCA1 and contributes to its DNA repair function. *Cell* 2001;105:149–160. [PubMed: 11301010]
- Celeste A, Petersen S, Romanienko PJ, Fernandez-Capetillo O, Chen HT, Sedelnikova OA, Reina-San-Martin B, Coppola V, Meffre E, Difilippantonio MJ, Redon C, Pilch DR, Oлару A, Eckhaus M, Camerini-Otero RD, Tessarollo L, Livak F, Manova K, Bonner WM, Nussenzweig MC, Nussenzweig A. Genomic instability in mice lacking histone H2AX. *Science (New York, NY)* 2002;296(5569):922–927.
- Celeste A, Fernandez-Capetillo O, Kruhlak MJ, Pilch DR, Staudt DW, Lee A, Bonner RF, Bonner WM, Nussenzweig A. Histone H2AX phosphorylation is dispensable for the initial recognition of DNA breaks. *Nat Cell Biol* 2003;5(7):675–679. [PubMed: 12792649]
- Chen JJ, Silver D, Walpita D, Cantor SB, Gazdar AF, Tomlinson G, Couch FJ, Weber BL, Ashley T, Livingston DM, Scully R. Stable interaction between the products of the BRCA1 and BRCA2 tumor suppressor genes in mitotic and meiotic cells. *Mol Cell* 1998;2(3):317–328. [PubMed: 9774970]
- Chen L, Nievera C, Lee AY, Wu X. Cell cycle-dependent complex formation of BRCA1/CtIP/MRN is important for DNA double-strand break repair. *J Biol Chem* 2008;283(12):7713–7720. [PubMed: 18171670]
- Consortium. Cancer risks in BRCA2 mutation carriers. The Breast Cancer Linkage Consortium. *J Natl Cancer Inst* 1999;91(15):1310–1316. [PubMed: 10433620]
- Cortez D, Wang Y, Qin J, Elledge SJ. Requirement of ATM-dependent phosphorylation of brca1 in the DNA damage response to double-strand breaks. *Science (New York, NY)* 1999;286(5442):1162–1166.see comments
- Cressman VL, Backlund DC, Avrutskaya AV, Leadon SA, Godfrey V, Koller BH. Growth retardation, DNA repair defects, and lack of spermatogenesis in BRCA1-deficient mice. *Mol Cell Biol* 1999a;19(10):7061–7075. [PubMed: 10490643]

