Functions of BRCA1 and BRCA2 in the biological response to DNA damage

Ashok R. Venkitaraman

University of Cambridge, CRC Department of Oncology and The Medical Research Council Cancer Cell Unit, Hutchison/MRC Research Centre, Hills Road, Cambridge CB2 2XZ, UK

(e-mail: arv22@cam.ac.uk)

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Summary

Inheritance of one defective copy of either of the two breast-cancer-susceptibility genes, *BRCA1* and *BRCA2*, predisposes individuals to breast, ovarian and other cancers. Both genes encode very large protein products; these bear little resemblance to one another or to other known proteins, and their precise biological functions remain uncertain. Recent studies reveal that the BRCA proteins are required for maintenance of chromosomal stability in mammalian cells and function in the biological response to DNA damage. The new work suggests that, although the phenotypic consequences of their disruption are similar, BRCA1 and BRCA2 play distinct roles in the mechanisms that lead to the repair of DNA double-strand breaks.

Key words: Cell cycle, Chromosomal stability, DNA double-strand break repair, Homologous recombination, Tumour suppression

Introduction

Approximately 10% of all cases of breast cancer exhibit a familial pattern of incidence. Efforts to identify the genetic basis of familial breast cancer reached fruition some five years ago, when the breast- cancer-susceptibility genes *BRCA1* and *BRCA2* were identified through positional cloning. Germline mutations in either of these genes account for 20-60% of breast cancer cases in families where multiple individuals are affected (~2-6% of all cases) (Nathanson et al., 2001). Mutations in a small number (~4) of other genes, some identified and some unknown, are predicted to account for the remainder of familial risk (Easton, 1999).

Epidemiological studies sparked by the discovery of *BRCA1* and *BRCA2* have made clear several features of inherited mutations in the genes. The mutations are highly penetrant, carrying a lifetime risk of 30-70% for cancer incidence (Ford et al., 1998), with variation related to genetic background (Nathanson et al., 2001). The majority are small insertions or deletions distributed throughout the genes, which are predicted to result in truncation of the encoded protein. Many mis-sense and nonsense alterations have also been described. A list of known mutations can be found in the Breast Information Core databases [http://www.nhgri.nih.gov/Intramural_research/Lab_transfer/Bic].

Attempts to draw a correlation between the inheritance of a particular mutation (or cluster of mutations) and the resulting disease phenotype have so far proven largely unsuccessful (with some exceptions; see below). In part, this is because the prevalence of specific *BRCA1* or *BRCA2* mutations is small, limiting the data set from which genotype/phenotype correlations can be drawn. Even where this hurdle can be overcome, there is evidence that the cancer risk associated with *BRCA1* or *BRCA2* mutations is modified by additional factors (Gayther et al., 1997; Struewing et al., 1997). This is best illustrated by studies of *BRCA2* founder mutations in the (genetically quite homogenous) Icelandic population (Thorlacius et al., 1996). The risk of cancer associated with one particular founder mutation, *BRCA2 999del5*, varies considerably in the families that inherit it, resulting in differences in the age of cancer onset, the degree of penetrance, and even the type of tumour to which individuals are predisposed. Identification of the genetic or environmental factors that modify the phenotypic effects of mutations in these genes is likely to provide important new insights into inherited cancer predisposition.

Mutations in *BRCA1* or *BRCA2* are not simply associated with increased breast cancer risk (Rahman and Stratton, 1998). Mutation carriers are also susceptible to cancers of the ovary, prostate, pancreas and male breast. Other associations may be revealed when more epidemiological information becomes available.

Inheritance of one defective *BRCA1* or *BRCA2* allele suffices to confer cancer predisposition. Breast and ovarian tumours from predisposed individuals almost invariably exhibit loss of heterozygosity while retaining the mutant allele (Collins et al., 1995; Cornelis et al., 1995), indicating that the protein products of the genes may behave in some respects as tumour suppressors. It is puzzling, therefore, that somatic mutations in *BRCA1* or *BRCA2* do not frequently occur in sporadic (non-familial) breast cancers (Rahman and Stratton, 1998). This weakens, but does not disprove, the notion that the gene products operate in a cellular pathway that is defective in the majority of breast cancers. One possible explanation for the discrepancy is that the genetic alterations in sporadic cancers target other molecules whose functions are linked to those of BRCA1 or BRCA2. Thus, hopes that delineation of the functions of BRCA1 and BRCA2 might reveal common mechanisms underlying pathogenesis have stimulated much interest in the cell biology of these proteins. Here, I discuss recent advances in our understanding of the functions of BRCA1 and BRCA2 in biological responses to DNA damage.

