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PERSPECTIVE

Cancer Suppression by the Chromosome Custodians, BRCA1 and BRCA2

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Germline mutations in *BRCA1* and *BRCA2* predispose to common human malignancies, most notably tumors of the breast and ovaries. The proteins encoded by these genes have been implicated in a plethora of biochemical interactions and biological functions, confounding attempts to coherently explain how their inactivation promotes carcinogenesis. Here, I argue that tumor suppression by BRCA1 and BRCA2 originates from their fundamental role in controlling the assembly and activity of macromolecular complexes that monitor chromosome duplication, maintenance, and segregation across the cell cycle. A tumor-suppressive role for the BRCA proteins as "chromosome custodians" helps to explain the clinical features of cancer susceptibility after their inactivation, provides foundations for the rational therapy of BRCA-deficient cancers, and offers general insights into the mechanisms opposing early steps in human carcinogenesis.

The landmark discovery that germline mutations affecting *BRCA1* or *BRCA2* trigger inherited susceptibility at high penetrance to cancers of the breast and other organs sparked intensive investigations into the mechanisms by which their protein products, localized primarily in the cell nucleus, work as cancer suppressors. Over the past 20 years, however, these studies have unearthed many physical and functional connections made by the BRCA proteins in diverse biological processes whose links to cancer pathogenesis remain uncertain. I argue here that the role of BRCA1 and BRCA2 as custodians of the structural and numerical integrity of chromosomes during the cell cycle underlies their tumor-suppressive function. I will discuss how this conceptual framework helps to explain the clinical features of cancer susceptibility in *BRCA* mutation carriers, reveals principles underlying new approaches for treatment, and offers a powerful experimental paradigm for understanding how chromosomal instability (*1*) contributes to human carcinogenesis.

Tumor Suppression and the Functions of BRCA1 and BRCA2

Discovery of an Essential Role in Chromosome Integrity

Key studies in the first few years after the discovery of *BRCA1* and *BRCA2*, which defined their essential function in preserving chromosome integrity during cell division, have been instrumental in guiding subsequent work. Targeted disruption of both copies of *BRCA1* or *BRCA2* in the mouse germ line was shown to provoke early embryonal lethality and impede cell proliferation (*2*–*7*). This is accompanied by hypersensitivity to genotoxins (*4*–*6*, *8*), consistent with the migration of BRCA1 and BRCA2 proteins (Fig. 1) to nuclear foci triggered by DNA damage (*9*, *10*), and their interaction with different proteins implicated in the cellular response to such lesions (*4*, *11*–*13*). It is remarkable that BRCA2-deficient cells spontaneously accumulate aberrations in chromosome structure and number during division (*6*). The structural aberrations typically include breaks affecting a single sister chromatid, as well as quadriradial and triradial chromosomes. Both types of abnormality signify defects in homologous DNA recombination and are also characteristic of two other cancer susceptibility syndromes, Bloom syndrome and Fanconi anemia (*6*). BRCA2-deficient cells exhibit translocations, large deletions, or fusions that involve multiple, nonhomologous chromosomes (*14*). These structural anomalies are accompanied by aberrations in chromosome number reflecting inaccurate chromosome segregation (*6*). Cells lacking BRCA1 exhibit similar defects (*15*). Collectively, these findings establish that BRCA1 and BRCA2 act as custodians of chromosome integrity during the cell cycle, in turn engendering a model (*16*, *17*) wherein *BRCA* inactivation fosters carcinogenesis by promoting chromosomal instability.

Protein "Hubs" Protecting Chromosome Integrity

The precise mechanisms by which BRCA1 and BRCA2 protect chromosome integrity during the cell cycle remain unclear. Notable confounding factors include the number and diversity of proteins that have been reported to physically interact with BRCA1 and BRCA2 (fig. S1), the localization of BRCA1 and BRCA2 to different intracellular compartments and structures during the cell cycle, and the shifting nature of these properties in response to cellular signals that trigger posttranslational modifications, such as phosphorylation or ubiquitylation. These features suggest that BRCA1 and BRCA2 may belong to a small subset of proteins that serve as dynamic "hubs" for multiple macromolecular complexes. Hub proteins typically contain one or more intrinsically disordered regions, which lack a defined three-dimensional structure in isolation but, instead, tend to acquire more stable conformations when they bind to other macromolecules (*18*). For instance, the 1863-residue human BRCA1 protein encodes a structured RING domain at its extreme amino (N) terminus and tandem BRCT domains at its carboxyl (C) terminus, but the long central region between residues 170 and 1649 is predicted to exhibit intrinsic disorder by in silico and experimental results (www.disprot.org) (*19*). Such analyses also suggest that the 3418-residue human BRCA2 protein likewise contains intrinsically disordered regions dispersed between more structured segments (www.disprot.org) (*20*, *21*). Some of these structured segments or motifs (for example, the RING or BRCT domains in BRCA1 and the BRC repeats and OB folds in BRCA2) (Fig. 1) also occur in proteins from simpler organisms with overlapping functions. However, these simpler proteins are typically smaller and less complex, and their functions are likely more limited than those of the corresponding BRCA protein, as illustrated by the BRCA2 ortholog Brh2 from *Ustilago maydis* (*22*).

