

BRCA1 Gene in Breast Cancer

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The *BRCA1* gene was identified and cloned in 1994 based on its linkage to early onset breast cancer and breast-ovarian cancer syndromes in women. While inherited mutations of *BRCA1* are responsible for about 40–45% of hereditary breast cancers, these mutations account for only 2–3% of all breast cancers, since the *BRCA1* gene is rarely mutated in sporadic breast cancers. However, *BRCA1* expression is frequently reduced or absent in sporadic cancers, suggesting a much wider role in mammary carcinogenesis. Since *BRCA1* was cloned in 1994, its molecular function has been the subject of intense investigation. These studies have revealed multiple functions of the *BRCA1* that may contribute to its tumor suppressor activity, including roles in: cell cycle progression, several highly specialized DNA repair processes, DNA damage-responsive cell cycle checkpoints, regulation of a set of specific transcriptional pathways, and apoptosis. Many of these functions are linked to protein/protein interactions involving different portions of the 1,863 amino acid (aa) *BRCA1* protein. *BRCA1* functions in cell cycle progression and the DNA damage response appear to be regulated by distinct and specific phosphorylation events, but the molecular pathways activated by these phosphorylations are only beginning to be unraveled. In addition, the reason that *BRCA1* mutation carriers develop specific tumor types (breast and ovarian cancers in women and possibly prostate cancers in men) is not clearly understood. Elucidation of the precise molecular functions of the *BRCA1* gene product will greatly enhance our understanding of the pathogenesis of hereditary as well as sporadic mammary carcinogenesis. J. Cell. Physiol. 196: 19–41, 2003.

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The *BRCA1* gene on chromosome 17q12-21 was identified and cloned by Miki et al. (1994) after an intensive international effort. Hereditary breast cancers account for 5–10% of all breast cancers, and inherited mutations of the *BRCA1* gene account for about 40–45% of these hereditary cancers (Hall and Lee, 1990; Ford et al., 1994, 1995; Rowell et al., 1994; Easton et al., 1995). However, *BRCA1* mutations account for about 80% of families whose members have a high incidence of both breast and ovarian cancers. The *BRCA1* gene fits the profile of a classical “tumor suppressor gene,” since the breast and ovarian cancers that develop in carriers of *BRCA1* gene mutations almost always exhibit loss of the wild-type *BRCA1* allele (Neuhausen and Marshall, 1994; Cornelis et al., 1995). In contrast, *BRCA1* gene mutations are rare in sporadic breast cancer cases and relatively uncommon in sporadic ovarian cancers (Futreal et al., 1994).

Over the last 8 years, much has been learned about the unique features of *BRCA1* mutant breast cancers and about the structure and function of the *BRCA1* gene product. In this article, we will review the unique clinical features of *BRCA1* mutant breast cancers and

particularly focus on the functional activities of the *BRCA1* protein that may account for its ability to suppress tumor formation. We will also review what has been learned about the functional role of *BRCA1* in breast cancer development based on experimental animal models. It is hoped that this review will provide a framework for understanding what the *BRCA1* gene normally does, and why mutations of this gene lead to cancer and, in particular, to breast cancer.

Hereditary breast cancers

Heredity (familial) breast cancer syndromes account for about 5–10% of all breast cancer cases, while the other 90–95% of breast cancers are considered to

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Received 18 November 2002; Accepted 10 December 2002

DOI: 10.1002/jcp.10257



be "sporadic." The hereditary early onset breast cancer syndrome is characterized by the following features:

- At least four or more affected members of a kindred;
- An increased frequency of early onset breast cancer cases (i.e., before age 40);
- Cancers are frequently multicentric and bilateral;
- Life-time (cumulative) risk of female breast cancer of well over 50%;
- Frequent association with ovarian cancer;

About 80% of the cases of hereditary breast cancer are due to mutations of either BRCA1 (40–45%) (chromosome 17q12–21) (Miki et al., 1994) or BRCA2 (13q12) (Wooster et al., 1995) (35–40%). Some of the remaining 20% of cases are associated with mutations of various genes: e.g., Cowden syndrome (the PTEN tumor suppressor), Li-Fraumeni syndrome (p53), and the androgen receptor (Harris et al., 1992; Cannon-Albright and Skolnick, 1996; Greene, 1997). These known genes do not account for 100% of hereditary breast cancers, suggesting there may another susceptibility gene (*BRCA3*) to be discovered (de Jong et al., 2002).

BRCA1 and BRCA2

About a year after the identification of BRCA1, a second breast cancer susceptibility gene (*BRCA2*, on human chromosome 13q12) was identified and cloned (Wooster et al., 1995; Tavtigian et al., 1996). The *BRCA2* gene product (3,418 aa, $M_r \approx 380$ kDa) is even larger than that of BRCA1 (1,863 aa, $M_r \approx 220$ kDa). Although *BRCA2* is structurally distinct from *BRCA1*, various studies suggest that the expression of *BRCA1* and *BRCA2* is co-regulated during cell cycle progression and in response to DNA damage (Monteiro et al., 1996; Rajan et al., 1996; Andres et al., 1998; Fan et al., 1998a,b, 1999b) and that *BRCA1* and *BRCA2* have overlapping functions (e.g., their roles in DNA repair and transcriptional regulation) (Connor et al., 1997; Milner et al., 1997; Sharan et al., 1997; Wong et al., 1997; Scully et al., 1997c). In addition, the expression patterns of *BRCA1* and *BRCA2* during mouse development are similar, although not identical (Rajan et al., 1997) (see "Brcal Deficient Animal Models"). These findings suggest that *BRCA1* and *BRCA2* may function in the same molecular pathways. Nevertheless the functions of these genes are not identical, since disruption of either gene by gene targeting of embryonic stem cells leads to early lethality of the homozygous mutant embryos (Gowen et al., 1996; Hakem et al., 1996; Liu et al., 1996; Sharan et al., 1997; Suzuki et al., 1997). Thus, the *Brcal* gene cannot take over all of the essential functions of the *Brcal* gene during early development, and vice versa.

Consistent with these observations, the clinical syndromes resulting from inherited mutations of *BRCA1* and *BRCA2* are similar but not identical. Both *BRCA1* and *BRCA2* mutations predispose to ovarian and breast cancers in women; and the relative proportions of each tumor type depends on the mutation site (see "Genotype–Phenotype Correlation"). However, while *BRCA2* mutations are frequently associated with male breast cancers, male *BRCA1* mutation carriers

rarely develop breast cancer (Couch et al., 1996; Thorlacius et al., 1996; Basham et al., 2002). Both *BRCA1* and *BRCA2* mutations appear to confer an increased risk of prostate cancer (Ford et al., 1994; Langston et al., 1996; Sigurdsson et al., 1997; Streuwing et al., 1997). But sporadic prostate cancers rarely show *BRCA1* or *BRCA2* mutations, suggesting that other factors contribute to most of these cancers (Hubert et al., 1999; Nastiuk et al., 1999; Wilkens et al., 1999). *BRCA2* mutation carriers are also at increased risk for a variety of other cancer types (e.g., pancreatic cancer), although the risk is not as high as that for breast cancer (Thorlacius et al., 1996; Easton et al., 1997). It was originally thought that *BRCA1* mutation carriers also had an excess risk for other common tumor types, such as colon cancer (Ford et al., 1994). However, recent studies suggest that this is probably not the case for *BRCA1* (Streuwing et al., 1997).

CLINICAL PERSPECTIVES

Hereditary BRCA1 mutant breast cancers

Studies of *BRCA1* breast cancer families suggest that an inherited mutation of *BRCA1* confers a near certainty of female breast cancer (85–90% by age 70) (Hall and Lee, 1990; Rowell et al., 1994; Easton et al., 1995). The risk of developing breast cancers at a very early age—before 40 or even 30 years—is substantially elevated in *BRCA1* mutation carriers. Thus, among women who developed breast cancer before age 30, about 10–15% were found to carry mutations of either *BRCA1* or *BRCA2* (Fitzgerald et al., 1996; Peto et al., 1999). Nevertheless, for *BRCA1* mutation carriers, the risk of developing breast cancer continues throughout life. In *BRCA1* breast–ovarian cancer families, the cumulative risk of developing ovarian cancer by age 70 was estimated to be about 55–60% (Easton et al., 1995).

Population-based studies

Within *BRCA1* breast cancer families, the cumulative risks by age 70 are about 85–90% for breast cancer and 60% for ovarian cancer (Hall and Lee, 1990; Easton et al., 1994; Narod et al., 1995). These estimates were based on studies of breast and ovarian cancer families and thus may select for women who have other mutations that further pre-dispose to cancer. Population-based studies revealed that specific germ-line mutations of *BRCA1* (185delAG and 5382insC) or *BRCA2* (6174delT) occurred relatively frequently in Ashkenazi Jews (up to 1% each); and their combined frequency exceeded 2% (Streuwing et al., 1995, 1997; Roa et al., 1996). This contrasts with an estimated *BRCA1* mutation frequency in the general population of about 0.06% (Ford et al., 1995). Among Ashkenazi women, one of these founder mutations was observed in 30% of women with early-onset breast cancer (before age 40) and 60% of women with ovarian cancer at any age (Abeliovich et al., 1997). Carriers of these mutations had an estimated cumulative risk by age of 70 of 56% for breast cancer and 16% for ovarian cancer (Streuwing et al., 1997). Among mutation carriers, only 45% had a history of breast cancer in a first degree relative, as compared with 20% for non-carriers (Streuwing et al., 1997). Thus, the cancer risk associated with *BRCA1* mutations is not as high as once

thought. It is not known if these risk estimates will hold for women of other ethnic origins.

Immunophenotype of BRCA1 mutant cancers

Histopathologic and genetic studies of BRCA1 mutant breast cancers suggest a characteristic immunophenotype, distinct from those of sporadic cancers and BRCA2 linked breast cancers. Thus, BRCA1 mutant tumors often exhibit highly poor nuclear grade, have a very high frequency of p53 mutations (84%, as compared with 20–25% for sporadic cancers), and are more frequently estrogen and progesterone receptor (ER and PR) negative (Eisinger et al., 1996; Breast Cancer Linkage Consortium, 1997; Johannsson et al., 1997; Karp et al., 1997; Osin et al., 1998; Armes et al., 1999; Greenblatt et al., 2001). Interestingly, the p53 mutation spectrum (i.e., mutation site and type) observed in BRCA1 breast cancers is distinct from that found in sporadic cancers (Buller et al., 2001; Greenblatt et al., 2001). Despite the fact that these three features usually connote a poor prognosis, stage for stage, women with BRCA1 mutations do not suffer an adverse prognosis relative to women with sporadic cancers. This observation is explained, in part, by a higher than expected frequency of the medullary histotype in women with BRCA1 mutant breast cancers (Armes et al., 1999). Medullary histology is usually associated with a more favorable prognosis than most other histologies (Harris et al., 1992). BRCA2 mutations are associated with breast cancers of higher architectural grade, p53 mutations, estrogen receptor positivity, and poor prognosis (van den Berg et al., 1996; Johannsson et al., 1997; Karp et al., 1997). These findings may reflect distinct genetic alterations in breast cancers due to BRCA1 vs. BRCA2 mutations (Tirkkonen et al., 1997).

Sporadic breast cancers exhibit a relatively high frequency of *HER2/neu* and *cyclin D1* gene amplification (20–25% and 15–20%, respectively), as demonstrated by FISH (fluorescent *in situ* hybridization) analysis (Pavelic et al., 1992; Dickson et al., 1995; Barnes and Gillett, 1998; Spyros et al., 2000; Lohrisch and Piccart, 2001). The incidence of over-expression of the *HER2/neu* and *cyclin D1* proteins is even higher (about 25–30% and 30–40%, respectively); and these two genes are thought to function as essential oncogenes during the development of a subset of human breast cancer cases (reviewed in Barnes and Gillett, 1998; Lohrisch and Piccart, 2001). In contrast, BRCA1 mutant breast cancers rarely exhibit amplification of the *HER2/neu* or *cyclin D1* genes and show a very low incidence of *HER2/Neu* and *cyclin D1* protein over-expression (Vaziri et al., 2001; Grushko et al., 2002; Lakhani et al., 2002). In addition to immunophenotypic characteristics, analysis of human breast cancers by DNA microarray analyses suggest a characteristic pattern of gene expression alterations in BRCA1 vs. BRCA2 cancers (Hedenfalk et al., 2001; van't Veer et al., 2002).