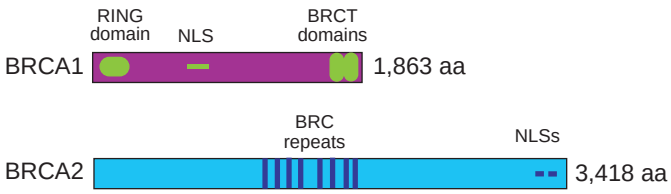

Fig. 1. Features of the BRCA proteins. The N-terminal RING domain, nuclear localisation signal (NLS) and C-terminal BRCT domains of BRCA1 are shown, as are the eight BRC repeat motifs in BRCA2. Modified from Venkitaraman, 2001 with the permission of Elsevier Science Ltd (Venkitaraman, 2001).

Proteins encoded by the breast cancer genes

BRCA1 and BRCA2 have very different primary sequences (Fig. 1). BRCA1 is a 1,863-residue protein of unknown structure and has a few identifiable features. An N-terminal RING domain has been implicated in several protein-protein interactions (for instance, with BARD1) (Wu et al., 1996). The C-terminus of BRCA1 contains two 95-residue (Koonin et al., 1996) BRCT (for BRCA1 C-terminal) domains, which are also found in many other proteins involved in DNA repair and cell cycle regulation. The crystal structure of one of the two BRCT domains from the DNA repair protein XRCC1 (Huyton et al., 2000; Zhang et al., 1998) reveals a central, four-stranded β-sheet, around which are three α-helices. The contacts that mediate asymmetric dimerisation of the two BRCT domains of XRCC1 are predicted to involve residues in one of the α helices. Similar contacts might also be important for heterodimerisation with other BRCT-domain proteins. The most C-terminal of the two BRCT domains of BRCA1 has sufficient sequence identity to be mapped onto the XRCC1 BRCT domain structure. Tumour-associated mutations in *BRCA1* are predicted to disrupt the folding or stability of the BRCT domain or to alter the putative interface for dimerization – this would be consistent with effects upon protein function (Zhang et al., 1998).

The 3,418 residue BRCA2 gene product does not exhibit significant similarity to any known protein (Fig. 1). Eight 30-40 residue motifs (Bork et al., 1996) – the so-called BRC repeats – are encoded in exon 11 and conserved between several mammalian species, which suggests they have an essential function (Bignell et al., 1997). In fact, the BRC repeats have been shown to mediate the binding of BRCA2 to RAD51 (see below), a mammalian protein essential for DNA repair and genetic recombination.

Interactions between RAD51 and the BRCA proteins

Despite the apparent dissimilarity in protein sequence and structure, there is considerable evidence that BRCA1 and BRCA2 have common biological functions. BRCA1 and BRCA2 exhibit similar patterns of expression and sub-cellular localisation. They are both expressed in many tissues in a cell-cycle-dependent manner (Bertwistle et al., 1997; Blackshear et al., 1998; Connor et al., 1997b; Rajan et al., 1996; Sharan and Bradley, 1997); their levels are highest during S phase, which is suggestive of functions during DNA replication. Both are localised to the nucleus in somatic cells, where they co-exist in characteristic subnuclear foci that redistribute following DNA damage. In meiotic cells, both proteins co-localise to the synaptonemal complexes of developing axial filaments (Chen et al., 1998a).

This pattern of expression and localisation is shared with RAD51, a mammalian homologue of the bacterial protein RecA, which is essential in *Escherichia coli* for the repair of DNA double-strand breaks (DSBs) by genetic recombination (Kowalczykowski, 2000). Indeed, both BRCA1 and BRCA2 have been reported to bind to RAD51. The BRCA2-RAD51 interaction is direct, in that it can be demonstrated in vitro with recombinant protein fragments (Chen et al., 1998b; Wong et al., 1997) and in the yeast two-hybrid system, and appears to be of relatively high stoichiometry. A C-terminal motif spanning residues 3196-3232 of murine BRCA2 has been shown to mediate binding of the protein to the first 98 residues of RAD51 in yeast two-hybrid assays (Sharan et al., 1997). However, an analogous region of human BRCA2, which is 95% identical to the murine sequence, does not bind RAD51 (Wong et al., 1997; Aihara et al., 1999).