These considerations suggest that the large BRCA1 and BRCA2 proteins act as segmental entities, in which distinct and sometimes intrinsically disordered regions enter into different physical interactions to perform distinct biological functions. In this way, the BRCA proteins may subsume and coordinate across the cell cycle the work performed by multiple protein complexes in simpler organisms. Not all of these functions are necessarily relevant to tumor suppression. Moreover, overexpression or cell-free biochemical studies on hub proteins like BRCA1 and BRCA2 may elicit false clues owing to the likelihood of promiscuous interactivity in such experimental settings. From this perspective, the phenotypes provoked by BRCA1 or BRCA2 deficiency in cells, model organisms, and patients provide a

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valuable yardstick against which to measure functional understanding.

Distinct BRCA1 and BRCA2 Functions Prevent Chromosome Structural Aberrations

The characteristic structural aberrations that accumulate spontaneously during the division of BRCA-deficient cells focus attention on double-strand DNA breaks (DSBs) or daughter-strand gaps (DSGs) created during chromosome duplication [reviewed in (*23*)]. DNA replication forks frequently stall at lesions in the template strands even during normal cell proliferation. Homologous DNA recombination (HR)—a mechanism for the error-free repair of DNA breakage by exchange with an intact, homologous sequence (such as the sister chromatid)—is central to the resolution of stalled forks. Stalled forks may be cleaved to generate a DSB that can be repaired by HR. Alternatively, a replication bypass around the arrested fork, leaving behind the arrest-inducing lesion, may create a DSG that can be resolved by HR without a DSB intermediate. BRCA1 and BRCA2 are individually essential for efficient HR in mammalian cells (*24*–*28*). In their absence, replication-associated DNA breaks can instead be repaired by error-prone mechanisms like nonhomologous end joining (NHEJ) and microhomology-mediated end joining (*6*, *14*, *26*–*28*), which promiscuously re-ligate broken ends, particularly across short microhomologies. Thus, BRCA deficiency reroutes replication-associated DSB or DSG repair down an error-prone pathway. Consistent with this notion, human cancers harboring homozygous mutations in BRCA1 or BRCA2 display not only extensive structural rearrangements in chromosomes but also many short deletions (≤50 bp) with overlapping microhomology at breakpoint junctions (*29*).

BRCA1 and BRCA2 have distinct functions that ensure the error-free resolution of replication-associated DNA damage (Fig. 1), although their