- Cressman VL, Backlund DC, Hicks EM, Gowen LC, Godfrey V, Koller BH. Mammary tumor formation in p53- and BRCA1-deficient mice. *Cell Growth Differ* 1999b;10(1):1–10. [PubMed: 9950212]
- Elledge SJ, Amon A. The BRCA1 suppressor hypothesis: an explanation for the tissue-specific tumor development in BRCA1 patients. *Cancer Cell* 2002;1(2):129–132. [PubMed: 12086871]
- Erkko H, Xia B, Nikkila J, Schleutker J, Syrjakoski K, Mannermaa A, Kallioniemi A, Pylkas K, Karppinen SM, Rapakko K, Miron A, Sheng Q, Li G, Mattila H, Bell DW, Haber DA, Grip M, Reiman M, Jukkola-Vuorinen A, Mustonen A, Kere J, Aaltonen LA, Kosma VM, Kataja V, Soini Y, Drapkin RI, Livingston DM, Winqvist R. A recurrent mutation in PALB2 in Finnish cancer families. *Nature* 2007;446(7133):316–319. [PubMed: 17287723]
- Evers B, Jonkers J. Mouse models of BRCA1 and BRCA2 deficiency: past lessons, current understanding and future prospects. *Oncogene* 2006;25(43):5885–5897. [PubMed: 16998503]
- Falck J, Coates J, Jackson SP. Conserved modes of recruitment of ATM, ATR and DNA-PKcs to sites of DNA damage. *Nature* 2005;434(7033):605–611. [PubMed: 15758953]
- Fernandez-Capetillo O, Chen HT, Celeste A, Ward I, Romanienko PJ, Morales JC, Naka K, Xia Z, Camerini-Otero RD, Motoyama N, Carpenter PB, Bonner WM, Chen J, Nussenzweig A. DNA damage-induced G2-M checkpoint activation by histone H2AX and 53BP1. *Nat Cell Biol* 2002;4(12):993–997. [PubMed: 12447390]
- Frank-Vaillant M, Marcand S. Transient stability of DNA ends allows nonhomologous end joining to precede homologous recombination. *Mol Cell* 2002;10(5):1189–1199. [PubMed: 12453425]
- Furuta T, Takemura H, Liao ZY, Aune GJ, Redon C, Sedelnikova OA, Pilch DR, Rogakou EP, Celeste A, Chen HT, Nussenzweig A, Aladjem MI, Bonner WM, Pommier Y. Phosphorylation of histone H2AX and activation of Mre11, Rad50, and Nbs1 in response to replication-dependent DNA double-strand breaks induced by mammalian DNA topoisomerase I cleavage complexes. *J Biol Chem* 2003;278(22):20303–20312. [PubMed: 12660252]
- Futreal PA, Liu Q, Shattuck-Eidens D, Cochran C, Harshman K, Tavtigian S, Bennett LM, Haugen-Strano A, Swensen J, Miki Y, et al. BRCA1 mutations in primary breast and ovarian carcinomas. *Science (New York, NY)* 1994;266(5182):120–122.
- Goggins M, Schutte M, Lu J, Moskaluk CA, Weinstein CL, Petersen GM, Yeo CJ, Jackson CE, Lynch HT, Hruban RH, Kern SE. Germline BRCA2 gene mutations in patients with apparently sporadic pancreatic carcinomas. *Cancer Res* 1996;56(23):5360–5364. [PubMed: 8968085]
- Goldberg M, Stucki M, Falck J, D'Amours D, Rahman D, Pappin D, Bartek J, Jackson SP. MDC1 is required for the intra-S-phase DNA damage checkpoint. *Nature* 2003;421(6926):952–956. [PubMed: 12607003]
- Gowen LC, Johnson BL, Latour AM, Sulik KK, Koller BH. Brca1 deficiency results in early embryonic lethality characterized by neuroepithelial abnormalities. *Nat Gen* 1996;12(2):191–194.
- Greenberg RA, Sobhian B, Pathania S, Cantor SB, Nakatani Y, Livingston DM. Multifactorial contributions to an acute DNA damage response by BRCA1/BARD1-containing complexes. *Genes Dev* 2006;20(1):34–46. [PubMed: 16391231]
- Guirouilh-Barbat J, Huck S, Bertrand P, Pirzio L, Desmaze C, Sabatier L, Lopez BS. Impact of the KU80 pathway on NHEJ-induced genome rearrangements in mammalian cells. *Mol Cell* 2004;14(5):611–623. [PubMed: 15175156]
- Hakem R, de la Pompa JL, Sirard C, Mo R, Woo M, Hakem A, Wakeham A, Potter J, Reitmaier A, Billia F, Firpo E, Hui CC, Roberts J, Rossant J, Mak TW. The tumor suppressor gene Brca1 is required for embryonic cellular proliferation in the mouse. *Cell* 1996;85(7):1009–1023. [PubMed: 8674108]
- Harper JW, Schulman BA. Structural complexity in ubiquitin recognition. *Cell* 2006;124(6):1133–1136. [PubMed: 16564007]
- Hoege C, Pfander B, Moldovan GL, Pyrowolakis G, Jentsch S. RAD6-dependent DNA repair is linked to modification of PCNA by ubiquitin and SUMO. *Nature* 2002;419(6903):135–141. [PubMed: 12226657]
- Huber LJ, Yang TW, Sarkisian CJ, Master SR, Deng CX, Chodosh LA. Impaired DNA damage response in cells expressing an exon 11-deleted murine Brca1 variant that localizes to nuclear foci. *Mol Cell Biol* 2001;21(12):4005–4015. [PubMed: 11359908]