While amplification of the Myb oncogene is relatively uncommon in sporadic breast cancers (2%), a recent study documents a high frequency of Myb amplification (5/17 or 29%) in BRCA1 cancers (Kauraniemi et al., 2000). Interestingly, *Drosophila* Myb was recently found to be required for G2/M cell cycle progression and its mutational inactivation leads to centrosomal

amplification and genomic instability (Fung et al., 2002). BRCA1 has been implicated as a regulator of the same processes [G2/M cell cycle checkpoint, centrosomal replication, and genomic stability (see below)], suggesting that Myb amplification in BRCA1 mutant breast cancers is not a random event. Taken together with the other immunophenotypic characteristics, these findings suggest a unique pathway for the development of BRCA1 mutant breast cancers, one which usually requires p53 mutations and the loss of ER, but which does not require *HER2/neu* or *cyclin D1* over-expression.

Genotype–phenotype correlation

Most BRCA1 mutations are frame-shift mutations resulting in truncated BRCA1 proteins; although point mutations in the C-terminal transcriptional activation domain or N-terminal ring domain are known (Friedman et al., 1994; Miki et al., 1994; Gayther et al., 1995). This contrasts with p53, for which most tumor-associated mutations are missense mutations resulting in an altered full-length protein (Runnebaum et al., 1991). Analysis of BRCA1 cancer families revealed a correlation between the mutation site and the relative risk of breast vs. ovarian cancer. 3' mutations, which cause truncation of the C-terminal region, yielded a higher proportion of breast than ovarian cancers; while 5' mutations, which delete a large segment of the protein, yielded a mixture of breast and ovarian cancers (Gayther et al., 1995). Based on statistical analysis of the relative breast/ovarian cancer incidence, it was predicted that the crossover point after which mutations yield mostly breast cancers rather than both tumors occurs at amino acid 1,435–1,443 (Gayther et al., 1995). A genotype–phenotype correlation was also described for BRCA2. Nearly all ovarian cancers in BRCA2 families were linked to mutations within a 3.3 kb region of exon 11 (Gayther et al., 1997). Mutations corresponding to the N- and C-termini of the BRCA2 protein were associated with breast cancer alone.

5'-Mutations in the regulatory region (which ablate BRCA1 expression) yielded almost all breast cancers (Gayther et al., 1997). These mutations comprise 10–15% of mutations in BRCA1 families. These findings were interpreted as suggesting an ovarian tumor suppressor function in the central/C-terminal region and N- and C-terminal breast cancer suppressor domains. This model does not explain the lack of ovarian cancers associated with 5' regulatory mutations. It is possible that certain truncated BRCA1 proteins are directly oncogenic to ovarian epithelium. Thus, p53 mutant proteins may act as dominant inhibitors of wild-type p53 and may be oncogenic in the absence of wild-type allele (Shaulian et al., 1992; Dittmer et al., 1993).

A similar mechanism (i.e., expression of oncogenic mutant proteins) could explain why BRCA2 mutations causing ovarian cancer are clustered in exon 11, and why ovarian cancer-associated mutations are not found in the N-terminus of BRCA2 (Gayther et al., 1997). One caveat is that these studies were performed using selected breast-ovarian cancer families; and population-based studies might not yield identical findings. Thus, in the population study of Ashkenazi Jewish women, N-terminal (185delAG) and C-terminal (5382insC) mutations of BRCA1 conferred similar risks for breast

and ovarian cancers (Streuwling et al., 1997), even though the 5382insC mutation is predicted to yield a lower risk for ovarian cancer (Gayther et al., 1995).

Breast cancer prevention trials

Recent breast cancer prevention trials have tested the ability of SERMs (selective estrogen response modifiers) Tamoxifen [NSABP (National Surgical Adjuvant Breast Cancer Program) P1 trial] and Raloxifene [MORE (Multiple Outcomes of Raloxifene Evaluation) trial] to reduce the incidence of breast cancer development in high-risk female study populations. These studies have thus far showed overall breast cancer risk reductions of about 50% and 65%, respectively (Dickler and Norton, 2001; King et al., 2001). The risk reduction was mostly for estrogen receptor positive tumors; most of the tumors that developed in Tamoxifen-treated women were estrogen receptor negative. Although the number of patients included in breast cancer chemoprevention studies with BRCA1 or BRCA2 mutations is small, analysis of the results to this point suggest that women with BRCA1 mutations derive little or no benefit from Tamoxifen, while women with BRCA2 mutations do benefit significantly from Tamoxifen (King et al., 2001; Duffy and Nixon, 2002). It is noted that the NSABP P1 trial only included women of age 35 years or older; so it is not known if earlier treatment of BRCA1 mutation carriers would result in a significant cancer risk reduction.

BRCA1 GENE AND PROTEIN STRUCTURE BRCA1 gene and mRNA

The *BRCA1* gene contains 24 exons (22 coding and two non-coding) and covers a span of ≈ 100 kb of genomic DNA (Miki et al., 1994; Smith et al., 1996). The *BRCA1* coding region comprises ≈ 5.5 kb and is predicted to encode a protein of 1,863 amino acids with relatively little overall amino acid sequence homology to known proteins. In addition to the full-length 8 kb mRNA transcript, various smaller *BRCA1* transcripts with a tissue specific expression pattern have also been described, including 7, 4.6, and 1.5–2.2 kb mRNAs. Several naturally occurring splice variants of *BRCA1* that encode truncated proteins missing all or part of exon 11 (the largest exon) have been identified (Thakur et al., 1997; Wang et al., 1997; Wilson et al., 1997; Cui et al., 1998). The best characterized of these is the 4.6 kb alternative splice product missing nucleotides 672–4,092, which encodes a truncated protein of about 97 kDa. The *BRCA1* Δ exon 11 isoform is missing two classical nuclear localization signals (NLS), but recent studies suggest this isoform can still localize to the nucleus and retains some *BRCA1* functional activity (Huber et al., 2001).

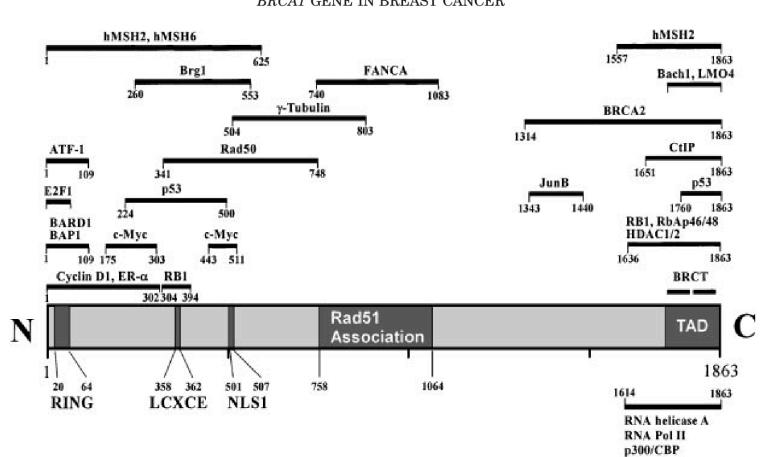
BRCA1 expression is regulated coordinately with *BRCA2* (Rajan et al., 1996). *BRCA2* mRNA and protein levels showed a pattern of cell cycle variation similar to that of *BRCA1* (Rajan et al., 1996; Vaughn et al., 1996a; Bervis et al., 1997). Expression of *BRCA1* and *BRCA2* decreased when breast epithelial or cancer cells were induced to become quiescent by confluence, serum starvation, or transforming growth factor- β (TGF- β) (Rajan et al., 1996). Conversely, serum stimulation of

quiescent cells caused re-expression of *BRCA1* and resumption of cell proliferation (Rajan et al., 1996). In estrogen-responsive breast cancer cell lines (e.g., MCF-7, BT-20), addition of estrogen (17 β -estradiol) to estrogen-starved cultures caused delayed up-regulation of *BRCA1* mRNA and protein (Gudas et al., 1995; Spillman and Bowcock, 1996; Marks et al., 1997) and coordinate up-regulation of *BRCA2* mRNA (Rajan et al., 1996; Spillman and Bowcock, 1996).

The up-regulation of *BRCA1* occurred by an indirect mechanism related to entry into S-phase, since it paralleled the induction of cyclin A and of DNA synthesis (Marks et al., 1997). Although the 5' region of the *BRCA1* gene contains several putative estrogen-responsive DNA sequences (Xu et al., 1995), estrogen failed to activate reporter plasmids containing these sequences in breast cancer cell lines (Marks et al., 1997). *Brca1* was expressed independently of estrogen or progesterone stimulation in proliferating granulosa-theca cell populations of the mouse ovary and was similarly expressed in estrogen receptor deficient mice (Phillips et al., 1997), providing further evidence that estrogen regulation of *BRCA1* is indirect. While exit from the cell cycle was associated with decreased expression, glucocorticoid-induced differentiation of mammary epithelial cells was associated with increased *BRCA1* expression (Rajan et al., 1996). This finding is consistent with the observation that *Brca1* expression is up-regulated in the mammary gland and other differentiating tissues during murine fetal and post-natal development (Lane et al., 1995; Marquis et al., 1995). Thus, *BRCA1* appears to play a role in mammary epithelial differentiation, although its particular function is not yet clear.

BRCA1 protein

Amino-terminal ring finger domain. One clue to *BRCA1* function is that the amino-terminal region (approximately aa 20–68) contains a ring finger domain containing the cys₃-his-cys₄ structure (Miki et al., 1994) characteristic of the ring finger family of transcriptional regulatory proteins (Saurin et al., 1996) (see Fig. 1). This segment of *BRCA1* has considerable homology to several other ring finger proteins, including the RET oncogene product, in addition to the conserved cysteines (Miki et al., 1994). The function of the ring finger domain in *BRCA1* is not definitively established; but a yeast two-hybrid screen identified another ring finger protein that interacts with the *BRCA1* ring domain, called the *BRCA1*-associated ring domain protein (BARD1) (Wu et al., 1996). Breast cancer-associated missense mutations of the *BRCA1* ring domain (e.g., C61G) disrupt the *BRCA1*:BARD1 interaction (Wu et al., 1996); and mutations in the *BARD1* gene, including a germ-line mutation, have been detected in a small percentage of breast, ovarian, and uterine cancers (Thai et al., 1998). The function of BARD1 is unclear, but a recent study indicates that BARD1 interacts with an RNA polyadenylation factor (Cst-50) and inhibits its activity, a finding that may implicate BARD1 in the regulation of RNA processing during transcription and DNA repair (Kleiman and Manley, 1999). Other activities involving the ring domains of *BRCA1* and BARD1 are described below ("BRCA1 Regulation of the Proteome").



BRCA1 Protein Interactions

Fig. 1. Schematic diagram summarizing BRCA1 protein:protein interactions. Abbreviations: ATF-1, activating transcription factor-1; BAP1, BRCA1-associated protein 1; BARD1, BRCA1-associated ring domain protein-1; BRCT, BRCA1 C-terminal repeat; CBP, p265 CREB binding protein; CtIP, CtBP (C-terminal binding protein) interacting

protein; ER- α , estrogen receptor- α ; HDAC1/2, histone deacetylase-1/2; LXXCE, consensus RB family protein binding motif; NLS1, primary nuclear localization signal; RB1, retinoblastoma-1; RbAp46/48, retinoblastoma-associated protein 46/48 kDa; RING, ring finger (zinc finger-like) domain; TAD, transcriptional activation domain.