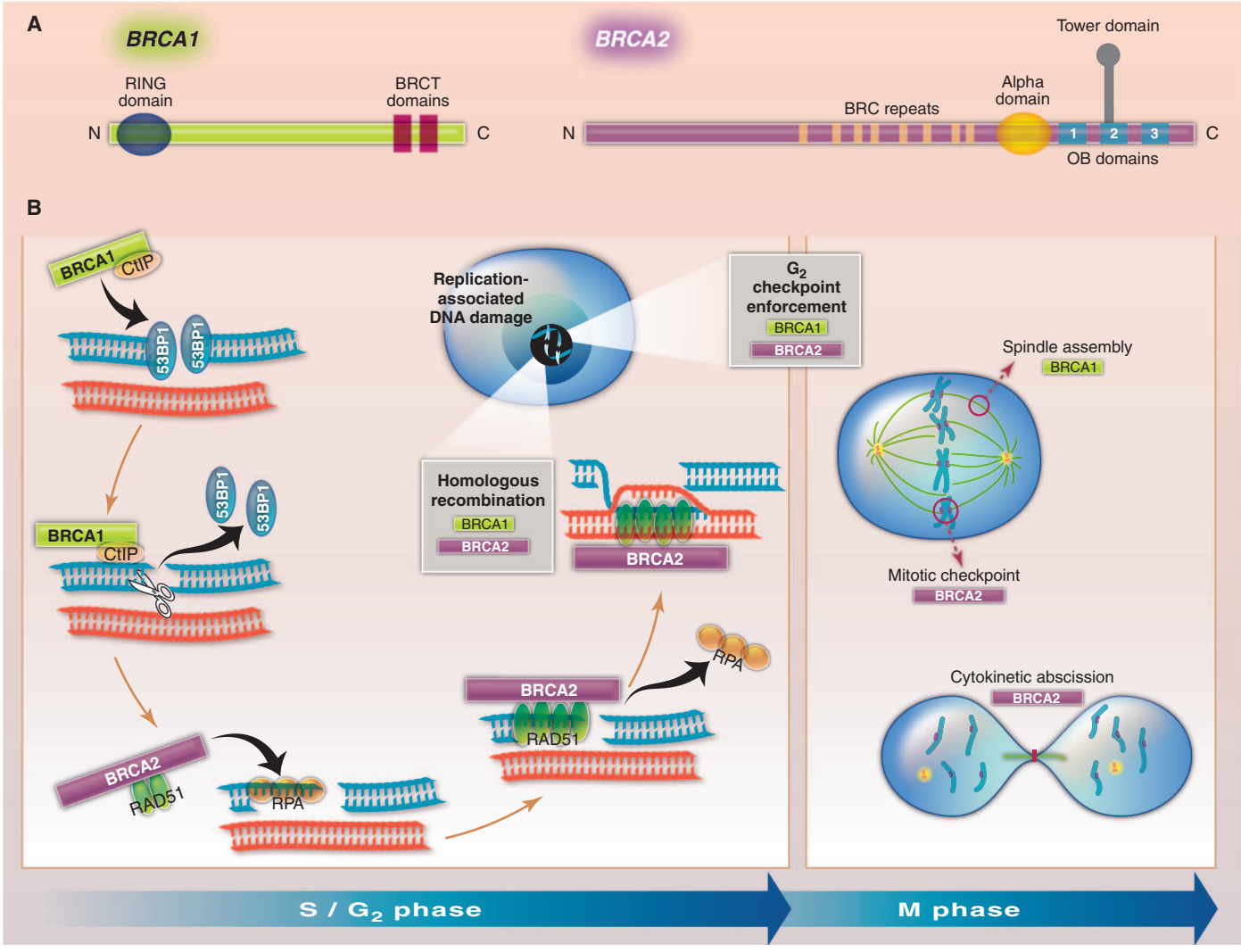

Fig. 1. BRCA1 and BRCA2: chromosome custodians. (**A**) The structural domains of human BRCA1 (1863 amino acids) and BRCA2 (3418 amino acids) proteins. An additional RAD51-binding region at the C terminus of BRCA2 is not marked, for simplicity. (**B**) Functions of BRCA1 and BRCA2 at different stages of the cell cycle discussed in the main text. During HR-mediated repair of replication-associated DNA damage, BRCA1 helps to initiate HR by displacing 53BP1 (p53 binding protein 1), thus triggering end resection (scissors). The C-terminal DNA binding domain of BRCA2 binds to the junction between ssDNA and dsDNA at the lesion, displacing RPA, an ssDNA-binding protein. The BRC repeats of BRCA2 bind to RAD51 recombinase and recruit it to the lesion. The BRC repeats promote nucleoprotein filament formation by stabilizing RAD51-ssDNA interactions while inhibiting RAD51-dsDNA binding. Both BRCA1 and BRCA2 participate in G_2 checkpoint enforcement. During mitosis, they promote mitotic spindle assembly (BRCA1) and regulate the mitotic checkpoint (BRCA2). BRCA2 participates in the abscission step of cytokinesis through functions at the midbody. Not all proposed functions of the BRCA proteins in chromosome maintenance are shown. Further detail is provided in the text.

partner protein PALB2 (*30*) bridges formation of a BRCA1-BRCA2 complex that assists in their localization there (*31*, *32*). A simplified view is that BRCA1 primarily acts at proximal steps that signal the presence of these lesions and helps initiate their repair by HR, whereas BRCA2 stabilizes the structure of replication-associated lesions and works directly to resolve them using HR, by controlling the activity and assembly of the essential recombination enzyme RAD51. Many studies not discussed here that add biochemical detail to this simplified view are more relevant to our understanding of HR than of tumor suppression by BRCA1 or BRCA2.

The C-terminal tandem BRCT domains of BRCA1 recruit it to damage sites. Initial recruitment likely proceeds via their ability to bind poly (ADP) ribose (PAR) chains conjugated to proteins at these sites (*33*), whereas recognition of phosphopeptide motifs with the consensus (pSer) XXPhe, created when protein kinases that signal DNA damage become activated, promotes continuing accumulation. Thus, BRCA1 BRCT domains engage phosphorylated Abraxas, a protein that bridges BRCA1 complex formation with RAP80, a protein that binds to ubiquitin conjugates at damage sites via multiple ubiquitin-interacting motifs (*34*–*36*). The BRCT domains also bind to the BRIP1 DNA helicase mutated in Fanconi anemia (*37*) and the CtIP protein (*38*), interactions that are mutually exclusive between themselves and with Abraxas (*34*, *35*). These findings suggest that BRCA1 uses the BRCT domains to manage the assembly and activity of several distinct macromolecular complexes (*39*) and performs varied functions at sites of DNA damage or replication stalling.