- Huen MS, Grant R, Manke I, Minn K, Yu X, Yaffe MB, Chen J. RNF8 transduces the DNA-damage signal via histone ubiquitylation and checkpoint protein assembly. *Cell* 2007;131:901–914. [PubMed: 18001825]
- Ira G, Malkova A, Liberi G, Foiani M, Haber JE. Srs2 and Sgs1-Top3 suppress crossovers during double-strand break repair in yeast. *Cell* 2003;115(4):401–411. [PubMed: 14622595]
- Ira G, Pellicioli A, Balijja A, Wang X, Fiorani S, Carotenuto W, Liberi G, Bressan D, Wan L, Hollingsworth NM, Haber JE, Foiani M. DNA end resection, homologous recombination and DNA damage checkpoint activation require CDK1. *Nature* 2004;431(7011):1011–1017. [PubMed: 15496928]
- Jasin M. Homologous repair of DNA damage and tumorigenesis: the BRCA connection. *Oncogene* 2002;21(58):8981–8993. [PubMed: 12483514]
- Jazaeri AA, Yee CJ, Sotiriou C, Brantley KR, Boyd J, Liu ET. Gene expression profiles of BRCA1-linked, BRCA2-linked, and sporadic ovarian cancers. *J Natl Cancer Inst* 2002;94(13):990–1000. [PubMed: 12096084]
- Joukov V, Chen J, Fox EA, Green JB, Livingston DM. Functional communication between endogenous BRCA1 and its partner, BARD1, during *Xenopus laevis* development. *Proc Natl Acad Sci U S A* 2001;98(21):12078–12083. [PubMed: 11593018]
- Kang J, Bronson RT, Xu Y. Targeted disruption of NBS1 reveals its roles in mouse development and DNA repair. *EMBO J* 2002;21(6):1447–1455. [PubMed: 11889050]
- Karpinen SM, Erkkö H, Reini K, Pospiech H, Heikkinen K, Rapakko K, Syvaöja JE, Winqvist R. Identification of a common polymorphism in the TopBP1 gene associated with hereditary susceptibility to breast and ovarian cancer. *Eur J Cancer* 2006;42(15):2647–2652. [PubMed: 16930991]
- Kim H, Chen J, Yu X. Ubiquitin-binding protein RAP80 mediates BRCA1-dependent DNA damage response. *Science* 2007a;316(5828):1202–1205. [PubMed: 17525342]
- Kim H, Huang J, Chen J. CCDC98 is a BRCA1-BRCT domain-binding protein involved in the DNA damage response. *Nat Struct Mol Biol* 2007b;14(8):710–715. [PubMed: 17643122]
- King MC, Marks JH, Mandell JB. Breast and ovarian cancer risks due to inherited mutations in BRCA1 and BRCA2. *Science* 2003;302(5645):643–646. [PubMed: 14576434]
- Kitagawa R, Bakkenist CJ, McKinnon PJ, Kastan MB. Phosphorylation of SMC1 is a critical downstream event in the ATM-NBS1-BRCA1 pathway. *Genes Dev* 2004;18(12):1423–1438. [PubMed: 15175241]
- Kolas NK, Chapman JR, Nakada S, Ylanko J, Chahwan R, Sweeney FD, Panier S, Mendez M, Wildenhain J, Thomson TM, Pelletier L, Jackson SP, Durocher D. Orchestration of the DNA-damage response by the RNF8 ubiquitin ligase. *Science* 2007;318:1637–1640. [PubMed: 18006705]
- Kruhlak MJ, Celeste A, Dellaire G, Fernandez-Capetillo O, Muller WG, McNally JG, Bazett-Jones DP, Nussenzweig A. Changes in chromatin structure and mobility in living cells at sites of DNA double-strand breaks. *J Cell Biol* 2006;172(6):823–834. [PubMed: 16520385]
- Lee JS, Collins KM, Brown AL, Lee CH, Chung JH. hCds1-mediated phosphorylation of BRCA1 regulates the DNA damage response. *Nature* 2000;404(6774):201–204. [PubMed: 10724175]
- Lieber MR, Ma Y, Pannicke U, Schwarz K. Mechanism and regulation of human non-homologous DNA end-joining. *Nat Rev* 2003;4(9):712–720.
- Lisby M, Barlow JH, Burgess RC, Rothstein R. Choreography of the DNA damage response: spatiotemporal relationships among checkpoint and repair proteins. *Cell* 2004;118(6):699–713. [PubMed: 15369670]
- Liu CY, Flesken-Nikitin A, Li S, Zeng Y, Lee WH. Inactivation of the mouse Brca1 gene leads to failure in the morphogenesis of the egg cylinder in early postimplantation development. *Genes Dev* 1996;10(14):1835–1843. [PubMed: 8698242]
- Liu Z, Wu J, Yu X. CCDC98 targets BRCA1 to DNA damage sites. *Nat Struct Mol Biol* 2007;14(8):716–720. [PubMed: 17643121]
- Longo JA, Nevaldine B, Longo SL, Winfield JA, Hahn PJ. An assay for quantifying DNA double-strand break repair that is suitable for small numbers of unlabeled cells. *Radiat Res* 1997;147(1):35–40. [PubMed: 8989367]