Another study suggests that the BRCA1 ring domain can bind to various cell cycle proteins, including cyclins (D1, A, B1), cyclin-dependent kinases (CDC2, CDK2, CDK4), and E2F-4, a member of the E2F transcription factor family (Wang et al., 1997). The ring domain, but not tumor-associated ring domain mutants, interacts with a novel protein (BRCA1 associated protein-1, or BAP1) homologous to C-terminal ubiquitin hydrolases (Jensen et al., 1998). Disruption of the ring domain (by the mutation C61G) also blocked the ability of BRCA1 to repress estrogen receptor- α (ER- α) signaling (Fan et al., 2001a) and to modulate DNA repair (Ruffner et al., 2001), chemosensitivity, and apoptosis (Fan et al., 2001b). These findings suggest that the ring domain mediates critical protein interactions, and disruption of which contributes to carcinogenesis.

Carboxyl-terminal acidic domain. A second clue to the function of BRCA1 is that the C-terminal region contains an acidic domain (Miki et al., 1994) that can function as a transcriptional activation domain (TAD) in yeast and mammalian cells, when linked to a DNA-binding domain (Gal4) (Chapman and Verma, 1996; Monteiro et al., 1996). TAD function requires the last 300 or so amino acids (aa 1,560–1,863) for maximal activity. However, partial transcriptional activity is retained by a “minimal TAD” (aa 1,760–1,863). Deletion of a short segment or cancer-associated point mutations in the minimal TAD led to ablation of activity. A third clue to BRCA1 function is that computer analysis suggests that the minimal TAD contains a tandem repeat of about 95 aa (called “BRCT” for BRCA1 C-terminus) similar to C-terminal domains found in a variety of proteins involved in cell cycle checkpoints and DNA repair (Koonin et al., 1996; Bork et al., 1997; Williams et al., 2001). As described below, the BRCT

domain of BRCA1 mediates protein interactions that are critical to its role in transcriptional regulation and the response to DNA damage.

BRCA1 subcellular localization. It was originally thought that BRCA1 was a cytoplasmic and extracellular protein, based on immunolocalization studies and the presence of a putative granin motif characteristic of secretory proteins (Jensen et al., 1996). However, it is now evident that the full-length 220 kDa BRCA1 protein is normally a nuclear protein, targeted to the nucleus via two nuclear localization signal sequences [NLS1 (⁵⁰¹KLKRKRR) and NLS2 (⁶⁰⁷KKNRLRRK)], one of which (NLS1) is more important for nuclear targeting (Thomas et al., 1996; Chen et al., 1996a,b; Thakur et al., 1997). Breast and ovarian cancer cell lines may also exhibit cytoplasmic localization of endogenous BRCA1 isoforms as well as exogenous epitope-tagged full-length BRCA1; although normal cells and other tumor types show primarily nuclear BRCA1 (Chen et al., 1995; Scully et al., 1996). Part of the discrepancy in the BRCA1 localization studies may be explained by: (1) splice variants missing all or part of exon 11, including NLS1; (2) the finding that low expression of full-length BRCA1 leads to nuclear localization, while high expression causes spill-over into the cytoplasm (Chen et al., 1995); (3) cross-reaction of certain BRCA1 antibodies with the epidermal growth factor receptor (Thomas et al., 1996); and (4) development of mechanisms of nuclear exclusion or cytoplasmic sequestration by breast and ovarian cancers as a means to escape the control of wild-type BRCA1. However, the significance of and mechanisms responsible for aberrant BRCA1 localization remain to be elucidated.

It was initially believed that a classic NLS1 was absolutely required for BRCA1 nuclear localization (Thakur

et al., 1997), but recent studies suggest that this may not be the case. Fan et al. (2001b) found that a truncated BRCA1 protein containing only the N-terminus (aa 1–302) enters the nucleus. Similarly, a BRCA1 mutant protein corresponding to a naturally occurring isoform (*Brc1* Δ exon 11) enters the nucleus despite the fact that this isoform is missing both NLS1 and NLS2 (Huber et al., 2001). These findings suggest the presence of a cryptic (i.e., non-classical) NLS within the first 300 or so aa of BRCA1. Alternatively, truncated or deleted forms of BRCA1 may enter the nucleus “piggy-backed” to another nuclear protein.

The BRCA1 protein was found to contain a functional HIV Rev-type nuclear export signal (NES) within its N-terminus [⁸¹QLVEELLKIICAFQLDTGGL] that facilitates its nuclear exit via the CRM1/exportin pathway (Rodriguez and Henderson, 2000). Mutation of the hydrophobic residues of this putative NES or treatment of cells with leptomycin B, a CRM1-dependent export inhibitor, resulted in nuclear accumulation of BRCA1. In an earlier study, it was demonstrated that heregulin could induce the phosphorylation of BRCA1 through HER2/neu-mediated activation of the serine/threonine kinase c-Akt (protein kinase B) (Altioik et al., 1999). The c-Akt-mediated phosphorylation of BRCA1 occurred on T508, which is part of an Akt consensus phosphorylation motif [RXRXX(S/T)], where R = arginine, S = serine, T = threonine, and X = any amino acid] (Datta et al., 1999) immediately adjacent to NLS1 [⁵⁰¹KLKRKRRPTS]. The phosphorylation of T508 resulted in cytoplasmic accumulation of BRCA1 (Altioik et al., 1999). Interestingly, BRCA1 has a second consensus Akt phosphorylation motif adjacent to NLS2 [⁶⁰⁷KKNRLRRKSST], although the physiological importance of this site has not been established. Taken together, these findings suggest that BRCA1 may shuttle back and forth between the nucleus and cytoplasm in a physiologically regulated fashion, and that cytoplasmic BRCA1 may have some, as yet unidentified, physiologic function (Rodriguez and Henderson, 2000).

The finding that Akt can phosphorylate and inactivate BRCA1 suggests a mechanism by which BRCA1 can be functionally inactivated during sporadic mammary carcinogenesis. Thus, heregulin induces BRCA1 phosphorylation via HER2/Neu receptor signaling (Altioik et al., 1999). Amplification and/or over-expression of HER2/Neu occurs in a significant proportion of breast cancers (≥20–25%) and has both prognostic and therapeutic implications (reviewed in Lohrisch and Piccart, 2001). Thus, HER2/Neu positivity generally connotes a poorer prognosis and is used to predict clinical responsiveness to the humanized anti-HER2 monoclonal Herceptin. The activation of the c-Akt cell-survival signaling pathway is a common event during breast cancer progression (Hutchinson et al., 2001). At this point, it is conjectural whether functional inactivation of BRCA1 through HER2/Neu and other pathways leading to c-Akt activation occurs and is of pathophysiologic importance during breast cancer progression. However, in contrast to sporadic tumors, BRCA1 mutant breast cancers show a very low incidence of HER2/Neu positivity, suggesting a lack of selection pressure for HER2/Neu expression in this setting (Grushko et al., 2002; Lakhani et al., 2002).

BRCA1 protein: protein interactions

BRCA1 is a large protein and is able to undergo many protein interactions—as judged by in vitro binding and in vivo association assays—that may contribute to its biological functions. Various classes of proteins interact with BRCA1, including: (1) components of the basal transcription machinery [e.g., RNA helicase A and RNA pol II (Anderson et al., 1998; Schlegel et al., 2000a)]; (2) generalized transcriptional coactivators [p300, CBP, Brg1 (Bochar et al., 2000; Pao et al., 2000)] and corepressors [e.g., RbAp46, RbAp48, histone deacetylases-1,2, and CtIP (Yarden and Brody, 1998; Yu et al., 1998)]; (3) tumor suppressors [e.g., p53, RB1, BRCA2 (Chen et al., 1998; Ouichi et al., 1998; Yarden and Brody, 1998; Zhang et al., 1998; Aprelikova et al., 1999; Chai et al., 1999; Fan et al., 2001c)]; (4) steroid hormone receptors, estrogen receptor- α , and androgen receptor (Yeh et al., 2000; Fan et al., 2001a); (5) DNA repair proteins [e.g., Rad51, Rad50, hMSH2 (Scully et al., 1997b; Zhong et al., 1999; Wang et al., 2001a)]; (6) other sequence-specific transcription factors [e.g., c-Myc, Oct-1, and NF-YA (Wang et al., 1999; Fan et al., 2002b)]; and (7) cell cycle regulatory proteins [e.g., BARD1, E2F1, cyclins (Wu et al., 1996; Wang et al., 1997)]. These interactions are summarized in Figure 1; and the significance of these interactions is discussed in “Functional Activities of BRCA1”.

Regulation of BRCA1 expression

Cell cycle control of BRCA1 expression and phosphorylation. BRCA1 expression in human mammary epithelial and cancer cell lines varies with the cell cycle (Gudas et al., 1996; Chen et al., 1996a; Vaughn et al., 1996b; Marks et al., 1997). BRCA1 mRNA and protein expression rise in mid-late G1, before the onset of DNA synthesis, coincident with the rise of cyclin A levels; peak at the G1/S interface or in early-mid S; and decline by late S or G2. As BRCA1 levels rise at the G1/S boundary, there is a concomitant increase in protein phosphorylation, demonstrated by the shift to a slower migrating form of BRCA1 that is abrogated by treatment with phosphatases (Chen et al., 1996a; Thomas et al., 1997; Scully et al., 1997c). Immunoprecipitation/in vitro kinase assays revealed that several cyclins and associated cyclin-dependent kinases (cyclins D and A, CDK2) can associate with and phosphorylate BRCA1 on tyrosine residues in human mammary cell line HBL100 (Wang et al., 1997). In addition, a CDK2/cyclin A or E complex phosphorylates BRCA1 at a CDK consensus phosphorylation site (serine-1497) both in vitro and in vivo (Ruffner et al., 1999).

Specific phosphorylation of the retinoblastoma (RB1) protein by G1 cyclin-dependent kinases causes the release of the E2F family transcription factors required in early S-phase (reviewed in Sherr, 1993; Weinberg, 1995; Cobrinik, 1996). BRCA1 contains the consensus RB1 family protein binding sequence LXCXE [aa 358–362; L = leucine, C = cysteine, E = glutamic acid, and X = any amino acid] and an atypical Rb binding motif LXCXXE (aa 439–444) (see Fig. 1), suggesting that a BRCA1:RB1 family protein interaction may regulate the transition from G1 into S. Recently, two protein interactions between BRCA1 and RB1 have been

described, one involving the C-terminal TAD of BRCA1 (Yarden and Brody, 1998) and the other involving an N-terminal domain between aa 300 and 400 (Aprelikova et al., 1999; Fan et al., 2001c). In the latter study, the ability of BRCA1 to cause growth arrest at G1/S was dependent upon the presence of RB1 in the cell. Neither the LXCXE nor the LXCXXE motif was required for in vitro binding or in vivo association of BRCA1 with RB1. However, expression of a full-length BRCA1 with a mutation of the LXCXE motif (³⁵⁸LXCXE → RXRXH) conferred an altered phenotype characterized by: (1) chemoresistance; (2) failure to down-regulate RB1 expression; and (3) inhibition of the wild-type BRCA1 mediated chemosensitization and down-regulation of RB1 in prostate and breast cancer cells (Fan et al., 2001c).

BRCA1 may also be involved in a G2/M cell cycle checkpoint. Expression in human mammary epithelial cells of a truncated BRCA1 protein containing the full TAD and sequences immediately N-terminal to it caused a decreased cell cycle transit time, increased proliferation rate, and reduction of the G2/M arrest induced by the spindle poison colcemid (Larson et al., 1997). The authors suggest that competitive inhibition of the TAD of endogenous BRCA1 by the truncated BRCA1 inhibited G2/M progression. Curiously, this truncated BRCA1 protein lacks NLS1 and is thus expected to remain in the cytoplasm. However, the authors provide evidence that this protein is phosphorylated; and nuclear localization may occur by interaction with another nucleoprotein. The mechanism by which BRCA1 regulates G2/M progression is unclear, but it is noteworthy that the truncated BRCA1 protein contains domains that interact with the RNA polymerase II holoenzyme (Scully et al., 1997a; Anderson et al., 1998; Schlegel et al., 2000a; Chiba and Parvin, 2002) as well as a region homologous to Rad9, a protein involved in the G2 damage-responsive cell cycle checkpoint (Weinert and Hartwell, 1988; Hirai and Wang, 2002; Yoshida et al., 2002). Roles for BRCA1 in several DNA damage-responsive checkpoints (including G2/M) have been established and are discussed (see "Caretaker Role: Maintenance of Genomic Integrity").