The interaction of RAD51 with human BRCA2 is mediated primarily, if not exclusively, by the eight BRC repeats (Wong et al., 1997). Each repeat, with the exception of BRC5 and BRC6, can bind individually to RAD51 in two-hybrid assays, as well as in vitro when expressed as a GST fusion protein. Their relative RAD51-binding capacities vary considerably (Chen et al., 1999), BRC4 being about four times as active as BRC1 in two-hybrid assays. PCR mutagenesis defines a binding consensus of about 30 residues present in both BRC1 and BRC4. Interestingly, despite the conservation of this core motif, different residues appear to be important in BRC1 and BRC4 for RAD51 binding. This, and the large difference in the affinity for RAD51, indicates that the mode of interaction of BRC1 or BRC4 with RAD51 could be quite distinct.

A region encompassing residues 758-1064 in BRCA1 was first reported to be involved in its interaction with RAD51 (Scully et al., 1997). It remains unclear, however, whether the two proteins can bind directly. Co-immunoprecipitation from cell extracts reveals an interaction of low stoichiometry, which has not yet been demonstrated in yeast two-hybrid assays or in vitro with recombinant proteins.

BRCA1 and BRCA2 co-localise in mitotic and meiotic cells (Chen et al., 1998a) and physically associate with one another through a region in BRCA1 (residues 1314-1863) distinct from that reported to bind to RAD51. Again, the interaction may not be direct, and it appears to involve a small fraction (perhaps 2-5%) of the total cellular pool of each protein. Moreover, recent efforts to characterise the protein complex associated with BRCA1 by biochemical purification and mass spectroscopy do not report the presence of appreciable amounts of either RAD51 or BRCA2 (Wang et al., 2000).

Thus, of the reported physical interactions between BRCA1, BRCA2 and RAD51, the BRCA2-RAD51 interaction appears to be the best established. Tantalising though existing evidence may be, it remains to be firmly demonstrated that BRCA1 functions together with BRCA2 and RAD51 in a multi-molecular complex, and the functional significance of their reported interactions is yet to be rigorously defined.

BRCA1 and BRCA2 participate in the biological response to DNA damage

Inevitably, the suggestion that BRCA1 and BRCA2 co-localise

with RAD51 provoked speculation that they participate in some aspect of the cellular response to DNA damage. Direct evidence for such a function has come from studies on cells that harbour mutations in the breast-cancer-susceptibility genes.

The cellular response to DNA damage involves the activation of cell cycle checkpoints and the recruitment of the machinery for DNA repair, processes that are intimately linked to one another. Failure to activate these checkpoints or DNA repair following DNA damage manifests as increased sensitivity to genotoxic agents. Indeed, *Brca1*-deficient and *Brca2*-deficient murine cells exhibit hypersensitivity to genotoxins such as X-rays (Connor et al., 1997a; Patel et al., 1998; Sharan et al., 1997; Xu et al., 1999b), confirming an essential role for the two proteins in the response to DNA damage.

The role of BRCA1 and BRCA2 in DNA double-strand break repair

The X-ray sensitivity of cells lacking BRCA1 or BRCA2 suggests they have a defect in the repair of DSBs, the major lesion inflicted by ionising radiation. Mammalian cells use several mechanisms (Karran, 2000) to repair DSBs, in particular non-homologous end joining (NHEJ) and homologous recombination. NHEJ, which culminates in the ligation of broken DNA fragments without regard to the homology of sequences at their ends, is critically dependent on the DNA-dependent protein kinase (DNA-PK) and its accessory molecules Ku70 and Ku80. By contrast, DSB repair by homologous recombination is achieved through the exchange of genetic information between the damaged template and a homologous DNA sequence, such as that found on a sister chromatid. Its mechanism in mammalian cells is poorly understood. In yeast, recombination is dependent upon the *RAD52* epistasis group of genes, which encode not only yeast Rad51p but also Rad52p, Rad54p, Rad55p, Rad57p, Rad59p and the Mre11p/Xrs2p-Rad50 complex. There is increasing evidence that the mammalian homologues of these yeast genes roles play roles similar to those of their yeast counterparts.