For HR to initiate, flush-ended broken DNA must be resected by a 5′ to 3′ nucleolytic activity to expose 3′-overhanging single-stranded (ss)DNA tracts that become the substrate for HR reactions. Regulation of this step by BRCA1 may help to restrict HR to the postreplicative G_2 phase of the cell cycle, during which a duplicated sister chromatid is available to template the repair of its damaged partner (*40*–*43*). HR is normally suppressed during the G_1 phase when the 53BP1 protein binds to broken DNA ends and recruits cofactors to prevent end resection. During G_2, a macromolecular complex containing BRCA1 displaces 53BP1 by an undefined mechanism, which facilitates end resection to initiate HR (Fig. 1). Thus, BRCA1 may help route the repair of replication-associated DNA damage via error-free HR, by displacing the HR-suppressing factor 53BP1 and its partners from broken ends. Supporting this view, 53BP1 inactivation partly reverses the structural chromosome aberrations induced by BRCA1 deficiency (*40*, *41*). How BRCA1 displaces 53BP1 to initiate HR remains controversial. CtIP binding via the BRCT domains has been implicated (*43*) and, in addition, recently was suggested to regulate, in competition with 53BP1, the extent of genetic exchange during later steps in HR (*44*). However, the recent demonstration that the BRCA1-CtIP interaction is dispensable for resection-mediated HR, DNA damage responses, and tumor suppression in transgenic mice (*45*) raises questions about its functional significance.

After end resection, the abundant ssDNA-binding protein RPA (replication protein A) coats exposed single-stranded (ss) DNA at chromosomal DSBs. RPA must be displaced to allow HR to proceed. This function is performed by an ~800-residue segment within BRCA2, which becomes ordered upon binding to the small acidic protein DSS1, and forms a protein complex that contains repeated oligonucleotide-oligosaccharide–binding (OB) folds capable of binding to ssDNA, in addition to a putative double-stranded DNA (dsDNA)–binding fold (*21*). This complex (Fig. 1) not only targets BRCA2 to dsDNA and/or ssDNA junctions typical of replication-associated lesions, DSBs, or DSGs but also displaces RPA to allow loading of the recombination enzyme RAD51 (*46*).

RAD51 must first form a helical nucleoprotein filament on ssDNA. The RAD51-ssDNA filament subsequently mediates synapsis with homologous dsDNA in the sister chromatid to initiate the strand exchange required for repair by HR. Getting these reactions to proceed in the right order presents a challenge, because under physiological ionic conditions, human RAD51 preferentially forms stable complexes on dsDNA rather than ssDNA.