Transcriptional regulation of the BRCA1 gene. As discussed below ("Cell Cycle Regulation and Growth Control"), the mRNA expression, protein levels, and phosphorylation state of BRCA1 are cell cycle dependent. For the most part, the molecular mechanisms of endogenous regulation of BRCA1 expression and BRCA1 regulation by DNA damage, xenobiotic agents, and nutrients (described below) are not well understood. However, over the last few years, several factors that can cause the down- or up-regulation of BRCA1 expression have been identified. Using a reverse ribozyme library based approach, Beger et al. (2001) identified Id4 as a potent inhibitor of BRCA1 expression. Id proteins contain helix-loop-helix (HLH) dimerization motifs, but do not have the DNA-binding basic domain found present in basic HLH proteins, thereby allowing them to function as inhibitors of bHLH transcription factors. A CREB/ATF-1 site within the BRCA1 proximal promoter (Atlas et al., 2001) and a transcriptional repressor element in the first intron of BRCA1 (Suen and Goss, 2001) function, respectively, as constitutive positive and

negative regulatory elements for BRCA1. Several studies indicate that activation of the p53 tumor suppressor protein inhibits BRCA1 gene expression (Arizi et al., 2000; MacLachlan et al., 2000), raising the possibility of a p53:BRCA1 negative feedback loop, since BRCA1 binds p53 and co-activates its transcriptional activity (see below, "Regulation of Transcription").

Several sequence-specific DNA binding transcription factors were identified as potent regulators of the BRCA1 promoter, including: (1) the GA binding protein GABP α/β [a transactivator in MCF-7 but to a lesser extent T47D cells (Atlas et al., 2000)]; and (2) the POU family transcription factor Brn-3b, which negatively regulates BRCA1 in mammary tumor cells and whose expression is inversely correlated with that of BRCA1 in breast cancers (Budhram-Mahadeo et al., 1999). Treatment of breast and prostate cancer cells with 1 α ,25-dihydroxyvitamin D₃ induced BRCA1 expression through the vitamin D receptor, a mechanism that may contribute to vitamin D receptor mediated growth arrest and apoptosis (Campbell et al., 2000). BRCA1 may also be regulated separately at the protein level (Blagosklonny et al., 1999; Ma et al., 2003).

Decreased BRCA1 expression in sporadic breast and ovarian cancers. Although inherited mutations of BRCA1 account for a small minority of all breast cancers (2.5–5%), BRCA1 may play a much wider role, in many sporadic cancers. Thus, 30–40% of sporadic breast cancers show reduced expression of BRCA1 mRNA and protein relative to the uninvolved normal breast, particularly tumors with high nuclear grade (Thompson et al., 1995; Taylor et al., 1998; Lee et al., 1999; Wilson et al., 1999; Yang et al., 2001). The mechanisms of the observed down-regulation of BRCA1 in sporadic human breast cancers is unclear, but may involve some of the transcriptional pathways described above. Alternatively, or in addition, about 10–15% of sporadic breast and ovarian cancer cases exhibit hyper-methylation of the BRCA1 promoter, associated with a reduction of BRCA1 mRNA expression (Baldwin et al., 2000; Esteller et al., 2000; Magdinier et al., 2000; Rice and Futscher, 2000; Rice et al., 2000; Chan et al., 2002). The repression of BRCA1 transcription was due to aberrant methylation of CpG islands, and some of the methylation sites have been mapped.

Down-regulation by DNA damaging agents and polycyclic hydrocarbons. DNA damaging agents. Finally, following certain forms of DNA damage (e.g., induced by the DNA topoisomerase II α inhibitor adriamycin and the topoisomerase I inhibitor camptothecin), BRCA1 mRNA expression is reduced within 4–8 h, and the protein disappears over 24–72 h (Andres et al., 1998; Fan et al., 1998a,b). As described below, the DNA damage-induced down-regulation of BRCA1 at the mRNA and protein levels is preceded by its rapid hyperphosphorylation and subnuclear translocation, which occurs in <1 h. In characterizing the disappearance of the BRCA1 following DNA damage, it was observed that the higher M_r (hyper-phosphorylated) species decreased more rapidly (starting at 2–8 h post DNA damage) than did the lower M_r (hypo-phosphorylated) species (Fan et al., 1999b). The physiological regulation of BRCA1 following DNA damage may be critical for cell survival, since enforced expression of a wild-type BRCA1 gene in

cells with endogenous functional BRCA1 conferred reduced DNA repair activity and increased sensitivity to apoptosis induced by adriamycin or camptothecin (Fan et al., 1998b, 2001b,c).

Polyyclic aryl hydrocarbons (PAHs). The carcinogen benzo(a)pyrene [B(a)P] and its metabolite, BPDE, were found to inhibit BRCA1 promoter activity and to down-regulate BRCA1 protein levels in estrogen receptor positive human breast cancer cell lines, such as MCF-7 (Jeffy et al., 1999, 2002). The inhibition of BRCA1 promoter activity required functional aryl hydrocarbon receptor (AhR) and p53. AhR is a ligand-dependent transcription factor that targets promoters containing the xenobiotic response element (XRE) (also known as the dioxin response element, DRE), which include drug-metabolizing enzymes such as CYP1A1 and CYP1B1. AhR activation was found to be necessary but not sufficient for the inhibition of BRCA1 promoter activity, since the non-metabolizable AhR ligand TCDD (dioxin) did not inhibit the BRCA1 promoter. These findings suggest that BRCA1 may be a molecular target through which B(a)P and related agents contribute to mammary carcinogenesis, although this hypothesis remains to be proven.

Up-regulation of BRCA1 expression by indole-3-carbinol (I3C). I3C is a phytochemical found in cruciferous (*Brassica* species) vegetables with potential cancer chemopreventive activity, particularly for estrogen-dependent cancer types, such as breast, cervical, and endometrial cancers (Bradlow et al., 1991, 1999; Kojima et al., 1994; Jin et al., 1999; Shertzer and Senft, 2000). In clinical studies, I3C caused regression of the cervical intraepithelial neoplasia (CIN) lesions (Bell et al., 2000) and caused regression or a decreased growth rate of recurrent laryngeal polyps (Rosen et al., 1998). The anti-cancer activity of I3C is attributed, in part, to its anti-estrogenic properties. Thus, I3C stimulates 2-hydroxylation and inhibits 16 α -hydroxylation of E2, activities which lead to inactive E2 metabolites (Michnovicz and Bradlow, 1990; Bradlow et al., 1991). Recently, we showed that I3C and its major active metabolite 2-(indol-3-ylmethyl)-3,3'-dindolylmethane (DIM) up-regulates the mRNA and protein levels of BRCA1 in various human breast and cervical cancer cell lines (Meng et al., 2000c,d; Carter et al., 2002). In addition, I3C in combination with exogenous wtBRCA1 synergistically inhibited estrogen receptor (ER- α) signaling in breast cancer cells (Meng et al., 2000d). These findings raise the possibility that BRCA1 is a target of I3C through which some of its chemopreventive activity (or at least its ability to inhibit ER- α signaling) is mediated.

Down-regulation of BRCA1 expression by alcohol. Epidemiological studies suggest that moderate alcohol consumption increases the risk for female breast cancer, but the molecular basis for this increased risk is unclear. Recent studies suggest that exposure of breast cancer cells to ethanol stimulates cell migration, invasion, and metastasis and enhances ER- α signaling (Meng et al., 2000a; Fan et al., 2000). Associated with the increase in ER- α transcriptional activity was a decrease in BRCA1 mRNA and protein levels (Fan et al., 2000). Since previous studies had documented that exogenous BRCA1 inhibits ER- α activity, these findings suggest

that an ethanol-induced down-regulation of BRCA1 expression could contribute to enhanced ER- α signaling. They also suggest that the down-regulation of BRCA1 by ethanol could contribute to breast cancer development, although this hypothesis remains to be proven.

FUNCTIONAL ACTIVITIES OF BRCA1

Cell cycle regulation and growth control

The role of BRCA1 in normal cell cycle regulation is not well understood. Virally mediated over-expression of BRCA1 abolished the proliferation of breast and ovarian cancer cells, while anti-sense knockdown caused increased proliferation (Thompson et al., 1995; Holt et al., 1996). In our experience, the expression of wtBRCA1 using a CMV promoter-driven expression plasmid caused a modest reduction of in vitro proliferation, but a marked reduction of the growth of wtBRCA1 cell clones as subcutaneous tumors in nude mice (Fan et al., 1998b, 2001b). A complete knockout of the murine *Brc1* gene led to early embryonic lethality (by day 7.5–8.5), due to a severe proliferation defect (Gowen et al., 1996; Hakem et al., 1996; Liu et al., 1996). But a partial deletion within exon 11 (*Brc1*^{Δex11/Δex11}) targeted to the mammary gland allowed survival with an increased incidence of breast cancer (Xu et al., 1999a) [see below]. BRCA1 causes transcriptional activation of the *p21^{WAF1/Cip1}* and *Gadd45* genes (Somasundaram et al., 1997; Harkin et al., 1999; Jin et al., 2000a), which contribute to proliferation inhibition and cell cycle blockade. Several studies, including our own, document a direct interaction between the BRCA1 and Rb1 proteins, through two sites within BRCA1: (1) the carboxyl-terminal TAD (Yarden and Brody, 1998); and (2) an amino-terminal site within aa 300–400 (Aprelikova et al., 1999; Fan et al., 2001c). Brca1 blocked entry into S-phase in Rb1^{+/+} cells but not in Rb1^{−/−} cells mouse embryo fibroblasts (MEFs), suggesting a requirement for Rb (Aprelikova et al., 1999).

Several reports describe the effect of genetic manipulation of BRCA1 on cell proliferation in vitro. Over-expression of BRCA1 by retroviral mediated gene transfer suppressed colony formation in human breast and ovarian cancer, but not colon cancer cells or fibroblasts (Thompson et al., 1995). Suppression of colony formation depended upon the segment of BRCA1 and the cell type. For breast cancer cells, a small C-terminal deletion abrogated the ability of BRCA1 to suppress colony formation; while for ovarian cancer cells, colony suppression was retained unless a larger segment was deleted. Intraperitoneal administration of BRCA1 retroviral vector inhibited the growth of MCF-7 human breast cancer tumors in athymic nude mice (Thompson et al., 1995). These findings suggest that the TAD of BRCA1 is required for suppression of breast but not ovarian cancer growth. On the other hand, antisense inhibition of BRCA1 enhanced the in vitro proliferation of MCF-7 and normal mammary epithelial cells, but not other epithelial cell types (Holt et al., 1996).

Caretaker role: Maintenance of genomic integrity

Clues to a major function for BRCA1 in the maintenance of genomic integrity have come from various

types of experiments demonstrating that BRCA1: (1) plays a role in several highly specialized types of DNA repair; (2) is a target of several upstream DNA damage signaling nuclear protein kinases; (3) is an essential component of several DNA damage-responsive cell cycle checkpoint mechanisms; and (4) is required for the proper replication and functioning of centromeres. As described below, mutational inactivation of BRCA1 results in defects in all of these processes. And consistent with these considerations, BRCA1 and BRCA2 mutant breast cancers showed a significantly greater frequency of chromosomal aberrations (e.g., deletions) than do sporadic breast cancers (Tirkkonen et al., 1997). The pattern of chromosomal loss associated with BRCA1 or BRCA2 mutations was non-random and was distinct from that found in sporadic breast cancers, suggesting preferred pathways of tumor development.

Clues to BRCA1 role in DNA damage response. The first clue to a role for BRCA1 in the DNA-damage response was the finding that BRCA1 associates and co-localizes with Rad51, a DNA recombinase homologous to the bacterial RecA protein (Shinohara et al., 1992), during mitotic and meiotic cell cycles (Scully et al., 1997b). The co-localization of BRCA1 and Rad51 in synaptonemal complexes implicates BRCA1 in genetic recombination events occurring during meiosis (Scully et al., 1997b). During the S-phase of mitotic cell cycles, BRCA1 co-localizes with Rad51 in subnuclear structures called "BRCA1 nuclear dots" (Scully et al., 1997b). BARD1 also localizes to these BRCA1 nuclear dots during S-phase; but unlike BRCA1, BARD1 expression is not cell cycle-dependent (Jin et al., 1997).