Several lines of evidence now indicate that BRCA2 is not essential for DSB repair by NHEJ. For instance, V(D)J rearrangement of antibody or T cell receptor genes in developing lymphocytes, an NHEJ reaction that requires DNA-PK, Ku70 and Ku80, proceeds to completion in murine cells that harbour a targeted truncation in *Brca2* (Patel et al., 1998). Moreover, protein extracts from human BRCA2-deficient cells retain the ability to carry out DNA-PK-dependent NHEJ reactions in vitro (Yu et al., 2000).

There is now good evidence that BRCA2 is essential for DSB repair by homologous recombination. Cells that contain truncated *Brca2* progressively accumulate aberrations in chromosome structure during passage in culture; these typically include tri-radial and quadri-radial chromosomes as well as chromosome breaks (Patel et al., 1998). The radial structures, pathognomonic of human diseases such as Bloom's syndrome and Fanconi's anaemia, mark defects in mitotic recombination. The formation of nuclear foci containing RAD51 after exposure to DNA damage, putative sites for recombination repair, is compromised by BRCA2 deficiency (Chen et al., 1999; Yu et al., 2000; Yuan et al., 1999). Finally, *Brca2*-negative cells exhibit deficient homologous recombinational (but not NHEJ) repair of DSBs introduced by the yeast endonuclease I-SceI into chromosomally integrated substrates (Moynahan et al., 2001).

Chromosomal aberrations similar to those reported in BRCA2-deficient cells are also induced by loss of BRCA1 (Xu et al., 1999b). Moynahan et al. have demonstrated that BRCA1 participates in DSB repair by homologous recombination, using *Brca1*-deficient cells (Moynahan et al., 1999). These experiments demonstrate that NHEJ is not compromised – and may in fact be increased – by the disruption of *Brca1*.

The roles played by BRCA1 and BRCA2 in DSB repair by homologous recombination appear to be somewhat different. Available evidence indicates a more direct role for BRCA2 in this mechanism. Indeed, recent results suggest that BRCA2 controls the intracellular transport and function of RAD51. In BRCA2-deficient cells, RAD51 (which does not contain a consensus nuclear localisation signal) is inefficiently transported into the nucleus, which suggests that one function of BRCA2 is to move RAD51 from its site of synthesis to its site of activity (Davies et al., 2001). In addition, BRCA2 also appears to control the enzymatic activity of RAD51.

RAD51 efficiently coats DNA substrates to form ordered nucleoprotein filaments, key intermediates in homologous-strand pairing and exchange reactions (Baumann et al., 1996; Sung and Robberson, 1995). Addition of peptides containing the RAD51-binding BRC repeat BRC3, BRC4 or BRC7 inhibits nucleoprotein filament formation in vitro (Davies et al., 2001). Cancer-associated mutants of the BRC peptides do not exhibit this inhibitory activity. Gel filtration experiments suggest that the binding of RAD51 to BRC peptides renders it incapable of undergoing multimerisation, an important step in filament formation. Collectively – and surprisingly – these observations suggest that the BRCA2-RAD51 complex in vivo us unable to promote homologous recombination.

How can we reconcile these in vitro findings with the genetic data indicating that BRCA2 is essential for DSB repair by recombination? One possible explanation (Fig. 2) is that the BRCA2-RAD51 complex exists in at least two states in vivo: an 'inactive' state, which prevents the promiscuous binding of single-strand DNA by RAD51; and an active state in which RAD51 can form nucleoprotein filaments and is delivered to sites of DNA damage by BRCA2. The necessity for an 'inactive' BRCA2-RAD51 complex may stem from the frequent creation of single-stranded DNA during normal DNA metabolism, which must not inappropriately evoke a DNA damage response. Transition from the inactive to the active state might involve release of RAD51 from BRCA2 through post-translational modifications, such as protein phosphorylation by DNA-damage-activated kinases (e.g. ATM and ATR; Fig. 2).