- Lou Z, Minter-Dykhouse K, Franco S, Gostissa M, Rivera MA, Celeste A, Manis JP, van Deursen J, Nussenzweig A, Paull TT, Alt FW, Chen J. MDC1 maintains genomic stability by participating in the amplification of ATM-dependent DNA damage signals. *Mol Cell* 2006;21(2):187–200. [PubMed: 16427009]
- Ludwig T, Fisher P, Ganesan S, Efstratiadis A. Tumorigenesis in mice carrying a truncating Brca1 mutation. *Genes Dev* 2001;15(10):1188–1193. [PubMed: 11358863]
- Lukas C, Falck J, Bartkova J, Bartek J, Lukas J. Distinct spatiotemporal dynamics of mammalian checkpoint regulators induced by DNA damage. *Nat Cell Biol* 2003;5(3):255–260. [PubMed: 12598907]
- Lukas C, Melander F, Stucki M, Falck J, Bekker-Jensen S, Goldberg M, Lerenthal Y, Jackson SP, Bartek J, Lukas J. Mdc1 couples DNA double-strand break recognition by Nbs1 with its H2AX-dependent chromatin retention. *EMBO J* 2004;23(13):2674–2683. [PubMed: 15201865]
- Luo G, Yao MS, Bender CF, Mills M, Bladl AR, Bradley A, Petrini JH. Disruption of mRad50 causes embryonic stem cell lethality, abnormal embryonic development, and sensitivity to ionizing radiation. *Proc Nat Acad Sci U S A* 1999;96(13):7376–7381.
- Mailand N, Bekker-Jensen S, Fastrup H, Melander F, Bartek J, Lukas C, Lukas J. RNF8 ubiquitylates histones at DNA double-strand breaks and promotes assembly of repair proteins. *Cell* 2007;131(5):887–900. [PubMed: 18001824]
- Manke IA, Lowery DM, Nguyen A, Yaffe MB. BRCT repeats as phosphopeptide-binding modules involved in protein targeting. *Science* 2003;302(5645):636–639. [PubMed: 14576432]
- Martin RW, Orelli BJ, Yamazoe M, Minn AJ, Takeda S, Bishop DK. RAD51 up-regulation bypasses BRCA1 function and is a common feature of BRCA1-deficient breast tumors. *Cancer Res* 2007;67(20):9658–9665. [PubMed: 17942895]
- Matsuoka S, Ballif BA, Smogorzewska A, McDonald ER III, Hurov KE, Luo J, Bakalarski CE, Zhao Z, Solimini N, Lerenthal Y, Shiloh Y, Gygi SP, Elledge SJ. ATM and ATR substrate analysis reveals extensive protein networks responsive to DNA damage. *Science* 2007;316(5828):1160–1166. [PubMed: 17525332]
- McCarthy EE, Celebi JT, Baer R, Ludwig T. Loss of Bard1, the heterodimeric partner of the Brca1 tumor suppressor, results in early embryonic lethality and chromosomal instability. *Mol Cell Biol* 2003;23(14):5056–5063. [PubMed: 12832489]
- Miki Y, Swensen J, Shattuck-Eidens D, Futreal PA, Harshman K, Tavtigian S, Liu Q, Cochran C, Bennett LM, Ding W, et al. A strong candidate for the breast and ovarian cancer susceptibility gene BRCA1. *Science* 1994;266(5182):66–71. [PubMed: 7545954]
- Morris JR, Solomon E. BRCA1: BARD1 induces the formation of conjugated ubiquitin structures, dependent on K6 of ubiquitin, in cells during DNA replication and repair. *Hum Mol Genet* 2004;13(8):807–817. [PubMed: 14976165]
- Moynahan ME, Chiu JW, Koller BH, Jasin M. Brca1 controls homology-directed DNA repair. *Mol Cell* 1999;4(4):511–518. [PubMed: 10549283]
- Moynahan ME, Pierce AJ, Jasin M. BRCA2 is required for homology-directed repair of chromosomal breaks. *Mol Cell* 2001;7(2):263–272. [PubMed: 11239455]
- Myung K, Chen C, Kolodner RD. Multiple pathways cooperate in the suppression of genome instability in *Saccharomyces cerevisiae*. *Nature* 2001;411(6841):1073–1076. [PubMed: 11429610]
- Nathanson KL, Wooster R, Weber BL. Breast cancer genetics: what we know and what we need. *Nat Med* 2001;7(5):552–556. [PubMed: 11329055]
- Nevaldine B, Longo JA, Hahn PJ. The scid defect results in much slower repair of DNA double-strand breaks but not high levels of residual breaks. *Radiat Res* 1997;147(5):535–540. [PubMed: 9146698]
- Osborn AJ, Elledge SJ, Zou L. Checking on the fork: the DNA-replication stress-response pathway. *Trends Cell Biol* 2002;12(11):509–516. [PubMed: 12446112]
- Peng J, Schwartz D, Elias JE, Thoreen CC, Cheng D, Marsischky G, Roelofs J, Finley D, Gygi SP. A proteomics approach to understanding protein ubiquitination. *Nat Biotechnol* 2003;21(8):921–926. [PubMed: 12872131]
- Peng M, Litman R, Xie J, Sharma S, Brosh RM Jr, Cantor SB. The FANCI/MutLalpha interaction is required for correction of the cross-link response in FA-J cells. *EMBO J* 2007;26(13):3238–3249. [PubMed: 17581638]