The Rad51 interaction domain is located in a segment of BRCA1 encompassing aa 758–1,064 (Scully et al., 1997b). BRCA2 also binds to Rad51, via a 59 amino acid minimal region present in eight conserved BRC motifs encoded by BRCA2 exon 11 (Wong et al., 1997); and the expression of Rad51 in murine Brca2^{-/-} cells confers increased sensitivity to γ -radiation (Sharan et al., 1997). Mice homozygous for a mutant Brca2 truncated within exon 11 survived to adulthood; but the mice exhibited a phenotype characterized by small size, developmental abnormalities, sterility, a defect in repair of double-stranded DNA breaks, and thymic lymphomas (Connor et al., 1997). Embryonic fibroblasts from Brca2(11)^{-/-} mice showed decreased cell proliferation and increased levels of p21^{WAF1/CIP1} and p53 (Connor et al., 1997). Like BRCA1, BRCA2 mediates a transcriptional activation function, through two TADs (Milner et al., 1997). These findings implicate BRCA2 in DNA repair and recombination; and they suggest that the functions of BRCA1 and BRCA2 may overlap.

In MCF-7 cells, DNA damage induced by ultraviolet radiation, the DNA cross-linking agent mitomycin C, and the DNA synthesis inhibitor hydroxyurea caused a rapid (≤ 1 h) translocation of BRCA1 and its binding partners (Rad51 and BARD1) to protein: DNA complexes containing proliferating cell nuclear antigen (PCNA) (Scully et al., 1997c). In addition to acting as a permissivity factor for replication-related DNA synthesis, PCNA plays a role in synthesis related to DNA damage repair (Torres-Ramos et al., 1996). Associated with translocation of BRCA1 to these PCNA-containing complexes, BRCA1 was phosphorylated, to a greater

extent than normally occurs during entry into S-phase (Scully et al., 1997c). The authors speculate that BRCA1 participates in an S-phase cell cycle checkpoint. Following the rapid subnuclear translocation and phosphorylation events, exposure of human breast, ovarian, and prostate cancer cell lines to certain DNA damaging agents (e.g., UV radiation, adriamycin, camptothecin) caused a subsequent loss of hyper-phosphorylated BRCA1 (2–8 h), down-regulation of BRCA1 and BRCA2 mRNA levels (4–8 h), and loss of BRCA1 and BRCA2 proteins (24–72 h) (Andres et al., 1998; Fan et al., 1998a, 1999b). Taken together, these findings suggest roles for BRCA1 in the signaling and/or repair of certain forms of DNA damage.

Role of BRCA1 in specialized DNA repair processes. Subsequent studies have documented roles for BRCA1 in two highly specialized DNA repair processes: (1) transcription coupled DNA repair (TCR); and (2) homology-directed repair (HDR). Studies of both Brca1^{-/-} mouse embryo fibroblasts (MEFs) and a human breast cancer cell line (HCC1937) that contains a single mutant BRCA1 allele (5682insC) revealed that BRCA1 deficient cells have a defect in TCR of oxidative DNA damage caused by ionizing radiation (Gowen et al., 1998; Abbott et al., 1999). Thus, when strand-specific DNA repair was evaluated for several genes, including DHFR (dihydrofolate reductase), BRCA1 competent cells exhibited greater ability to repair the transcribed strand than the non-transcribed strand; whereas BRCA1 deficient cells showed equal repair of both strands, with a reduced rate of repair of the transcribed strand, as compared with BRCA1 competent cells. This defect in TCR was rescued (reversed) by a wild-type BRCA1 gene.

Wang et al. (2001d) identified an interaction between BRCA1 and hMSH2 [a DNA mismatch repair (MMR) protein] that was modulated by adenosine nucleotide. BRCA1 was also found to associate with hMSH3 and hMSH6, the heterodimeric partners of hMSH2; and the BRCA1-interacting protein BARD1 was found to interact with hMSH2. The authors postulate that BRCA1/BARD1 may function as downstream effectors of the adenosine-activated hMSH2–hMSH6 signaling complex. These findings have implications for understanding the role of BRCA1 in TCR, since hMSH2 as well as several other MMR proteins appear to be essential components of the TCR pathway (Leadon and Avrutskaya, 1997, 1998). A role for BRCA1 in TCR is consistent with its ability to recruit transcriptional cofactors and regulate transcription ("Regulation of Transcription"). Interestingly, a histone acetyltransferase (HAT) enzymatic function was mapped to the N-terminus of BRCA2 (Siddique et al., 1998). Although the BRCA1 C-terminus can recruit histone deacetylases (Yarden and Brody, 1998), it is not clear whether BRCA1 can directly regulate histone acetylation.

Chromosomal double strand DNA breaks (DSBs) can be repaired by two processes: (1) homology-directed repair (HDR), in which the chromosomal DNA is restored to its original state; and (2) non-homologous end joining (NHEJ), a process that prevents cell death by preserving genomic DNA, but at the expense of potential mutagenesis (reviewed in Pastink et al., 2001). Several studies indicate that BRCA1 deficient cells have a severe

defect in HDR, which is partially rescued by providing an exogenous wt*BRCA1* gene (Moynahan et al., 1999, 2001). *BRCA1* appears to be essential for two types of HDR: (1) gene targeting (akin to the process used to create embryonic stem cell lines with specific gene mutations); and (2) DSB repair utilizing the homologous undamaged gene as a template. Both of these processes are severely defective in *BRCA1* mutant cell lines; and both are partially corrected by supplying exogenous wt*BRCA1*. The fact that wt*BRCA1* only partially rescued these defects was attributed to the lack of physiologic regulation of the transfected *BRCA1* gene. Thus, when wt*BRCA1* under its own promoter was “knocked into” cells lacking functional *BRCA1*, the defects in HDR was nearly fully corrected (Moynahan et al., 2001). Similar to *BRCA1*, human *BRCA2* deficiency also conferred a defect in HDR (Xia et al., 2001).

A clue to the mechanism by which *BRCA1* participates in DSB repair is the finding that *BRCA1* interacts directly with *BACH1*, a new member of the DEAH helicase family, through its C-terminal BRCT repeats (Cantor et al., 2001). A functional role for the *BRCA1*:*BACH1* interaction in DNA repair was suggested by the finding that a *BACH1* mutant with defective catalytic function interfered with DSB repair in a manner that was dependent on its ability to bind *BRCA1*. The DEAH box family (of which *Rad3* is a member) includes DNA and RNA helicases, which participate in DNA repair, meiotic recombination, and various aspects of RNA processing and editing (Luking et al., 1998). It is interesting to speculate whether *BACH1* may participate in the transcription-coupled repair function of *BRCA1*.

Two recent studies suggest that in addition to its role in DSB repair, *BRCA1* may also participate in a form of nucleotide excision repair (NER) referred to as global genomic repair (GGR) (Hartman and Ford, 2002; Takimoto et al., 2002). The ability of *BRCA1* to stimulate NER may be due, in part, to its ability to mediate p53-independent induction of several NER genes: *XPC*, *DDB2*, and *Gadd45*. Several studies suggest a role for *BRCA1* in DNA damage repair and survival following treatment with *cis*-platinum (a DNA cross-linking agent), although the mechanism is unclear (Bhattacharya et al., 2000).

DNA damage signaling. Members of the family of nuclear phosphoinositide (PI) kinases have been implicated in DNA damage signaling through their protein kinase activities. These proteins include: ATM (ataxia-telangiectasia mutated), ATR (the ATM and *Rad3* related protein), and DNA-PK (DNA-dependent protein kinase). In the setting of DNA damage, these proteins participate in a signaling pathway leading to the activation of down-stream components, including p53, c-Abl, Chk1, Chk2/hCds1, and other proteins (reviewed in Shiloh, 2001; Durocher and Jackson, 2001). Previously, it had been found that following various forms of DNA damage, *BRCA1* is rapidly phosphorylated and undergoes subnuclear translocation to macromolecular complexes containing DNA, PCNA (proliferating cell nuclear antigen), the DNA recombinase *Rad51*, BARD1 (*BRCA1* ring domain-associated protein), and *BRCA2* (Scully et al., 1997c; Thomas et al., 1997). Over the last few years, significant progress has been made in understanding the mechanisms and consequences of the DNA damage-induced *BRCA1* phosphorylation and translocation events.

Over the last few years, *BRCA1* has been identified as a target for several upstream nuclear PI-like kinases: ATM and ATR, (Cortez et al., 1999; Chen, 2000; Tibbetts et al., 2000; Gatei et al., 2001). After DNA damage, *BRCA1* is phosphorylated in vitro and in vivo on S1423 and S1524 by both ATM and ATR (see Fig. 2). However, a study utilizing a series of *BRCA1* phospho-specific antibodies and cells deficient for ATM or ATR revealed that ATM mediates these phosphorylations in response to ionizing radiation (IR), while ATR mediates the phosphorylations in response to ultraviolet-C (UV) irradiation (Gatei et al., 2001). In addition, S1387 was specifically phosphorylated after IR, while S1457 was phosphorylated predominantly after UV treatment. These findings suggest that to some extent, ATM/ATR signaling to *BRCA1* is dependent on the particular type of DNA lesion. Recently, Chk2 (also known as hCds1)—which itself is thought to be a tumor suppressor and mutation of which is linked to a Li-Fraumeni-like syndrome—was found to interact with *BRCA1* in discrete nuclear foci after IR and to phosphorylate *BRCA1* on S988 (Lee et al., 2000). The ability of *BRCA1* to rescue cell survival following IR appears to be

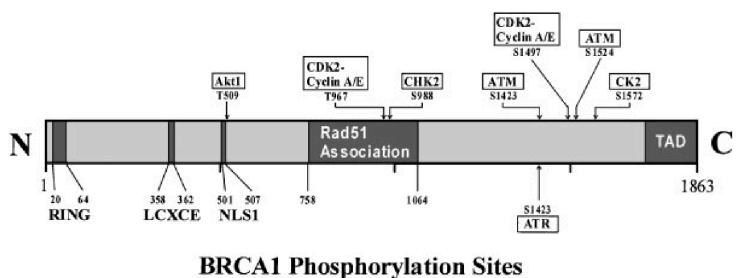


Fig. 2. Schematic diagrams showing sites of *BRCA1* protein phosphorylation. Abbreviations: ATM, ataxia-telangiectasia mutated; ATR, ATM- and *rad3*-related; CDK2, cyclin-dependent kinase-2; CHK2, human homolog of yeast Chk2 (checkpoint kinase-2), also known as hCds-1; CK2, casein kinase-2; also see Figure 1 legend. References: O'Brien et al., 1999; see text for other reference citations.

dependent, in part, on the S988 phosphorylation. Finally, it was demonstrated that functional BRCA1 is required for the activation of Chk1 kinase, a major component of the DNA damage-responsive G2/M cell cycle checkpoint (Yarden et al., 2002) (see below).

Li et al. (2000) have described another potential functional interaction between ATM and BRCA1 in response to IR and other genotoxic stresses. They reported that in response to IR, CtIP [C-terminal binding protein (CtBP)-interacting protein] is phosphorylated by ATM (at S664 and S745), causing its dissociation from BRCA1. The dissociation of CtIP from BRCA1 allowed the IR-induced, BRCA1-dependent induction of Gadd45. However, another study challenged the view that phosphorylated CtIP dissociates from BRCA1 as a mechanism of BRCA1 induction of DNA damage response genes (Wu-Baer and Baer, 2001). Recent studies suggest that in response to double-strand DNA breaks (DSBs) induced by IR, BRCA1 translocates to sub-nuclear foci ("dots") containing Mre11, hRad50, and p95NBS1 (Nijemeyer breakage syndrome protein) (Zhong et al., 1999; Wu et al., 2000), an enzyme complex implicated in DSB repair. BRCA1 can bind directly to DNA, particularly to branched DNA structures, where it inhibits the nucleolytic activity of the Mre11-hRad50-p95NBS1 (Paull et al., 2001). The significance of this observation is unclear at present, but the recruitment of BRCA1 to this complex may favor DSB repair by homologous recombination, at the expense of the more mutagenic non-homologous end-joining (NHEJ). A study by Celeste et al. (2002) has linked the histone H2AX [which is phosphorylated in response IR] to genomic stability. H2AX^{-/-} mice exhibited radiation sensitivity and immunodeficiency, associated with chromosomal instability, DNA repair defects, and impaired recruitment of Brca1 and p95Nbs1 to IR-induced foci. These findings suggest a role for H2AX in the formation of Brca1-containing repair complexes at the sites of damaged DNA.