This model is hypothetical, and numerous limitations are evident. Not all intracellular RAD51 is in complex with BRCA2; several distinct RAD51 pools, whose activity and localisation are regulated in distinct ways might therefore exist. The mode by which inactive RAD51 bound to BRCA2 could be converted to an active form at sites of DNA damage is not apparent. Does the transition involve release of RAD51 from BRCA2 binding or a change in the nature of their interaction? Finally, this model is based on biochemical studies using a

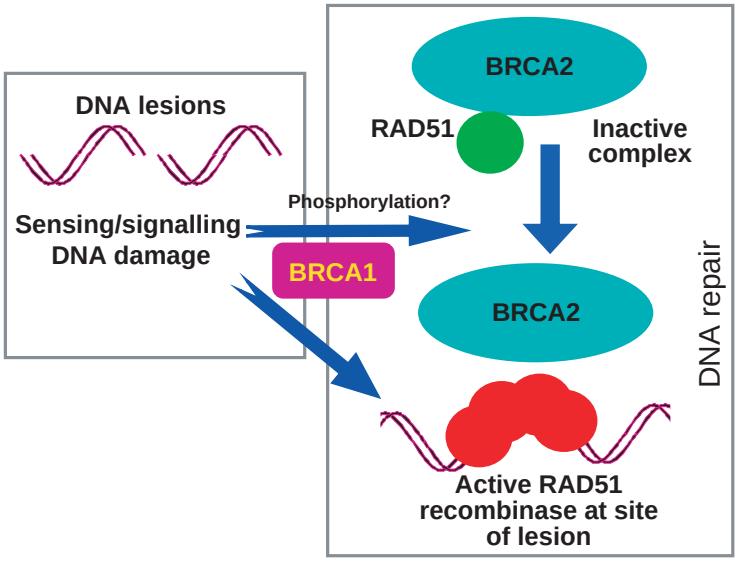

Fig. 2. A model for the role of BRCA2 in DSB repair based on Davies et al. (Davies et al., 2001). As discussed in the text, an 'inactive' complex between BRCA2 and RAD51 in the nucleus undergoes transition to a form in which RAD51 is 'active' in forming nucleoprotein filaments at the sites of DNA damage. The transition may be induced after phosphorylation by kinases such as ATM or ATR activated by DNA damage. The precise role of BRCA1 in these events is unclear, but it is likely to have multiple functions in DNA damage responses. A single RAD51 molecule is shown bound to a single BRCA2 molecule for simplicity. Modified from Venkitaraman, with permission from Elsevier Science (Venkitaraman, 2001).

single BRC-repeat peptide. However, the BRCA2 molecule contains at least six BRC repeats that can bind RAD51. The in vitro results might therefore have only limited relevance. In particular, cancer-associated BRCA2 mutations affecting only a single BRC repeat occur, and it is difficult to see how they might affect RAD51 function in vivo. These limitations are not necessarily fatal, but underline the need for further studies on the cell biology of the BRCA2-RAD51 interaction.

BRCA1 is also essential for DSB repair by homologous recombination, but its mode of action is far less clear (Figs 2 and 3). It might not directly control RAD51 function, since the stoichiometry of their interaction is possibly low and does not appear to be greatly altered following DNA damage.

BRCA1 also physically associates, directly or indirectly, with proteins other than RAD51 whose yeast homologues are known to participate in recombination (Wang et al., 2000; Zhong et al., 1999). RAD50, together with its partners MRE11 and NBS1, have been reported (Zhong et al., 1999) to co-localise and co-immunoprecipitate with BRCA1, although it is not universally accepted that localisation to foci is dependent upon BRCA1 (Wu et al., 2000). RAD50 can also be detected in appreciable amounts in the complex of proteins that co-purify with BRCA1 in large-scale biochemical experiments (Wang et al., 2000).

Recent work suggests that BRCA1 regulates the activity of the RAD50-MRE11-NBS1 complex (Paull et al., 2001). The exonucleolytic activity of the complex – mediated by MRE11 – may be important for the creation of resected single-stranded DNA at sites of DSB repair by homology-directed mechanisms (Haber, 1998). BRCA1 binds DNA directly and, when it does so, it becomes an inhibitor of MRE11 activity (Paull et al., 2001). How such a mechanism might assist in DNA repair remains to be elucidated. However, the RAD50-MRE11-NBS1 complex works proximally in the biological pathways that sense and signal the presence of DSBs, resulting in the activation of cell cycle checkpoints. The interaction of BRCA1 with this complex suggests functions at a similar, proximal level (Fig. 3).