- Pickart CM, Fushman D. Polyubiquitin chains: polymeric protein signals. *Curr Opin Chem Biol* 2004;8(6):610–616. [PubMed: 15556404]
- Pierce AJ, Hu P, Han M, Ellis N, Jasin M. Ku DNA end-binding protein modulates homologous repair of double-strand breaks in mammalian cells. *Genes Dev* 2001;15(24):3237–3242. [PubMed: 11751629]
- Polanowska J, Martin JS, Garcia-Muse T, Pcorin MI, Boulton SJ. A conserved pathway to activate BRCA1-dependent ubiquitylation at DNA damage sites. *EMBO J* 2006;25(10):2178–2188. [PubMed: 16628214]
- Potts PR, Porteus MH, Yu H. Human SMC5/6 complex promotes sister chromatid homologous recombination by recruiting the SMC1/3 cohesin complex to double-strand breaks. *EMBO J* 2006;25(14):3377–3388. [PubMed: 16810316]
- Rahman N, Seal S, Thompson D, Kelly P, Renwick A, Elliott A, Reid S, Spanova K, Barfoot R, Chagtai T, Jayatilake H, McGuffog L, Hanks S, Evans DG, Eccles D, Easton DF, Stratton MR. PALB2, which encodes a BRCA2-interacting protein, is a breast cancer susceptibility gene. *Nat Genet* 2007;39(2):165–167. [PubMed: 17200668]
- Rebbeck TR, Levin AM, Eisen A, Snyder C, Watson P, Cannon-Albright L, Isaacs C, Olopade O, Garber JE, Godwin AK, Daly MB, Narod SA, Neuhausen SL, Lynch HT, Weber BL. Breast cancer risk after bilateral prophylactic oophorectomy in BRCA1 mutation carriers. *J Natl Cancer Inst* 1999;91(17):1475–1479. [PubMed: 10469748]
- Rebbeck TR, Lynch HT, Neuhausen SL, Narod SA, Van't Veer L, Garber JE, Evans G, Isaacs C, Daly MB, Matloff E, Olopade OI, Weber BL. Prophylactic oophorectomy in carriers of BRCA1 or BRCA2 mutations. *N Engl J Med* 2002;346(21):1616–1622. [PubMed: 12023993]
- Rodrigue A, Lafrance M, Gauthier MC, McDonald D, Hendzel M, West SC, Jasin M, Masson JY. Interplay between human DNA repair proteins at a unique double-strand break in vivo. *EMBO J* 2006;25(1):222–231. [PubMed: 16395335]
- Rogakou EP, Pilch DR, Orr AH, Ivanova VS, Bonner WM. DNA double-stranded breaks induce histone H2AX phosphorylation on serine 139. *J Biol Chem* 1998;273(10):5858–5868. [PubMed: 9488723]
- Rogakou EP, Boon C, Redon C, Bonner WM. Megabase chromatin domains involved in DNA double-strand breaks in vivo. *J Cell Biol* 1999;146(5):905–916. [PubMed: 10477747]
- Sartori AA, Lukas C, Coates J, Mistrik M, Fu S, Bartek J, Baer R, Lukas J, Jackson SP. Human CtIP promotes DNA end resection. *Nature* 2007;450(7169):509–514. [PubMed: 17965729]
- Scully R, Livingston D. In search of the tumour-suppressor functions of BRCA1 and BRCA2. *Nature* 2000;408:429–432. [PubMed: 11100717]
- Scully R, Chen J, Ochs RL, Keegan K, Hoekstra M, Feunteun J, Livingston DM. Dynamic changes of BRCA1 subnuclear location and phosphorylation state are initiated by DNA damage. *Cell* 1997a;90(3):425–435. [PubMed: 9267023]
- Scully R, Chen J, Plug A, Xiao Y, Weaver D, Feunteun J, Ashley T, Livingston DM. Association of BRCA1 with Rad51 in mitotic and meiotic cells. *Cell* 1997b;88(2):265–275. [PubMed: 9008167]
- Scully R, Ganesan S, Vlasakova K, Chen J, Socolovsky M, Livingston DM. Genetic analysis of BRCA1 function in a defined tumor cell line. *Mol Cell* 1999;4(6):1093–1099. [PubMed: 10635334]
- Seal S, Thompson D, Renwick A, Elliott A, Kelly P, Barfoot R, Chagtai T, Jayatilake H, Ahmed M, Spanova K, North B, McGuffog L, Evans DG, Eccles D, Easton DF, Stratton MR, Rahman N. Truncating mutations in the Fanconi anemia J gene BRIP1 are low-penetrance breast cancer susceptibility alleles. *Nat Genet* 2006;38(11):1239–1241. [PubMed: 17033622]
- Sharan SK, Morimatsu M, Albrecht U, Lim DS, Regel E, Dinh C, Sands A, Eichele G, Hasty P, Bradley A. Embryonic lethality and radiation hypersensitivity mediated by Rad51 in mice lacking Brca2. *Nature* 1997;386(6627):804–810. [PubMed: 9126738]
- Shroff R, Arbel-Eden A, Pilch D, Ira G, Bonner WM, Petrini JH, Haber JE, Lichten M. Distribution and dynamics of chromatin modification induced by a defined DNA double-strand break. *Curr Biol* 2004;14(19):1703–1711. [PubMed: 15458641]
- Snouwaert JN, Gowen LC, Latour AM, Mohn AR, Xiao A, DiBiase L, Koller BH. BRCA1 deficient embryonic stem cells display a decreased homologous recombination frequency and an increased frequency of non-homologous recombination that is corrected by expression of a brca1 transgene. *Oncogene* 1999;18(55):7900–7907. [PubMed: 10630642]