Wang et al. (2000a) utilized a proteomics approach to identify proteins that associate with BRCA1 *in vivo*. They identified a BRCA1 supercomplex of about 40 proteins, which was termed the BRCA1 genome surveillance complex (or BASC). Components of BASC included: the Mre11-HRad50-p95NBS1 complex (implicated in DSB repair pathways, HDR and NHEJ); the MSH2–MSH6/MLH1–PMS2 complexes (required for transcription-coupled repair); the DNA replication factor C complex (RFC, 5 subunits, which facilitate the loading of PCNA onto DNA and participate in replication repair); the Bloom's syndrome helicase (BLM, post-replication repair, resolution of stalled replication forks); several known BRCA1-binding proteins (ATM, BARD1, and HDAC1); and the rest, as yet, uncharacterized protein components.

Fanconi's anemia is an hereditary (autosomal recessive) disorder characterized by spontaneous chromosomal instability and hypersensitivity to DNA cross-linking agents (e.g., mitomycin C) and to ionizing radiation. While a number of Fanconi (FA) complementation genes have been identified, their roles in DNA repair are not known. Recently, Garcia-Higuera et al. (2001) reported that a nuclear complex containing FANCA, FANCC, FANCF, and FANCG mediates the

activation of FANCD2 to a monoubiquitinated form in response to DNA damage, resulting in its targeting to nuclear foci. They showed that activated FANCD2 colocalizes with BRCA1 in radiation-induced nuclear foci. These findings provide a linkage between FA proteins and DNA damage-induced BRCA1 containing foci that function in the DNA repair process.

DNA damage responsive cell cycle checkpoints. DNA damage activated nuclear kinases (e.g., ATM, ATR, chk1, chk2) promote survival, in part, by activating specific DNA damage-responsive cell cycle checkpoints, including those in G1/S, intra-S, and G2/M (reviewed in McGowan, 2002; Melo and Toczyzski, 2002). These checkpoints play an important protective function by preventing the propagation of DNA damage by cell cycle progression occurring before the DNA is repaired. Thus, an unrepaired single strand break would be converted to a double strand break as the cells pass through S-phase, while an unrepaired DSB would result in loss of the acentric fragment as the cells pass through M. By the use of cells defective for different DNA damage response proteins, it was shown that both ATM and BRCA1 are required for effective S-phase and G2/M checkpoints, while p95NBS1 (nibrin) is required for the S-phase but not G2/M checkpoint (Xu et al., 2001a, 2002). Mutation of S1423 of BRCA1, which had been identified as a target as a target for phosphorylation by ATM, abolished the ability of BRCA1 to cause G2/M arrest. In contrast, the ATM-dependent IR inducible S-phase checkpoint required S1387 of BRCA1 but not S1423. And, as noted above, BRCA1 regulation of the G2/M DNA damage checkpoint occurred through its ability to activate the Chk1 kinase and, thereby, induce molecular events downstream of Chk1 (Yarden et al., 2002).

Consistent with these findings, mouse embryonic fibroblasts (MEFs) carrying a targeted homozygous deletion of exon 11 (which contains $\geq 60\%$ of the Brca1 coding sequence) exhibited a defective G2/M checkpoint and extensive chromosomal anomalies (Huber et al., 2001). These Brca1-defective MEFs showed centrosome amplification with multiple functional centrosomes, which led to unequal chromosome segregation and aneuploidy (Xu et al., 1999b). Consistent with this finding, an earlier study had shown that BRCA1 localizes to the centrosome during mitosis and associates with γ -tubulin, a centrosomal protein necessary for the nucleation of microtubules (Hsu and White, 1998; Hsu et al., 2001). These studies suggest a role for BRCA1 in the regulation of centrosome duplication as a component of its function in maintaining genomic integrity. Taken together, these findings suggest the complexity of BRCA1 functions during the DNA damage response, which may be specific to the type of lesion as well as the specific compartment of the cell cycle.

As will be discussed below ("p53-Mediated Transcription"), BRCA1 is a coactivator of p53-mediated gene transcription. A study of Brca1 mutant mouse embryonic stem cells revealed loss of expression of 14-3- σ , a p53 target gene that functions as a major G2/M checkpoint control gene (Aprelikova et al., 2001). Brca1-deficient cells showed a similar defect in ionizing radiation-induced G2/M growth arrest as did cells with decreased 14-3- σ expression. The ability of BRCA1 to

induce 14-3-3 σ expression was dependent upon the presence of wild type p53 and was regulated by a minimal p53 response element. In addition to DNA damage, Mullan et al. (2001) adduced evidence that BRCA1 participates in G2/M cell cycle arrest caused by antimicrotubule agents (taxol and vincristine). The G2/M block correlated with BRCA1-mediated induction of Gadd45 and could be reproduced by the inducible expression of Gadd45 in the presence of taxol.

Regulation of transcription

A role in transcription was suggested by the finding that BRCA1 has a conserved acidic domain with transcriptional activity in yeast and mammalian cells (Chapman and Verma, 1996; Monteiro et al., 1996). Subsequently, it was found that BRCA1 regulates a variety of transcriptional pathways. BRCA1 transcription regulatory activity may be mediated, in part, by interaction with the basal transcriptional machinery (RNA helicase A and RNA pol II) (Anderson et al., 1998; Schlegel et al., 2000a) and with transcriptional coactivators [p300, CBP (Pao et al., 2000)] and corepressors [RbAp46/48, HDAC-1/2, and CtIP (Yarden and Brody, 1998; Yu et al., 1998)] (see Fig. 1). The C-terminal BRCT domains of BRCA1 can interact with both CtIP and LMO4 [a LIM-only (LMO) transcriptional regulator]; and LMO4 represses BRCA1-mediated transcriptional activity (Sun et al., 2002). BRCA1 can also interact with Brg1, a component of the SWI/SNF chromatin remodeling complex with ATPase activity; and a proteomic analysis revealed that BRCA1 is associated with a SWI/SNF-like macromolecular complex (Bochar et al., 2000). A chromatin unfolding activity has recently been ascribed to the C-terminus of BRCA1 (Ye et al., 2001). This activity is not linked to histone hyper-acetylation, but may be related to the ability of BRCA1 to recruit a novel cofactor (COBRA1) to the chromosome site. As described below, BRCA1 can interact with and activate or repress a variety of sequence-specific transcription factors, providing selectivity to its transcription regulatory function.

Growth inhibitory genes: Gadd45, p21^{WAF1/Cip1}, and p27^{Kip1}. Members of the MyD/Gadd (growth arrest and DNA damage response) gene family function to regulate transcription and cell proliferation (Zhan et al., 1994). Gadd45 is a DNA damage-responsive gene that occurs in three isoforms (α , β , and γ) and functions in DNA repair, cell cycle checkpoint mechanisms, and apoptosis pathways (Kastan et al., 1992; Jin et al., 2000b; Sheikh et al., 2000; Tran et al., 2002). Several studies suggest that BRCA1 up-regulates the expression of Gadd45 (=Gadd45 α), in part, by activating the Gadd45 promoter (Harkin et al., 1999; Jin et al., 2000a). BRCA1-mediated activation of the Gadd45 promoter occurred through association with Oct-1 and CAAT sites within the Gadd45 promoter and involved interactions between BRCA1 and the corresponding site-specific transcription factors, Oct-1 and NF-YA, respectively (Fan et al., 2002b). The significance of BRCA1 inducible Gadd45 expression is unclear, but one study suggests that BRCA1 over-expression stimulates the JNK/SAPK (c-Jun N-terminal kinase/stress-activated protein kinase) pathway of cellular apoptosis via the induction of Gadd45 expression (Harkin et al., 1999). Although

BRCA1 is predominantly a positive regulator of Gadd45, a recent study suggests that BRCA1 can also function as a corepressor of Gadd45, through its interaction with a novel zinc finger protein, ZBRK1, which targets a specific sequence within Gadd45 intron 3 in a BRCA1-dependent fashion (Zheng et al., 2000).

p21^{WAF1/Cip1} was originally identified as a G1 cyclin-dependent kinase inhibitor, a target of p53 transcriptional activity (like Gadd45), and a growth inhibitor that accumulates during cellular senescence; subsequently, p21^{WAF1/Cip1} has been proposed as a tumor suppressor in its own right (Harper, 1997). Exogenous wild-type BRCA1 was found to transactivate the p21^{WAF1/Cip1} promoter in human colon cancer cells (Somasundaram et al., 1997). Transactivation of p21^{WAF1/Cip1} did not require the sequence-specific p53 binding site. Expression of exogenous wild-type BRCA1 blocked entry into S in a manner dependent on p21^{WAF1/Cip1}, since it did not occur in the same colon cancer cell line genetically engineered to be p21 null. And cancer associated mutant BRCA1 cDNAs failed to activate the p21^{WAF1/Cip1} promoter (Somasundaram et al., 1997).

BRCA1 has also been found to transactivate the cyclin-dependent kinase inhibitor p27^{Kip1} promoter (Williamson et al., 2002). A potential BRCA1-responsive element was localized to position -615 to -511 of the p27^{Kip1} promoter. p27^{Kip1} is thought to function as a tumor suppressor and to be a target for cell cycle dysregulation during mammary carcinogenesis (Zafonte et al., 2000). Thus, low p27^{Kip1} expression in breast cancer specimens is correlated with histological aggressiveness and poor prognosis (Chiarle et al., 2001); and mice that are genetically heterozygous for p27^{Kip1} show an increased susceptibility to tumorigenesis (Fero et al., 1998). Interestingly, a study of primary invasive breast cancers from Ashkenazi Jewish women revealed a significant association between the presence of a BRCA1 or BRCA2 mutation and low expression of p27^{Kip1} (Chappuis et al., 2000). But it remains to be determined if this association is causal or casual.

c-Myc-mediated transcription. c-Myc is a proto-oncogene that functions in diverse cellular processes, including cell proliferation, development, apoptosis, and cellular transformation (reviewed in Nass and Dickson, 1997; Liao and Dickson, 2000). Through its two DNA-binding domains (Myc box 1 and Myc box 2), c-Myc can either stimulate or repress transcription. BRCA1 can associate with c-Myc in vivo and bind directly to c-Myc in vitro (Wang et al., 1999). The BRCA1:c-Myc interaction is mediated by aa 175–303 and aa 443–511 of BRCA1 and by the HLH (helix-loop-helix) region of c-Myc, a domain involved in the formation of Myc–Max heterodimers (Wang et al., 1999). This interaction resulted in the inhibition of the c-Myc-mediated transactivation function and of c-Myc-mediated cellular transformation. Subsequently, it was reported that BRCA1 can inhibit the promoter activity of hTERT (telomerase reverse transcriptase, the catalytic subunit of the telomerase holoenzyme), a major target of c-Myc-mediated transactivation, through the formation of a three-way complex involving BRCA1, c-Myc, and Nmi (N-terminal Myc interactor) (Li et al., 2002). It has not yet been established whether BRCA1 can also block the c-Myc transrepression function.

In a recent study using serial analysis of gene expression (SAGE) in human umbilical vein endothelial cells, exogenous c-Myc induced expression of several DNA repair genes including BRCA1 (Menssen and Hermekeing, 2002). Interestingly, hMSH2 (whose protein product interacts with BRCA1) as well as APEX were also up-regulated. These findings raise the possibility of a feedback loop, in which c-Myc-mediated induction of BRCA1 leads to BRCA1-mediated inhibition of c-Myc transcriptional activity.