Activation of DNA damage checkpoints

Much evidence supports the possibility that the products of the breast-cancer-susceptibility genes are involved in activation of DNA damage checkpoints. Disruption of *Brca1* in murine cells results in abnormalities of checkpoint enforcement in G2/M phase of the cell cycle (Xu et al., 1999b). Studies (Carr, 2000) in yeast models have identified a group of four protein kinases

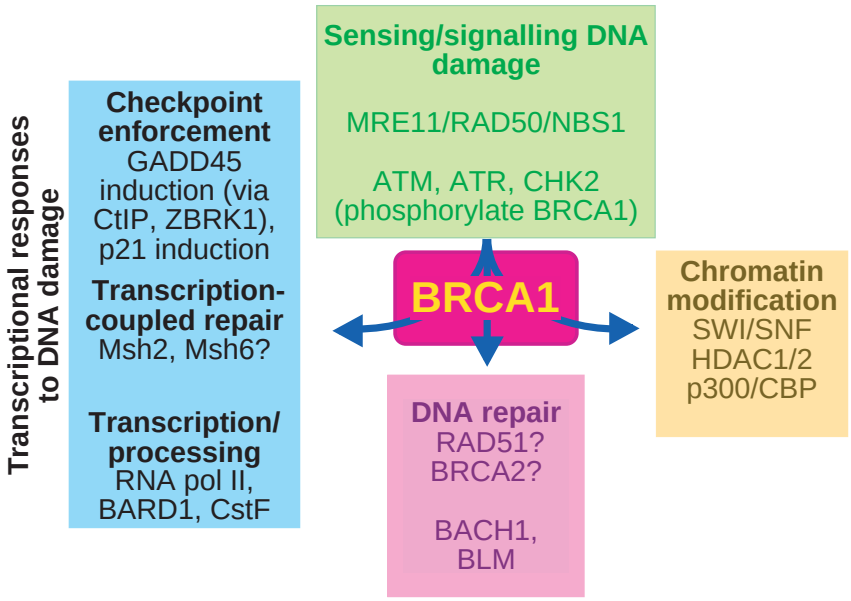

Fig. 3. Protein partners of BRCA1 in DNA damage responses. There is accumulating evidence that BRCA1 performs multiple functions in the cellular response to DNA damage through its interactions with different protein partners. The list of BRCA1-interacting proteins indicated here is not exhaustive but illustrates points made in the text.

whose activation is essential for these checkpoints: Rad3p/Mec1p, Tel1p, Chk1p and Cds1p/Rad53p. Each has a well-conserved mammalian homologue, ATM, ATR, Chk1 and Chk2, respectively. BRCA1 is a target for phosphorylation by several checkpoint kinases, and these modifications are essential for the proper response to DNA damage induced by γ-radiation (Fig. 3).

ATM, the gene mutated in the human disease ataxia telangiectasia, encodes a molecule related to the phosphoinositide 3-kinases (Shiloh, 2001). Several of the manifestations of ATM deficiency – at the cellular and the organismal level – are remarkably similar to those induced by loss of *BRCA1*. ATM patients and heterozygotes are predisposed to cancer, including breast cancer. ATM-deficient cells exhibit chromosomal instability and have compromised abilities to respond to genotoxic agents. After γ-radiation, ATM phosphorylates BRCA1 on several serine residues clustered in its C-terminal region (Cortez et al., 1999; Gatei et al., 2001). Replacement of two of these residues (Ser1423 and Ser1524) with alanine is enough to abrogate the function of *BRCA1* in the radiation response. That BRCA1 phosphorylation is defective in ATM-deficient cells provides a rationale for the similarity in the clinical and cellular manifestations caused by dysfunction of either of these proteins.

The cellular effects of ATM activation are mediated at least in part through a downstream effector, the Chk2 kinase. Chk2 directly phosphorylates a serine residue in BRCA1, Ser988, distinct from those that are ATM targets (Lee et al., 2000). Again, this modification appears to be essential in the cellular response to ionising radiation, which is compromised by the Ser988Ala substitution. Interestingly, the redistribution of BRCA1 that usually follows DNA damage does not occur in the Ser988Ala mutant. Thus, Chk2 phosphorylation may exert its effect on BRCA1 function by modifying its intracellular localisation.