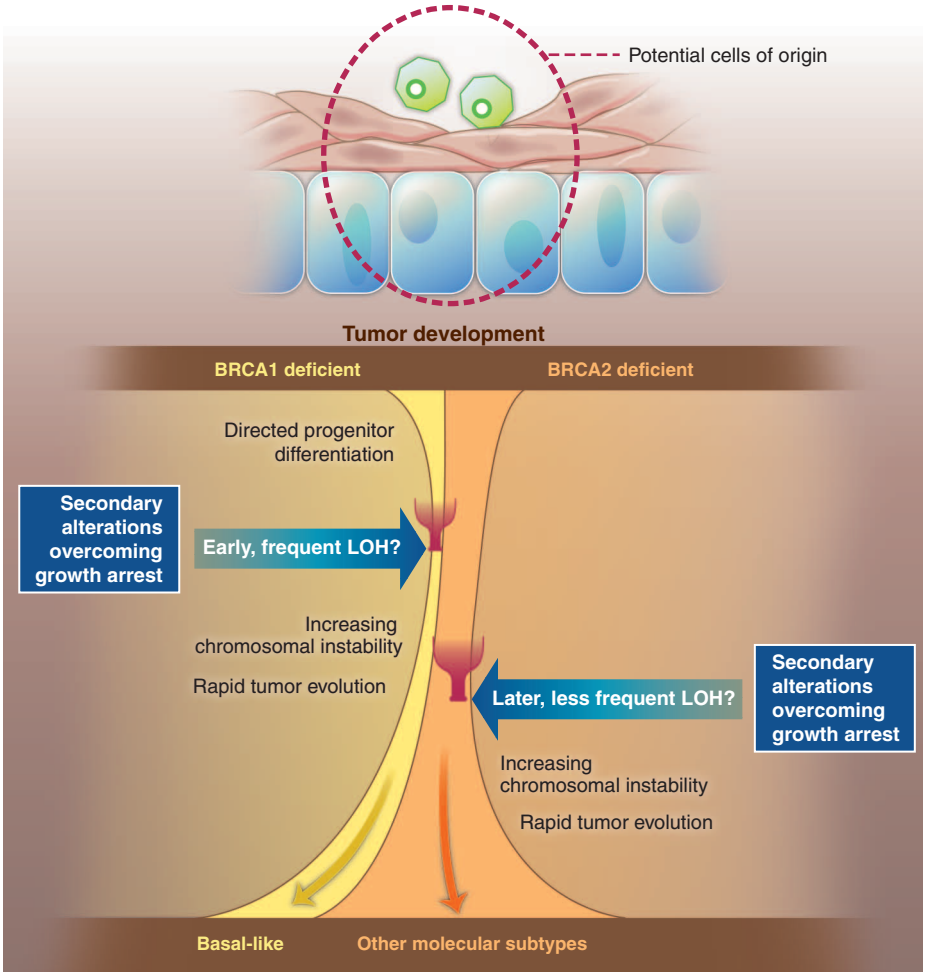

Fig. 2. The chromosomal instability model for the pathogenesis of BRCA-deficient breast cancer. The potential cell of origin depicted in the red dotted circle (blue, luminal epithelium; brown, myoepithelium; green, progenitor cell) is debated. BRCA1 deficiency forces differentiation toward basal-like characteristics, whereas BRCA2 deficiency does not. LOH provokes chromosomal aberrations, which lead to cell growth arrest (red bottleneck). Further genetic alterations, such as those inactivating cell cycle checkpoints, allow cells to bypass this bottleneck and resume growth. Chromosomal instability in the absence of functional BRCA proteins allows rapid evolution of tumors with basal-like (BRCA1-deficient) or different (BRCA2-deficient) characteristics. In contrast to BRCA1, there is evidence that BRCA2 LOH may be intratumorally heterogeneous and dispensable for carcinogenesis, which leads to the speculation that LOH occurs later in BRCA2-deficient tumor evolution. [Figure adapted from (*65*)]

BRCA2 binds directly to RAD51, and regulates RAD51's DNA substrate selectivity, to overcome this challenge. RAD51 binding is mediated by the BRC repeats in BRCA2 (*8*, *12*). These are motifs of ~30 to 40 residues each (Fig. 1), present in eight copies in all known mammalian BRCA2 molecules, whose sequence and spacing is evolutionarily conserved (*47*, *48*). Binding of the BRC repeats to RAD51 via an interface comprising two distinct modules (*20*, *49*) stabilizes RAD51 filament formation on ssDNA while inhibiting RAD51-dsDNA binding (*50*, *51*). These opposing activities bolster one another to correctly order the HR reaction, first targeting RAD51 to ssDNA for presynaptic filament formation before synapsis with the homologous duplex (Fig. 1). This RAD51-targeting activity is exhibited by single BRC repeat peptides, a segment of human BRCA2 containing all eight BRC repeats, or the full-length human BRCA2 protein (*50*–*54*), which exemplifies the segmental nature of BRCA2 structure-function relations and suggests that the biochemistry of these functional segments accurately reflects that of the full-length protein.

Observations made more than 10 years ago (*55*) show that stalled replication forks in BRCA2-deficient cells collapse into DSBs after genome-wide replication fork stalling induced by prolonged exposure to hydroxyurea (HU), an agent that depletes nucleotide pools required for DNA synthesis. This suggests that BRCA2 stabilizes the structure of arrested forks to allow their error-free resolution by HR. More recent observations extend these prior findings (*56*), by showing that a C-terminal segment of BRCA2—dispensable for chromosomal DSB repair by HR—protects nascent DNA strands at stalled forks, after brief HU exposure, from degradation by the MRE11 nuclease.

BRCA1 acquires E3 ubiquitin ligase enzymatic activity (*57*–*59*) by heterodimerization with BARD1 (*60*), through their N-terminal RING domains. In humans, BRCA1 mutations in the RING domain that disengage it from BARD1 and lead to the loss of E3 ligase activity are frequently associated with cancer predisposition. At first glance, these observations suggest that BRCA1 and BARD1 may exert their functions primarily through their conjoint E3 ligase activity. However, an engineered mutation Ile(or Val)26Ala in the BRCA1 RING domain that removes E3 ligase activity without perturbing the BARD1 interaction reveals that DNA damage responses triggered by certain agents (e.g., irradiation and topoisomerase inhibitors) are independent of E3 ligase activity (*61*, *62*), as is breast cancer suppression in mouse strains that harbor such an engineered mutation (*63*). On the other hand, a cancer-associated BRCA1 RING mutation Cys61Gly that reduces both E3 ligase activity and BARD1 binding is essential for breast cancer suppression (*64*). These varied observations highlight gaps in our understanding of the relevance of BRCA1-BARD1 E3 ligase activity to chromosome integrity and tumor suppression.