Estrogen receptor- α (ER- α) and androgen receptor (AR)-mediated transcription. In 1999, we reported that BRCA1 inhibits the transcriptional activity of ER- α , the major estrogen receptor in breast epithelial cells (Fan et al., 1999a). This inhibition was selective, since BRCA1 failed to inhibit the activities of other sequence-specific transcription factors, including Sp1, E2F-1, c-Fos, and c-Jun. ER- α is a member of the nuclear receptor superfamily of ligand-activated transcription factors (Parker, 1998) and is thought to play a major role in stimulation of mammary epithelial cell proliferation during the multi-step process of carcinogenesis (Dickson and Stancel, 2000). BRCA1 targets the conserved C-terminal activation domain (AF-2) of ER- α , since it blocked the ligand-dependent activation of AF-2 in a specific assay of AF-2 dependent transactivation. These findings suggest a mammary tissue-specific function for BRCA1 (Fan et al., 1999a).

We found that BRCA1 interacts directly with the AF-2 region of ER- α via its N-terminus (aa 1–302) and that tumor-associated mutants of BRCA1 failed to inhibit ER- α activity or showed quantitatively reduced inhibition (Fan et al., 2001a). The BRCA1: ER- α interaction occurred in the presence or absence of ligand (17 β -estradiol). A second pathway of BRCA1 inhibition of ER- α activity is the down-regulation of p300, a coactivator of ER- α (Hanstein et al., 1996), other nuclear receptors, and other transcription factors (Fan et al., 2002a). Exogenous p300 (or its functional homolog CBP) rescued the BRCA1 inhibition of ER- α activity; and the rescue activity mapped to the CH₃ domain, a conserved cysteine- and histidine-rich region that interacts with various transcription factors, including ER- α . Interestingly, BRCA1 can also interact directly with p300/CBP (Pao et al., 2000). This interaction involves the CREB domain of p300/CBP and the N- and C-termini of BRCA1. These findings suggest a complex set of interactions governs the ability of BRCA1 to regulate ER- α activity.

BRCA1 was found to interact directly with the AR and to stimulate the activity of several androgen response element (ARE) driven promoters in prostate cancer cells (Yeh et al., 2000). In particular, BRCA1 up-regulated the AR-mediated expression of p21^{WAF/Cip1}.

Subsequently, it was reported that the absence of Brca1 in Brca1^{-/-}p53^{-/-} mouse embryonic fibroblasts (MEFs) conveyed ligand-independent activation of transfected ER- α or progesterone (PR-B) receptor, which did not occur in Brca1^{+/+}p53^{-/-} MEFs (Zheng et al., 2001). The p53^{-/-} background was required because MEFs with a complete knockout of Brca1 exhibit a severe proliferation defect (Hakem et al., 1996, 1997). In our hands, wild-type BRCA1 failed to inhibit the ligand-inducible activity of PR-B, PR-A, PR-

A plus PR-B, or ER- β (unpublished data), suggesting selective transcriptional regulatory activity within the steroid hormone receptor family. Based on chromatin immunoprecipitation assays, it was found that in MCF-7 cells, estrogen caused a rapid egress of BRCA1 from the promoters of estrogen-responsive genes (pS2 and cathepsin D), suggesting that endogenous BRCA1 blocks ligand-independent activation of these genes (Zheng et al., 2001).

STATs and interferon-mediated transcription.

The activities of interferon- γ are mostly mediated by the signal transducer and activator of transcription-1 (STAT1). Recently, it was reported that BRCA1 interacts with STAT1 and causes the activation of a subset of interferon- γ -regulated genes (Ouchi et al., 2000). BRCA1 stimulation of this transcriptional pathway involved an interaction between BRCA1 aa 502–802 and the C-terminal activation domain of STAT1, including S727, phosphorylation of which is critical for transcriptional activation. In DU-145 prostate cancer cells, exogenous BRCA1 caused the constitutive activation of STAT3 and its upstream activators, JAK1 and JAK2 (Janus kinase-1 and -2) (Gao et al., 2001). Immunoprecipitation-Western blotting assays revealed that BRCA1 associates with JAK1 and JAK2 in vivo. These findings are of interest, since STAT3 activation has been implicated in tumorigenesis, via stimulation of proliferation and inhibition of apoptosis (Bowman et al., 2000; Buettnner et al., 2002).

p53-mediated transcription. Recently, BRCA1 was found to bind directly to p53, via an interaction involving the DNA-binding domain of p53 and an N-terminal site localized to aa 224–500 of BRCA1 (Ouchi et al., 1998; Zhang et al., 1998). A second p53 binding site was identified within the C-terminal second BRCT domain (aa 1,760–1,863) of BRCA1 (Chai et al., 1999). The BRCA1:p53 interaction results in enhanced p53 transactivational activity. BRCA1 was also found to stabilize the p53 protein and increase its activity through a mechanism involving the tumor suppressor p14/ARF (Somasundaram et al., 1999). Thus, p53 may be a target for some of the biologic functions of BRCA1. A recent study suggests that BRCA1 does not enhance expression of all p53 target genes but instead induces a subset of genes involved in DNA repair and growth arrest, but not apoptosis (MacLachlan et al., 2002).

However, contrary to some earlier suggestions, BRCA1 tumor suppressor activity is not dependent upon p53, since various BRCA1 functional activities can be observed equally in cells with wild-type, mutant, or deleted p53 genes (Fan et al., 1998b, 2001b,c). The relationship between BRCA1 and p53 is an interesting one, since clinical and experimental studies suggest that inactivation of the p53 gene is an essential component of the BRCA1 tumorigenesis pathway. BRCA1 can also transactivate two p53 inducible gene promoters [p21^{WAF/Cip1} and Gadd45] independently of p53 (Somasundaram et al., 1997; Fan et al., 2002b). And as mentioned earlier, p53 inhibits BRCA1 expression, raising the possibility of a negative feedback loop that normally maintains low basal activities of BRCA1 and p53 (Arizti et al., 2000; MacLachlan et al., 2000).

A second BRCA1 transactivation domain. In addition to the C-terminal TAD of BRCA1 (\leq aa

1,560–1,863), Hu et al. (2000) described a second activation domain (designated AD1, aa 1,293–1,560) that can independently activate transcription in yeast and human cells by itself and could also act to synergistically stimulate transcription with the C-terminal TAD. In a subsequent study, they showed that BRCA1 interacts with Jun family proteins via a coiled-coil motif in AD1 and the Jun basic leucine zipper region (Hu and Li, 2002). BRCA1 AD1-mediated transactivation was dependent upon the Jun-interacting domain and on the presence of the JunB protein. Interestingly, it was also found that JunB mRNA expression was down-regulated in many ovarian cancer specimens. These findings raise the interesting possibility that the loss of JunB expression in ovarian cancers could impair the BRCA1 tumor suppressor function in this tissue.

Other BRCA1-regulated transcriptional pathways. The BRCA1 splice variants BRCA1a and BRCA1b—which suppress the growth of breast cancer cells—interact with Elk-1, an Ets-domain transcription factor that participates in the induction of immediate early gene expression (Chai et al., 2001). Over-expression of BRCA1a/b inhibited the expression of Elk-1 target genes, such as c-Fos, suggesting a mechanism of growth inhibition.

Apoptosis

Apoptosis (programmed cell death) is a common mode of cell death in response to developmental and environmental factors, including DNA damaging agents (Wertz and Hanley, 1996; Bergman and Harris, 1997). Following certain stresses (e.g., DNA damage, chromosomal aberrations, depletion of nucleotide pools, hypoxia), p53 is transcriptionally activated, leading to expression of genes involved in cell cycle inhibition (p21^{WAF1/CIP1}), apoptosis induction (Bax), and DNA repair (Gadd45) (reviewed in Levine, 1997). p53 induces apoptosis by several mechanisms, including transcription of apoptosis-regulatory genes (*Bax* and *IGF-BP3*) and another mechanism that does not require transcription (Haupt et al., 1995). Through mechanisms not well understood, the “cell fate” pathway (apoptosis vs. cell cycle arrest and repair) is selected.

Like p53, BRCA1 may participate in an apoptosis pathway and in the cell fate decision. Both unregulated BRCA1 expression and genetic deletion of the mouse *Brca1* gene can cause increased susceptibility to apoptosis. In *Brca1*^{-/-} cells, apoptosis induction may be due, in part, to accumulation of DNA damage and p53 activation. Thus, a p53^{-/-} background conferred an increased survival of *Brca1*^{-/-} mouse embryos (Hakem et al., 1997). In our experience, the over-expression of BRCA1 in various cancer cell types (e.g., DU-145 prostate, MCF-7 and T47D breast) does not induce apoptosis or loss of viability, but confers an increased susceptibility to apoptosis induction by DNA damaging agents (e.g., adriamycin and camptothecin) and the spindle poison taxol (Fan et al., 1998a, 2001b,c).

Over-expression of BRCA1 in NIH/3T3 fibroblasts and human breast cancer cells conferred increased susceptibility to induction of apoptosis by a calcium ionophore (A23187) (Shao et al., 1996); and inhibition of BRCA1 expression in NIH/3T3 cells by constitutive expression of antisense RNA promoted cell transformation and

resistance to apoptosis (Rao et al., 1996). The increased susceptibility to apoptosis may be due, in part, to BRCA1-induced down-regulation of Bcl-2 and the transcriptional co-factor (p300) (Fan et al., 1998b, 2001c), up-regulation of a Gadd45/JNK signaling pathway (Harkin et al., 1999), and up-regulation of the FasL/Fas interaction (Yan et al., 2002). BRCA1-mediated apoptosis is also negatively regulated by activation of the ERK1/2 pathway (Yan et al., 2002). The dual roles of BRCA1 in DNA damage repair and apoptosis may be reconciled by postulating a “caretaker” function, in which BRCA1 mediates DNA damage signaling/repair when possible, but pushes cells into apoptosis when the damage is too great to repair.

BRCA1 regulation of the proteome

While most studies have focused on the role of BRCA1 in transcription, several recent studies have suggested a role of BRCA1 in protein regulatory processes, including protein ubiquitination and the heat shock response, which involves the protection of proteins by chaperones.

BRCA1 ubiquitin protein ligase activity. An advance in understanding the molecular function of ring finger domain containing proteins is the observation that the ring domains of otherwise diverse proteins carry ubiquitin protein ligase activity, thereby implicating them in the modulation of protein levels by ubiquitination (Lorick et al., 1999). Subsequently, it was established that that the BRCA1 protein has endogenous ubiquitin ligase activity through its ring domain and that cancer-associated mutations within the ring domain disrupt the BRCA1 ubiquitin ligase activity (Ruffner et al., 2001). The BRCA1-associated ring domain protein BARD1, itself a potential tumor suppressor, also exhibited ubiquitin protein ligase activity; and co-expression of the N-terminal ring domain containing regions of BRCA1 plus BARD1 yielded a synergistic increase in ubiquitin ligase activity (Hashizume et al., 2001). Brzovic et al. (2001a,b) have investigated the structural basis for the BRCA1/BARD1 interaction and report that cancer-associated missense mutations of the BRCA1 ring domain do not affect helices required for heterodimerization but instead cause a local perturbation in a region required for the interaction with ubiquitin-conjugating enzymes.

Another study suggests that BARD1 may also act to facilitate nuclear entry of BRCA1 and block its exit (by covering up the BRCA1 nuclear export signal) (Fabbro et al., 2002). It has been suggested that the ubiquitin protein ligase activity of the BRCA1/BARD1 contributes to many of the biologic functions of the BRCA1 protein, including its breast and ovarian cancer suppressor activity (Baer and Ludwig, 2002). However, the principal protein targets of the BRCA1/BARD1 ubiquitin ligase activity have not been identified, nor have the mechanisms by which this enzymatic activity mediates BRCA1 biological functions.