Emerging evidence, which cannot be reviewed here owing to space limitations, indicates that the mammalian checkpoint kinases ATM, ATR, Chk1 and Chk2 phosphorylate an overlapping set of target proteins. Distinct patterns of target phosphorylation are triggered by different stimuli, such as ionising radiation and UV light. The functional significance of differential phosphorylation is not yet clearly understood but is perhaps best characterised in the case of the tumour suppressor protein p53 (Banin et al., 1998; Canman et al., 1998; Tibbetts et al., 1999). BRCA1 is probably also phosphorylated in a similar manner, by different combinations of the four checkpoint kinases in response to different stimuli. Recent data are consistent with this prediction (Gatei et al., 2001; Tibbetts et al., 2000).

Disruption of *Brca2*, in contrast to *Brca1*, does not appear to have a marked effect on cell cycle checkpoint enforcement (Patel et al., 1998). As yet, it is unclear whether BRCA2 undergoes phosphorylation by checkpoint kinases following DNA damage, but this seems likely. There is, however, some evidence that BRCA2 is involved in the regulation of metaphase progression, although it is unclear whether this proposed role is relevant to DNA damage responses. BRCA*2* can be co-immunoprecipitated with the BubR1 kinase involved in regulating the metaphase-to-anaphase transition during mitosis and can be phosphorylated by BubR1 in vitro, although it remains to be established whether this modification is functionally relevant (Futamura et al., 2000). Inactivation of the metaphase-to-anaphase surveillance mechanism mediated by BubR1 reverses proliferative arrest and fosters tumorigenesis in *BRCA2*-deficient cells (Lee et al., 1999). Finally, BRCA2 exists (Marmorstein et al., 2001) in a high-molecular-weight protein complex with a novel DNA-binding protein, BRAF35, which binds preferentially to branched DNA structures. BRAF35 and BRCA2 co-localise to condensing chromosomes, and injection of antibodies against BRCA2 delays metaphase progression.

Modulation of chromatin and DNA structure

Recent data indicate that BRCA1 contributes to DNA damage responses through its interaction with enzymes that alter chromatin and DNA structure (Fig. 3). Biochemical characterisation of BRCA1-containing protein complexes reveals an association with SWI/SNF proteins that remodel chromatin (Bochar et al., 2000), with regulators of histone acetylation/deacetylation (Pao et al., 2000; Yarden and Brody, 1999) and with two DNA helicases – BLM (the product of the gene mutated in the disease Bloom's syndrome, which predisposes to cancer (Wang et al., 2000) and the novel helicase BACH1 (Cantor et al., 2001). How these interactions are relevant to the function of BRCA1 in DNA damage responses is currently the subject of speculation. Chromatin changes mediated in part by histone modification could affect the accessibility of DNA lesions to the repair machinery. BLM is believed to participate in DNA replication and recombination, whereas disruption of the BACH1-BRCA1 interaction impairs DNA repair (Cantor et al., 2001). There are intriguing but preliminary hints that these interactions are relevant to cancer predisposition. Bloom's syndrome is associated with cancer predisposition in multiple tissues (German, 1993), and heterozygous mutations in *BACH1* occur in a small number of breast cancers (Cantor et al., 2001).

Transcriptional responses to DNA damage and transcription-coupled DNA repair

BRCA1 has been implicated in the transcriptional regulation of several genes activated in response to DNA damage (Fig. 3). These include those encoding the p21 CIP1 cyclin-dependent kinase inhibitor (Li et al., 1999; Somasundaram et al., 1997) and the GADD45 tumour suppressor (Harkin et al., 1999; Li et al., 2000; Zheng et al., 2000), both of which are downstream targets of the p53 pathway. *GADD45* regulation illustrates the complexity of the connections between BRCA1 and the cellular machinery for the response to DNA damage (Harkin et al., 1999; Li et al., 2000; Zheng et al., 2000). BRCA1 in association with a novel transcription factor, ZBRK1, which contains six Zn fingers and an N-terminal KRAB domain, forms a co-repressor complex at a sequence motif within the *GADD45* gene. X-irradiation triggers relief from this co-repression by provoking the dissociation of BRCA1 from the repressor complex, an event dependent upon ATM. Thus, BRCA1 serves to connect role of ATM in the sensing/signalling of DNA damage to the transcriptional regulation of *GADD45*.