Protection Against Aneuploidy During Chromosome Segregation

The numerical chromosomal aberrations (aneuploidy) found in BRCA-deficient cells likely emanate from several causes. Transgression of the G_2 checkpoint for DNA damage, anomalies in spindle formation and the mitotic checkpoint at the metaphase-anaphase transition, and defects in the completion of cell division by cytokinesis have all been reported after the inactivation of BRCA1 or BRCA2 in several different experimental models [reviewed in (*65*)].

Although BRCA1-deficient cells can complete mitosis, the fidelity of chromosome segregation is frequently compromised, which leads to the appearance of aneuploid progeny. The BRCA1-BARD1 heterodimer (*66*) regulates mitotic spindle assembly, localizing at the spindle poles where its E3 ligase activity recruits the essential spindle assembly factor TPX2. BRCA1-BARD1 has also been implicated in spindle microtubule organization via attenuation of HMMR, a putative motility receptor for hyaluronan, in which polymorphisms have been identified that correlate with an increased risk of breast cancer in an Israeli population (*67*). Phosphorylation of BRCA1 by the checkpoint kinase CHK2 may regulate these functions (*68*).

In BRCA2-deficient cells, aneuploid divisions likely arise from defects in the mitotic spindle assembly checkpoint (*69*), as well as the defective completion of cell division by cytokinesis at the stage of daughter cell abscission (*70*–*72*). Moreover, BRCA2, like BRCA1, has been implicated in controlling mitotic entry during recovery from the G_2 checkpoint for DNA damage (*73*, *74*), which raises the possibility that DNA damage persisting into mitosis affects later events during chromosome segregation. At the spindle assembly checkpoint, BRCA2 recruits the PCAF acetyltransferase to the kinetochores of metaphase chromosomes (*69*), which enables the acetylation of the checkpoint protein BubR1. BRCA2 deficiency decreases BubR1 acetylation and the stability of BubR1, which leads to weakened checkpoint activity and aneuploidy. BRCA2 inactivation in zebrafish, murine, or human cells (*70*–*72*, *75*, *76*) increases the frequency of failure to complete cell division by cytokinesis, which engenders multinucleate structures with ≥4N DNA content. This defect occurs at a low frequency in murine cells heterozygous for a germline truncation in Brca2 (*70*), as well as in human cells compound-heterozygous for *BRCA2* mutations found in Fanconi anemia patients (*76*). Filamin A recruits BRCA2 to the cytokinetic midbody, where it controls assembly of the ESCRT (endosomal sorting complex required for transport) protein complex implicated in cytokinetic abscission, a function impaired by a human cancer–associated *BRCA2* mutation (*72*).

Collectively, these observations suggest that BRCA1 and BRCA2 act in distinct mechanisms to enhance the fidelity of chromosome segregation, although they are not essential components of the mitotic machineries for spindle assembly, kinetochore-microtubule attachment, or cytokinesis. Segregation defects provoked by BRCA deficiency may not only generate the copy number variations and whole-chromosome aneuploidies thought to promote tumorigenesis (*77*) but also cooperate with the subtler, but frequent, structural variations triggered by HR defects (*29*). Thus, BRCA1 and BRCA2 may be particularly effective as tumor suppressors through their extended role as chromosome custodians across interphase as well as mitosis.

Emerging Functions

Additional functions have been proposed, particularly for BRCA1 but also for BRCA2. These include roles [reviewed in (*60*)] in chromatin remodeling and gene expression, in RNA polymerase II–associated complexes implicated in DNA damage responses, in the regulation of mRNA polyadenylation, in an E3 ligase complex that modifies p53, in protecting telomeres that cap chromosome ends, and in heterochromatin maintenance and retrotransposon silencing by the control of site-specific DNA methylation (*78*). Whether these functions are salient to tumor suppression by the BRCA proteins is not yet clear.

A recent report (*79*) proposes that decreased ubiquitylation of histone H2A in BRCA1-deficient cells alters the structure of constitutive heterochromatin that flanks the centromeres of chromosomes, activating transcription from normally silent genomic regions (called "satellite sequences") therein. Ectopic expression of satellite transcripts reproduces several effects of BRCA1 deficiency, such as DNA breakage and abnormal chromosome segregation. However, an Ile26Ala BRCA1 mutant protein lacking E3 ligase activity that does not support H2A ubiquitylation or satellite repression in vitro (*79*) nevertheless effectively suppresses tumorigenesis in vivo (*63*).