BRCA1 role in heat shock response. The heat shock response is a highly conserved mechanism that protects cells against thermal and other environmental stresses, by preserving the structural integrity of key cellular regulatory proteins. Our recent studies suggest a role for BRCA1 in the heat shock response as: (1) a target for rapid heat-induced protein degradation; and

(2) a potent effector of protection against heat (Ma et al., 2003). Thus, exogenous wild-type BRCA1 blocked heat-induced cytotoxicity in prostate (DU-145) and breast (MCF-7) cancer cells, by a mechanism independent of apoptosis; and Brca1 mutant mouse embryo fibroblasts were more sensitive to heat than Brca1 wild-type fibroblasts. The thermoprotective activity of BRCA1 may be due, in part, to its ability to activate the HSP27 (heat shock protein 27) promoter and to stimulate HSP27 expression. Both the transactivation of the HSP27 promoter and the heat-mediated degradation of BRCA1 required the N-terminal half of the protein but did not require the C-terminus.

Brca1 deficient animal models

Brca1 expression during murine development. Several investigators have examined the expression pattern of the *Brca1* gene during embryonic and postnatal growth and development in mice (Lane et al., 1995; Marquis et al., 1995; Phillips et al., 1997; Rajan et al., 1997). These studies indicate that Brca1 is expressed in rapidly proliferating cells undergoing differentiation. For example, in the mammary gland, Brca1 expression is up-regulated in ductal and alveolar epithelial cells during puberty, pregnancy, and after treatment of ovariectomized mice with estrogen (17 β -estradiol). Particularly increased Brca1 expression was observed in mammary cells during pregnancy, suggesting a mammary tumor suppressor function that may be linked to pregnancy and lactation. Studies of Brca2 expression during mouse development revealed a similar spatial and temporal pattern of expression to Brca1, including developmental regulation of Brca2 expression in the mammary gland, with induction of expression during puberty and pregnancy (Rajan et al., 1997). Despite the overlap in the expression patterns, some differences were noted. Thus, differential regulation of Brca2 vs. Brca1 expression was observed during the development of specific endocrine tissues, including the testis during spermatogenesis and the breast during pregnancy. And in estrogen-treated ovariectomized animals, the up-regulation of Brca1 in the breast was significantly greater than that of Brca2.

As cited above, several studies suggest concordant regulation of BRCA1 and BRCA2 during the cell cycle and in response to DNA damage in cultured cells (Andres et al., 1998; Fan et al., 1999b). When cultured mammary epithelial cells were rendered quiescent by serum starvation, BRCA1 expression was down-regulated, consistent with the finding that BRCA1 expression is linked to cell proliferation *in vivo* (Rajan et al., 1996). However, when cultured mammary epithelial cells were induced to differentiate via treatment with a specific hormonal cocktail, BRCA1 expression was up-regulated, again suggesting a function for BRCA1 in mammary differentiation that is distinct from its putative role in cell cycle progression (Rajan et al., 1996).

Bennett et al. (2000) studied the effects of the synthetic estrogen diethylstilbestrol (DES) on mice heterozygous for either Brca1 or Brca2. They found that mammary ductal branching was reduced in DES-treated Brca1 $^{+/-}$ mice, as compared with Brca1 $^{+/+}$ animals; and most of the Brca1 $^{+/-}$ mice exhibited ovarian atrophy, as compared with the Brca1 $^{+/+}$ mice,

which showed arrested follicular development. On the other hand, DES-treated Brca2 $^{+/-}$ mice showed a more subtle defect in mammary branching and no difference in ovarian pathology, as compared with Brca2 $^{+/+}$ mice. These findings suggest that Brca1 is haploinsufficient for mediating the proliferative endocrine responses of mouse tissues to DES treatment.

Phenotypes of Brca1 $^{-/-}$ mice. The murine homolog of BRCA1 ("Brca1") encodes a 1,812 aa protein with 58% sequence identity to human BRCA1 (Abel et al., 1995). The greatest homology between the human and murine proteins was found in the N- and C-termini, corresponding to the ring domain and the TAD. Targeted deletion of exons 5–6 of Brca1 (which encode the ring domain) revealed that Brca1 is essential for embryonic cell proliferation (Hakem et al., 1996). Brca1(5–6) $^{+/-}$ mice developed normally and were fertile, but Brca1(5–6) $^{-/-}$ mice died by embryo day 7.5–8.5. Defects were observed in embryonic and extra-embryonic cell proliferation, but death was not prevented by aggregation with wild-type tetraploid embryos. Similar findings were reported by other investigators, confirming the importance of Brca1 in early development (Liu et al., 1996; Gowen et al., 1996). Recent studies indicate that a "knock-in" of the human *BRCA1* gene can rescue the embryonic lethality due to a homozygous Brca1 mutation in mice (Lane et al., 2000; Chandler et al., 2001).

Brca1(5–6) $^{-/-}$ embryos exhibited decreased expression of the p53 inhibitor Mdm-2, normal p53 levels, and increased p21^{WAF1/Cip1} levels, suggesting that impaired growth might be due to the transcriptional activation of p53 (Hakem et al., 1996). Accordingly, p53 $^{-/-}$ Brca1 $^{-/-}$ and p21 $^{-/-}$ Brca1 $^{-/-}$ embryos showed increased survival time, but these double mutant embryos died by embryo day 10 (Hakem et al., 1997). Like Brca1, targeted mutations of Brca2, the mouse homolog of human BRCA2, conferred embryonic lethality due to a severe proliferation defect (Suzuki et al., 1997; Sharan et al., 1997). Brca2 $^{-/-}$ embryos survived to embryo day 8.5, one day longer than Brca1 $^{-/-}$ embryos, and the morphologic abnormalities were similar to those observed in Brca1 $^{-/-}$ embryos. The p53 $^{+/-}$ mutation also partially rescued the phenotype of Brca2 $^{-/-}$ embryos (Ludwig et al., 1997). These findings have established roles for Brca1 and Brca2 during embryogenesis. However, they also indicate that the functions of Brca1 and Brca2 are not completely interchangeable, since a wild-type Brca2 gene cannot compensate for a defective Brca1 gene, or vice versa. In contrast, BRCA1 and BRCA2 are not required for human breast or ovarian cancer cell proliferation, since cancers in women with these mutations usually exhibit loss of the wild-type allele (Neuhausen and Marshall, 1994; Cornelis et al., 1995).

Partial deletion of the Brca1 gene. Deng and colleagues developed an animal model to study the role of Brca1 in mammary tumorigenesis. This model features mammary targeted mutation of a *Brca1* gene containing a floxed exon 11 (which contains about 60% of the Brca1 coding sequence) via an MMTV-Cre transgene (Xu et al., 1999a; Deng, 2002). Animals homozygous for Brca1 Δ exon 11 (Brca1 Δ Ex11/ Δ Ex11) developed breast cancers after a long latent period, associated with mammary epithelial cell apoptosis and a defect in ductal development (Xu et al., 1999a). The latent period for

mammary tumorigenesis was significantly decreased in mice heterozygous for p53 ($p53^{+/+}$). Subsequent studies revealed that a truncated (92 kDa) $\text{Brca1}^{\Delta\text{Ex11}/\Delta\text{Ex11}}$ protein was expressed in the Brca1 mutant mouse embryo fibroblasts (MEFs) and that this protein localized to the nucleus (Huber et al., 2001). The Brca1 mutant MEFs proliferated normally in culture but displayed a defect in the Brca1 -mediated DNA damage response.

The mammary tumors that developed in the $\text{Brca1}^{\Delta\text{Ex11}/\Delta\text{Ex11}}$ animals showed an interesting phenotype characterized by: evidence of gross genomic instability (aneuploidy and chromosomal rearrangements), p53 mutations, over-expression of ErbB2 (=HER2/neu), c-Myc, p27^{Kip1}, and cyclin D1, and underexpression or absence of ER- α and p16 in most tumors (Brodie and Deng, 2001; Brodie et al., 2001). Interestingly, the pattern of chromosomal rearrangements in the mouse tumors resembled those found in human BRCA1 mutant cancers (Weaver et al., 2002). Cultured tumor cells were resistant to the anti-estrogen Tamoxifen but were very sensitive to the DNA damaging agents adriamycin and ionizing radiation. As noted above, this phenotype recapitulates some features of BRCA1 mutant human breast cancers (e.g., p53 mutations and ER- α negativity), but not others (i.e., absence of HER2/neu, cyclin D1, and p27^{Kip1} over-expression).

In a subsequent study, Xu et al. (2001b) examined the phenotype of the non-targeted homozygous deletion of Brca1 exon 11. $\text{Brca1}^{\Delta\text{Ex11}/\Delta\text{Ex11}}$ mice all died during late embryogenesis, due to widespread apoptosis. However, when mated into a p53 heterozygous background, the $\text{Brca1}^{\Delta\text{Ex11}/\Delta\text{Ex11}}/\text{p53}^{+/+}$ mice survived to adulthood. These mice exhibited normal mammary development, a high incidence of mammary tumors (by the age of 6–12 months), and lower incidences of other tumor types (lymphomas and ovarian tumors). The authors suggest that the abnormality in mammary development previously observed in the MMTV-targeted Brca1 Δ exon 11 mutant mice was due to the activation of p53 during mammary development in these animals.

UNANSWERED QUESTIONS AND FUTURE RESEARCH DIRECTIONS

Data from clinical and experimental studies indicate that BRCA1 mutant breast cancers develop via a unique molecular pathway, although there are differences between the human and animal cancers. As described above, BRCA1 mutant human breast cancers are typically high-grade lesions, ER/PR negative, with p53 mutations. They show a non-random pattern of chromosomal anomalies, suggesting a preferred pathway of tumorigenesis. In contrast to sporadic cancers, BRCA1 tumors rarely show HER2/neu or cyclin D1 amplification; but they frequently exhibit Myb amplification. The molecular pathogenesis of BRCA1 mutant breast cancers is poorly understood. For example, we do not know whether the loss of hormone receptors is a late event related to genomic instability or whether the tumors originate from ER negative cells. This question is of importance in understanding the role of selective estrogen receptor modulators (e.g., Tamoxifen and Raloxifene) as chemopreventive agents in BRCA1 mutation carriers. We do neither know when in the

tumorigenesis pathway the p53 mutations develop, or for that matter, when the wild-type BRCA1 allele is lost.

A role for BRCA1 in sporadic (non-hereditary) tumorigenesis is suspected, but not proven. As described above, a significant proportion of sporadic breast cancers show reduced BRCA1 expression; and BRCA1 can be functionally inactivated by c-Akt, a kinase that is commonly activated in breast cancers. These observations suggest means by which BRCA1 can be inactivated in sporadic tumors. Further evidence supporting a role for BRCA1 in sporadic carcinogenesis are the observations that BRCA1 expression is down-regulated by several mammary carcinogens [alcohol and benzo(a)pyrene] and is up-regulated by a chemopreventive agent (indole-3-carbinol). The findings that BRCA1 can inhibit the activity of a mammary oncogene (c-Myc) and augment the activity of tumor suppressors (p53 and RB1) are also consistent with a role for BRCA1 in sporadic cancers. Finally, BRCA1 suppresses ER- α transcriptional activity, which is important because most sporadic breast cancers (about two-thirds) are ER positive and estrogen-dependent.

It should be appreciated that there is a fundamental difference in the biology of tumors that develop in the setting of an inherited mutation vs. those that develop in the presence of wild-type BRCA1 or an acquired mutation. In the case of a germline mutation, every cell has a defective BRCA1 allele, which could influence stromal (e.g., fibroblast or adipocyte): epithelial interactions in the mammary gland and other tissues. The studies of Deng and colleagues ("Brca1 Deficient Animal Models") using a mouse model with a mammary (MMTV) targeted Brca1 mutation may serve as a model for sporadic tumorigenesis, because the Brca1 inactivation is selectively targeted to the mammary epithelium, although the MMTV promoter can become activated in other tissues.

Here, we propose several major questions which need to be answered in order to better understand the role of BRCA1 in mammary tumorigenesis: (1) which specific BRCA1 functional activities, when disrupted due to mutation, lead to mammary cancer; (2) what is the role of BRCA1 in sporadic mammary carcinogenesis; (3) is BRCA1 inactivated during the development of sporadic mammary cancers and, if so, by what mechanism? Some of these issues can be addressed by the development of more sophisticated transgenic animal models: e.g., models featuring targeted inducible gene expression and knock-in of a mutant Brca1 gene selectively deficient in one BRCA1 function but retaining all or most other functional activities.

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