BRCA1 might also be connected to the basal transcriptional machinery. It co-purifies with the RNA polymerase II

holoenzyme through an interaction with a helicase component (Anderson et al., 1998). The biological relevance of this interaction is uncertain. One possibility is that it reflects a role for BRCA1 in transcription-coupled DNA repair, a process in which base lesions such as those following X-irradiation or oxidative base damage are removed preferentially from the transcribed DNA strand. BRCA1 is essential for transcription-coupled repair in murine (Gowen et al., 1998; Le Page et al., 2000) and human (Le Page et al., 2000) cells, although the nature of its contribution to the process is not understood. The notion that BRCA1 functions as a sensor/signaller of DNA damage is consistent with the observed interaction (Wang et al., 2000) between BRCA1 and the mismatch proteins Msh2 and Msh6, also essential for transcription-coupled repair, which bind to aberrant DNA structures including base mismatches or loops.

BARD1, which interacts with BRCA1 through the N-terminal RING domain, has been implicated in the control of RNA processing following DNA damage (Kleiman and Manley, 2001). The endonucleolytic cleavage of newly transcribed RNA that precedes polyadenylation is strongly but transiently inhibited after DNA damage in in vitro assays. In vivo, this might serve as a mechanism to avoid the expression of damaged genetic information. This inhibition is dependent upon BARD1 and, potentially, upon formation of a complex with BRCA1 and the polyadenylation factor CstF50 (Kleiman and Manley, 1999; Kleiman and Manley, 2001). Interestingly, the inhibition of RNA cleavage induced by DNA damage in vitro is prevented by a tumour-associated BARD1 mutation (Kleiman and Manley, 2001). These intriguing results suggest another way in which BRCA1 could modulate the transcriptional response to DNA damage. It is unclear whether the mechanism is in any way linked to the ability of the BARD1-BRCA1 complex to function as a ubiquitin ligase of (as yet) indeterminate specificity (Hashizume et al., 2001; Ruffner et al., 2001).

No unified theory for BRCA protein function

Clearly, although compelling evidence implicates both BRCA1 and BRCA2 in the response of mammalian cells to DNA damage, there is little sign that a single property or protein-protein interaction underpins the essential function of either protein in this response. This is particularly so for BRCA1, for which there is evidence of involvement at multiple levels in the DNA damage response (Fig. 3). It might, by contrast, be reasonable to posit that the role of BRCA2 in control of the RAD51 recombinase, and through it in DNA repair by homologous recombination, constitutes a key function. This view will also probably prove simplistic if current circumstantial evidence that the roles of BRCA2 in normal mitotic progression and transcriptional regulation are relevant to DNA repair and chromosomal stability becomes more definitive.

Is there a single biological role that explains tumour suppression by the BRCA proteins? At least in the case of BRCA1, cancer-associated mutations affecting a number of different regions spanning the length of the protein have the common property of being unable to reconstitute DSB repair when expressed in BRCA1-deficient cells (Scully et al., 1999). This finding indicates that many of the protein-protein interactions of BRCA1 promote a common function. Equivalent data are not yet available for the BRCA2 protein. However, over 90% of cancer-associated mutations in *BRCA2* result in truncation of the protein. The identification of two conserved nuclear localisation signals in the extreme C-terminal 156 residues of BRCA2 suggests that most of these disease-causing mutants will be non-functional owing to cytoplasmic mis-localisation (Spain et al., 1999).

Other important issues connected with cancer predisposition by *BRCA* mutations are equally enigmatic. In humans, inheritance of one defective *BRCA* allele is enough to increase cancer predisposition. No defects have yet been identified in heterozygous cells that could explain their susceptibility to transformation. In fact, heterozygosity for *Brca* gene mutations causes no apparent increase in cancer risk in mouse models (Connor et al., 1997a; Cressman et al., 1999; Friedman et al., 1998; Xu et al., 1999a).

Perhaps the least understood feature of disease connected with *BRCA* mutations is its tissue specificity. Why should disruption of proteins expressed in many different tissues, which perform functions apparently fundamental to all cells, result in cancer predisposition in the breast and ovaries? Although it has been suggested that DSB repair by HR is particularly important in such tissues to remove DNA adducts known to be induced by hormones such as oestrogen, it is equally possible that the BRCA proteins have alternative functions unconnected to DNA repair whose loss fosters transformation. There are already some intriguing but highly speculative hints that BRCA1 is essential for ductal morphogenesis (Xu et al., 1999a) in the developing breast and that BRCA1 or BRCA2 regulates the growth response to oestrogen receptor signaling (Bennett et al., 2000; Fan et al., 1999). Further studies to address these issues will be essential to reveal how the BRCA proteins can function as tissue-specific tumour suppressors.

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