- Sobhian B, Shao G, Lilli DR, Culhane AC, Moreau LA, Xia B, Livingston DM, Greenberg RA. RAP80 targets BRCA1 to specific ubiquitin structures at DNA damage sites. *Science* 2007;316(5828):1198–1202. [PubMed: 17525341]
- Sorlie T, Tibshirani R, Parker J, Hastie T, Marron JS, Nobel A, Deng S, Johnsen H, Pesich R, Geisler S, Demeter J, Perou CM, Lonning PE, Brown PO, Borresen-Dale AL, Botstein D. Repeated observation of breast tumor subtypes in independent gene expression data sets. *Proc Natl Acad Sci U S A* 2003;100(14):8418–8423. [PubMed: 12829800]
- Stelter P, Ulrich HD. Control of spontaneous and damage-induced mutagenesis by SUMO and ubiquitin conjugation. *Nature* 2003;425(6954):188–191. [PubMed: 12968183]
- Stewart GS, Wang B, Bignell CR, Taylor AM, Elledge SJ. MDC1 is a mediator of the mammalian DNA damage checkpoint. *Nature* 2003;421(6926):961–966. [PubMed: 12607005]
- Stiff T, O'Driscoll M, Rief N, Iwabuchi K, Lobrich M, Jeggo PA. ATM and DNA-PK function redundantly to phosphorylate H2AX after exposure to ionizing radiation. *Cancer Res* 2004;64(7):2390–2396. [PubMed: 15059890]
- Stucki M, Clapperton JA, Mohammad D, Yaffe MB, Smerdon SJ, Jackson SP. MDC1 directly binds phosphorylated histone H2AX to regulate cellular responses to DNA double-strand breaks. *Cell* 2005;123(7):1213–1226. [PubMed: 16377563]
- Sung PA, Libura J, Richardson C. Etoposide and illegitimate DNA double-strand break repair in the generation of MLL translocations: new insights and new questions. *DNA Repair* 2006;5(9–10):1109–1118. [PubMed: 16809075]
- Thai TH, Du F, Tsan JT, Jin Y, Phung A, Spillman MA, Massa HF, Muller CY, Ashfaq R, Mathis JM, Miller DS, Trask BJ, Baer R, Bowcock AM. Mutations in the BRCA1-associated RING domain (BARD1) gene in primary breast, ovarian and uterine cancers. *Hum Mol Genet* 1998;7(2):195–202. [PubMed: 9425226]
- Tibbetts RS, Cortez D, Brumbaugh KM, Scully R, Livingston D, Elledge SJ, Abraham RT. Functional interactions between BRCA1 and the checkpoint kinase ATR during genotoxic stress. *Genes Dev* 2000;14(23):2989–3002. [PubMed: 11114888]
- Unal E, Arbel-Eden A, Sattler U, Shroff R, Lichten M, Haber JE, Koshland D. DNA damage response pathway uses histone modification to assemble a double-strand break-specific cohesin domain. *Mol Cell* 2004;16(6):991–1002. [PubMed: 15610741]
- van Heemst D, Brugmans L, Verkaik NS, van Gent DC. End-joining of blunt DNA double-strand breaks in mammalian fibroblasts is precise and requires DNA-PK and XRCC4. *DNA Repair* 2004;3(1):43–50. [PubMed: 14697758]
- Venkitaraman AR. Cancer susceptibility and the functions of BRCA1 and BRCA2. *Cell* 2002;108(2):171–182. [PubMed: 11832208]
- Walsh T, King MC. Ten genes for inherited breast cancer. *Cancer Cell* 2007;11(2):103–105. [PubMed: 17292821]
- Wang Y, Cortez D, Yazdi P, Neff N, Elledge SJ, Qin J. BASC, a super complex of BRCA1-associated proteins involved in the recognition and repair of aberrant DNA structures. *Genes Dev* 2000;14(8):927–939. [PubMed: 10783165]
- Wang B, Matsuoka S, Ballif BA, Zhang D, Smogorzewska A, Gygi SP, Elledge SJ. Abraxas and RAP80 form a BRCA1 protein complex required for the DNA damage response. *Science* 2007;316(5828):1194–1198. [PubMed: 17525340]
- Ward IM, Chen J. Histone H2AX is phosphorylated in an ATR-dependent manner in response to replicational stress. *J Biol Chem* 2001;276(51):47759–47762. [PubMed: 11673449]
- Weinstock DM, Jasin M. Alternative pathways for the repair of RAG-induced DNA breaks. *Mol Cell Biol* 2006;26(1):131–139. [PubMed: 16354685]
- Weinstock DM, Richardson CA, Elliott B, Jasin M. Modeling oncogenic translocations: distinct roles for double-strand break repair pathways in translocation formation in mammalian cells. *DNA Repair* 2006;5(9–10):1065–1074. [PubMed: 16815104]
- White MA, Nicolette C, Minden A, Polverino A, Van Aelst L, Karin M, Wigler MH. Multiple Ras functions can contribute to mammalian cell transformation. *Cell* 1995;80(4):533–541. [PubMed: 7867061]