The "Chromosomal Instability" Model for Carcinogenesis in *BRCA* Mutation Carriers

Genetic Progression and Clinical Features

How well does a "chromosomal instability" model (Fig. 2) explain the clinical features of cancer susceptibility in individuals who carry *BRCA* gene mutations? As predicted by a model in which chromosomal instability provoked by BRCA inactivation accelerates the process of genetic variation and selection that drives carcinogenesis, *BRCA* mutation carriers develop breast and ovarian cancers at an earlier age than noncarriers. Moreover, primary cancers of the contralateral breast occur very frequently in mutation carriers (*80*), which suggests that ongoing chromosomal instability generates a "field" of cells susceptible to transformation. One puzzle in this regard is

that *BRCA* gene heterozygosity suffices for cancer predisposition, although it does not provoke the high levels of chromosomal instability typical of cells lacking both copies of either *BRCA1* or *BRCA2* (*5*, *6*, *81*). This raises the possibility that heterozygosity has a dosage effect sufficient to trigger low, but quantitatively significant, levels of genome instability that accumulates over many cell divisions. Alternatively, certain heterozygous mutations may act in a dominant manner [for example, (*82*)].

Biallelic inactivation of *BRCA1* or *BRCA2* not only triggers profound chromosomal instability but also quickly leads to cell cycle arrest or apoptosis because of the activation of checkpoint mechanisms that monitor the cell cycle (Fig. 2). Indeed, in murine models, concordant inactivation of checkpoint mechanisms mediated by *Tp53* is essential for tumorigenesis in this setting (*83*–*86*). Thus, an important implication of the chromosomal instability model is that homozygous loss of both copies of either *BRCA1* or *BRCA2* in premalignant cells must be preceded (or at least, quickly followed) by inactivation of checkpoint mechanisms that would otherwise eliminate the BRCA-deficient cells (Fig. 2). Consistent with this notion, several studies report an increased frequency of *p53* mutations in *BRCA*-deficient cancers [e.g., (*87*, *88*)]. Not all BRCA-deficient tumors harbor *p53* mutations, however, which suggests that alternative routes to checkpoint inactivation in this setting probably exist. Indeed, BRCA1 and BRCA2 have themselves been implicated in checkpoint control.

Whereas germline inheritance of a single mutant allele of *BRCA1* or *BRCA2* predisposes to cancer, somatic deletion of the remaining wild-type allele (also termed "loss of heterozygosity" or LOH) is considered essential for carcinogenesis. However, emerging evidence suggests that germline heterozygous *BRCA2* mutations may suffice for carcinogenesis in several tissues, with LOH either absent or occurring relatively late in disease progression. Only 13 of 25 high-grade serous ovarian cancer samples from *BRCA2* mutation carriers exhibited LOH, defined as a >20% increase in mutant allele frequency compared to paired germline samples (*89*). In invasive prostate carcinomas from men carrying a *BRCA2* mutation, LOH was detected in only 5 of 10 samples (and none of the high-grade intraepithelial neoplasia from the same samples) (*90*). Heterogeneous intratumoral LOH was observed in invasive breast cancers from *BRCA* mutation carriers (*91*). Only one out of four invasive pancreatic ductal adenocarcinomas arising in $BRCA2^{999delC}$ carriers (*85*) and three out of four in $BRCA2^{6174delT}$ carriers (*92*) exhibited LOH. Although these small sample numbers warrant due caution, these analyses suggest that a varying, but perhaps substantial, fraction of cancers arising in *BRCA2* mutation carriers may retain an intact second allele, with the frequency of LOH increasing with advancing disease. By comparison, *BRCA1* LOH was detected in 30 of 32 serous ovarian carcinomas, and 2 of 4 advanced pancreatic cancers, from patients carrying *BRCA1* germline mutations. Epigenetic silencing of *BRCA1* has also been reported.

Tissue Specificity

Observed differences in the frequency of LOH in cancers from *BRCA1* versus *BRCA2* mutation carriers draw attention to other distinguishing features. *BRCA1* mutation carriers primarily experience a marked excess of ovarian and female breast cancers, whereas inherited *BRCA2* mutations also cause significant predisposition to cancers of the male breast, pancreas, prostate, and other organs (*93*, *94*). Moreover, BRCA1-deficient breast cancers predominantly exhibit characteristics of the "basal-like" subgroup (*95*), such as the lack of estrogen receptor (ER) expression, which is not evident in the majority of BRCA2-deficient tumors (Fig. 2). These clinical characteristics are consistent with laboratory experiments (*96*–*99*) suggesting that *BRCA1* mutations affect the early differentiation of breast tissue from ER-negative progenitors of debated lineage and so promote carcinogenesis through tissue-specific functions distinct from those responsible for chromosome integrity. Such tissue-specific functions of BRCA1 may also help to explain the predominance of breast and ovarian cancer susceptibility in mutation carriers.