- Williams RS, Green R, Glover JN. Crystal structure of the BRCT repeat region from the breast cancer-associated protein BRCA1. *Nat Struct Biol* 2001;8(10):838–842. [PubMed: 11573086]
- Wong AK, Ormonde PA, Pero R, Chen Y, Lian L, Salada G, Berry S, Lawrence Q, Dayananth P, Ha P, Tavtigian SV, Teng DH, Bartel PL. Characterization of a carboxy-terminal BRCA1 interacting protein. *Oncogene* 1998;17(18):2279–2285. [PubMed: 9811458]
- Wooster R, Neuhausen SL, Mangion J, Quirk Y, Ford D, Collins N, Nguyen K, Seal S, Tran T, Averill D, et al. Localization of a breast cancer susceptibility gene, BRCA2, to chromosome 13q12–13. *Science* 1994;265(5181):2088–2090. [PubMed: 8091231]
- Wu L, Hickson ID. The Bloom's syndrome helicase suppresses crossing over during homologous recombination. *Nature* 2003;426(6968):870–874. [PubMed: 14685245]
- Wu LC, Wang ZW, Tsan JT, Spillman MA, Phung A, Xu XL, Yang MC, Hwang LY, Bowcock AM, Baer R. Identification of a RING protein that can interact in vivo with the BRCA1 gene product. *Nat Genet* 1996;14(4):430–440. [PubMed: 8944023]
- Xia Y, Pao GM, Chen HW, Verma IM, Hunter T. Enhancement of BRCA1 E3 ubiquitin ligase activity through direct interaction with the BARD1 protein. *J Biol Chem* 2003;278(7):5255–5263. [PubMed: 12431996]
- Xia B, Sheng Q, Nakanishi K, Ohashi A, Wu J, Christ N, Liu X, Jasin M, Couch FJ, Livingston DM. Control of BRCA2 cellular and clinical functions by a nuclear partner, PALB2. *Mol Cell* 2006;22(6):719–729. [PubMed: 16793542]
- Xia B, Dorsman JC, Ameziane N, de Vries Y, Rooimans MA, Sheng Q, Pals G, Errami A, Gluckman E, Llera J, Wang W, Livingston DM, Joenje H, de Winter JP. Fanconi anemia is associated with a defect in the BRCA2 partner PALB2. *Nat Genet* 2007;39(2):159–161. [PubMed: 17200672]
- Xie A, Puget N, Shim I, Odate S, Jarzyna I, Bassing CH, Alt FW, Scully R. Control of sister chromatid recombination by histone H2AX. *Mol Cell* 2004;16(6):1017–1025. [PubMed: 15610743]
- Xu X, Weaver Z, Linke SP, Li C, Gotay J, Wang XW, Harris CC, Ried T, Deng CX. Centrosome amplification and a defective G2-M cell cycle checkpoint induce genetic instability in BRCA1 exon 11 isoform-deficient cells. *Mol Cell* 1999;3(3):389–395. [PubMed: 10198641]
- Xu B, Kim S, Kastan MB. Involvement of Brca1 in S-phase and G(2)-phase checkpoints after ionizing irradiation. *Mol Cell Biol* 2001a;21(10):3445–3450. [PubMed: 11313470]
- Xu X, Qiao W, Linke SP, Cao L, Li WM, Furth PA, Harris CC, Deng CX. Genetic interactions between tumor suppressors Brca1 and p53 in apoptosis, cell cycle and tumorigenesis. *Nat Genet* 2001b;28(3):266–271. [PubMed: 11431698]
- Xu B, Kim ST, Lim DS, Kastan MB. Two molecularly distinct G(2)/M checkpoints are induced by ionizing irradiation. *Mol Cell Biol* 2002;22(4):1049–1059. [PubMed: 11809797]
- Yang H, Jeffrey PD, Miller J, Kinnucan E, Sun Y, Thoma NH, Zheng N, Chen PL, Lee WH, Pavletich NP. BRCA2 function in DNA binding and recombination from a BRCA2-DSS1-ssDNA structure. *Science* 2002;297(5588):1837–1848. [PubMed: 12228710]
- Yang H, Li Q, Fan J, Holloman WK, Pavletich NP. The BRCA2 homologue Brh2 nucleates RAD51 filament formation at a dsDNA–ssDNA junction. *Nature* 2005;433(7026):653–657. [PubMed: 15703751]
- Yu X, Wu LC, Bowcock AM, Aronheim A, Baer R. The C-terminal (BRCT) domains of BRCA1 interact in vivo with CtIP, a protein implicated in the CtBP pathway of transcriptional repression. *J Biol Chem* 1998;273(39):25388–25392. [PubMed: 9738006]
- Yu X, Chini CC, He M, Mer G, Chen J. The BRCT domain is a phospho-protein binding domain. *Science* 2003;302(5645):639–642. [PubMed: 14576433]
- Yu X, Fu S, Lai M, Baer R, Chen J. BRCA1 ubiquitinates its phosphorylation-dependent binding partner CtIP. *Genes Dev* 2006;20(13):1721–1726. [PubMed: 16818604]
- Zhao GY, Sonoda E, Barber LJ, Oka H, Murakawa Y, Yamada K, Ikura T, Wang X, Kobayashi M, Yamamoto K, Boulton SJ, Takeda S. A critical role for the ubiquitin-conjugating enzyme Ubc13 in initiating homologous recombination. *Mol Cell* 2007;25(5):663–675. [PubMed: 17349954]
- Zhuang J, Zhang J, Willers H, Wang H, Chung JH, van Gent DC, Hallahan DE, Powell SN, Xia F. Checkpoint kinase 2-mediated phosphorylation of BRCA1 regulates the fidelity of nonhomologous end-joining. *Cancer Res* 2006;66(3):1401–1408. [PubMed: 16452195]

- Zou L, Elledge SJ. Sensing DNA damage through ATRIP recognition of RPA-ssDNA complexes. *Science* 2003;300(5625):1542–1548. [PubMed: 12791985]
- Zou L, Liu D, Elledge SJ. Replication protein A-mediated recruitment and activation of Rad17 complexes. *Proc Natl Acad Sci U S A* 2003;100(24):13827–13832. [PubMed: 14605214]

Fig. 1.

Models for DSB repair and for recognition of different DSB regions by repair proteins. **a** DNA DSBs can be repaired by either homologous recombination or non-homologous end joining mechanisms. Homologous recombination proceeds by a series of depicted DNA processing events to allow repair of a DSB by copying of the homologous region on the sister chromatid. Non-homologous end joining can repair DSBs by ligating contiguous (*red* or *blue*) stretches of DNA or non-contiguous regions (*red* and *blue*), resulting in chromosomal translocations. **b** Several different regions exist at the site of a DNA DSB. Nucleosomal clearing occurs directly adjacent to the DSB, followed by long stretches of chromatin modifications characterized by γ H2AX-MDC1. BRCA1-BARD1 heterodimers accumulate at both the DNA

and chromatin regions at DSB sites. BRCA1-BARD1 DSB chromatin accumulation is dependent on γ H2AX-MDC1. Ubiquitin chains are also abundant at chromatin regions flanking DSBs

Fig. 2.

The BRCA1 tumor suppressor network. **a** Domain structure of the BRCA1-BARD1 heterodimer. Characteristic domains for BRCA1 include the amino terminal RING domain, Exon 11 region, and the BRCT domains. Loss of function, cancer predisposing mutations occur in each of these domains. **b** BRCA1 supercomplexes displaying characteristic, unique binding partners. A schematic is shown that displays DSB recognition elements and repair activities for each BRCA1-BARD1 containing complex in the DNA damage response

Fig. 3.

Model for the role of ubiquitin dynamics in BRCA1-directed DSB repair. RNF8-Ubc13 synthesizes K63-Ub on chromatin-associated substrates at DSB sites in a manner dependent on γ H2AX-MDC1. Rap80 binds DSB-associated K63-Ub to target the BRCA1-BARD1 E3 Ub ligase and the BRCC36 DUB to DSBs. RNF8-Ubc13-dependent ubiquitination is also required for 53BP1 DSB recruitment. These opposing Ub synthesizing and degrading activities by RNF8-Ubc13 and Rap80-BRCC36, respectively, may alter ubiquitination events and chromatin structure to influence appropriate DSB repair. Alternatively, Rap80-BRCC36 DUB activity may represent a negative feedback mechanism to terminate RNF8-Ubc13 recruitment signals

Table 1

The BRCA1 tumor suppressor network in breast cancer

Gene	Associated malignancy
BRCA1	Breast/Ovarian
BRCA2	Breast/Ovarian, Prostate, Fanconi Anemia D1
Brip1	Breast Cancer, Fanconi Anemia J
PalB2	Breast Cancer, Fanconi Anemia N
TopBP1	Breast/Ovarian
MLH1, MSH2, MSH6	HNPCC, Ovarian Cancer
Blms Helicase	Breast Cancer, Blooms Syndrome
Mre11/NBS1	Breast Cancer, ATL-D/NBS syndromes
ATM	Breast Cancer, Ataxia Telangiectasia
CHK2	Breast Cancer

Genes encoding BRCA1-associated proteins in inherited cancer syndromes. A list of genes encoding validated BRCA1-associated proteins (Wang et al. 2000; Greenberg et al. 2006) and the cancer predisposition syndromes associated with mutation of each gene. It is proposed that loss of function mutations in any of these genes predisposes to malignancy, at least in part, by disrupting the function of specific elements of BRCA1-dependent DNA damage responses.