Why cancer predisposition associated with *BRCA* gene mutations should occur in specific tissues such as the breast, ovary, pancreas, or prostate has not yet been satisfactorily explained. Tissue-specific functions affecting gene expression or differentiation—such as those discussed above for BRCA1—have been proposed. Others may become apparent when BRCA disruption renders cells in particular tissues more sensitive to the effects of local mutagens or to periods of rapid cell division. An alternative hypothesis is that tumors can arise only in tissues that are permissive for the prolonged survival of cells lacking both *BRCA* alleles (*65*, *100*), which allows time for secondary mutations affecting genes like *p53* that permit tumor progression and outgrowth. Prophylactic oophorectomy in women who carry *BRCA* mutations reduces the risk of breast cancer, which suggests that ovary-derived factors may foster the outgrowth of malignant epithelial cells (*101*, *102*), particularly in *BRCA2* mutation carriers. However, recent work suggesting that a significant fraction of BRCA2-deficient tumors arising in the target tissues of germline mutation carriers retains an intact second allele diminishes the likelihood that this hypothesis will provide a satisfying general explanation for tissue specificity, at least in the case of BRCA2.

Rationalizing Approaches to Therapy

The role of the BRCA proteins as custodians of chromosome integrity during the cell cycle offers the potential for new approaches to therapy. The inability of BRCA-deficient cells to deal correctly with stalled DNA replication forks underpins their sensitivity to classical cancer chemotherapies that arrest replication. These drugs include well-established DNA cross-linking agents, such as platinum compounds, which are showing clinical promise in BRCA1-deficient cancer patients (*103*, *104*). In addition, new targeted agents have been developed that inhibit the enzyme poly (ADP)-ribose polymerase 1 (PARP1) and block the DNA repair pathway that removes damaged nitrogen bases by base excision repair to leave behind a ssDNA gap that effectively arrests replication forks. These drugs have shown excellent efficacy in laboratory studies (*105*, *106*) and are being extensively tested in different clinical settings.

Clinical trials of highly potent PARP1 inhibitors (PARP1i) have thus far produced a mix of encouraging (*107*–*109*) and disappointing (*110*, *111*) results. Further optimization of treatment regimes and better selection of patients are required to maximize their potential. Three major issues warrant consideration. First, there is evidence that a fraction of tumors arising in mutation carriers retain a wild-type *BRCA2* allele [for example, (*86*, *90*, *91*)], which confers de novo primary resistance to PARP1i. Patients with such tumors would be unlikely to benefit from the drugs. Second, chromosomal instability in BRCA-deficient cancer cells may enhance the risk of further mutations that induce secondary treatment resistance, including reversions that partially restore inactive *BRCA* gene function (*112*, *113*) particularly in patients previously treated with platinum compounds (*114*). Finally, diminished (but not absent) HR in the nontumorous somatic tissues of heterozygous *BRCA* mutation carriers could potentiate the toxicity of regimes combining PARP1i with systemic genotoxins like anthracyclines or nucleoside analogs. It could also potentiate iatrogenic carcinogenesis after long-term (potentially preventative) PARP1i treatment. These problems are raised by the observation that DNA breakage marked by γH2AX is transiently induced even in nontarget tissues in *BRCA* mutation carriers treated with PARP1i (*115*). Treatment regimes that better limit exposure or new methods that target drug delivery to tumor tissues could help to address these problems.

Emerging evidence that essential HR proteins other than BRCA1 and BRCA2 may be inactivated by somatic mutations or epigenetic silencing in certain tumor types has begun to impact the treatment of non-*BRCA* mutant sporadic cancers [reviewed in (*116*)]. For instance, it has been proposed that certain sporadic ovarian cancers or triple-negative breast cancers devoid of *BRCA* mutations may nevertheless exhibit defects in HR. If correct, this premise could help to explain variations in clinical sensitivity to replication-arresting chemotherapies, such as

platinum compounds, or targeted agents like PARP1 inhibitors.

Conclusion

In summary, the past 20 years have witnessed remarkable advances in our understanding of the biological functions of BRCA1 and BRCA2 as custodians of chromosome integrity across the cell cycle that underlie their potent tumor-suppressor activity. In a broader context, this body of work has revealed causal relations between chromosomal instability and susceptibility to common forms of epithelial cancer, has provided a powerful experimental landscape in which to explore the biological mechanisms that preserve chromosome integrity, and has allowed the formulation of a conceptual framework to interpret the role of these mechanisms in carcinogenesis and to exploit them for cancer therapy. There is, however, clearly so much more to do that perhaps the best is yet to come.

References and Notes

1. "Chromosomal instability" throughout this article refers to structural, as well as numerical, aberrations in chromosomes. Some workers use the term exclusively for numerical aberrations (typically, whole-chromosome aneuploidy). That is not the case here.
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Supplementary Materials

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Fig. S1
Reference (*117*)

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