Commentary

BRCA1 at a branch point

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The breast- and ovarian-specific tumor suppressor protein, BRCA1, has been implicated in regulating the nuclear processes of repair of damaged DNA, chromatin remodeling, and transcription. Although many have sought specific DNA sequences bound by BRCA1, no such sequences have been reported. In new research described in this issue of PNAS (1), BRCA1 protein is shown to bind to DNA with high affinity in a sequence-nonspecific fashion. Purified recombinant BRCA1 bound to DNA as a multimer, and the BRCA1 protein generated DNA loops by grabbing multiple lengths of DNA (Fig. 1, reprinted from ref. 1). Long DNA molecules were bound by BRCA1 with higher affinity than shorter molecules. For a given length of DNA, branched DNA molecules bound with higher affinity than did the linear molecules. The higher affinity for branched DNAs and the absence of sequence specificity support a direct role for BRCA1 in the repair of damaged DNA. With this new finding, the BRCA1 field is perhaps a step closer toward learning how mutation of this protein can lead to breast and ovarian cancer.

The role for BRCA1 in the maintenance in the genome was first suggested by experiments in which cells were stained by using BRCA1-specific antibodies. BRCA1 had been noted to form foci in nuclei during the S phase of the cell cycle and on tracts of DNA neighboring active meiotic recombination (2), although exactly how these foci correspond with the repair process is not well understood. The BRCA1 foci colocalize with Rad51 or with Rad50 as well as with other known repair factors, such as PCNA (2–4). These findings and the current data fit in the framework of BRCA1 function in repair being developed by Paull, Gellert, and collaborators (1). Damage to the genome results in the very rapid phosphorylation of histone H2A family member H2AX to γ-H2AX (5), which then recruits BRCA1 to the site of damage (3). Taking into account the new findings of Paull *et al.* (1), BRCA1 then may bind to DNA at the site of the DNA break and recruit either the Mre11/Rad50/Nbs1 (MRN) complex or Rad51. In some cells in the same tissue culture dish, MRN is recruited to the foci of BRCA1, and in some cells Rad51 is recruited, but never both. Thus, BRCA1 may act at a branch point in the choice of repair pathway. The timing of focus formation suggests that the MRN or Rad51 associate with these foci of damaged DNA after 2–5 hours (3, 4), but most double-strand break repair is completed in the first hour (6). Data collected after *in situ* fractionation of irradiated cells suggest that the MRN complex associates with the DNA lesions at earlier time points (10 min), and the foci that become apparent without fractionation represent DNA lesions that were refractory to repair (7).

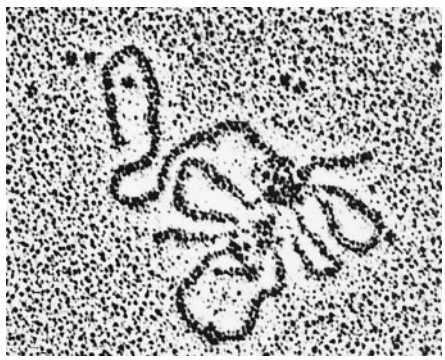

Fig. 1. Electron micrograph of BRCA1 bound to linear plasmid DNA at the base of DNA loops. [Reproduced with permission from ref. 1 (Copyright 2001, National Academy of Sciences).]

The MRN complex participates in the repair of double-stranded breaks in the DNA via two mechanisms, nonhomologous end joining (NHEJ) and homologous recombination (HR) (8). BRCA1, present in the foci of damaged DNA, is presumed to interact with either MRN or Rad51 to stimulate the repair of the lesion. The repair process, as mediated by MRN, has been shown to require the exonuclease activity of one of its subunits, Mre11. However, Paull *et al.* (1) report that the BRCA1 binding to DNA inhibited the exonuclease activity of MRN as measured by decreased amounts of DNA fragmentation in the presence of BRCA1. Thus, BRCA1 may be expected to inhibit the repair process mediated by MRN. Consistent with BRCA1 inhibition of NHEJ, other studies have shown that the absence of BRCA1 activity in a cell results in a nearly complete loss in the HR process and an enhancement of the MRN-mediated NHEJ (9, 10). It is thus possible that BRCA1-mediated inhibition of the exonuclease activity of MRN alters the balance between NHEJ and HR, thus yielding a net enhancement of HR. However, MRN also functions in the HR process (8), suggesting that this dichotomy of choice between NHEJ and HR is simplistic. In addition, the current experiments do not yet provide definitive evidence that the inhibition of Mre11 is relevant to the biology of BRCA1. Paull *et al.* (1) reported that DNA-bound BRCA1 inhibited not only the exonuclease activity of the MRN complex but also the exonuclease activity of the isolated Mre11 subunit, indicating that BRCA1 inhibition is independent of specific contacts with Rad50 or Nbs1. Certainly it would be important to determine the mechanism by which the BRCA1 is inhibiting exonuclease activity and whether it is specific for Mre11. For example, many proteins protect DNA from nuclease activity by physically blocking nuclease access to its target (e.g., as demonstrated in DNase footprint assays).

Much BRCA1 research has focused on proteins with which BRCA1 has been found associated. For example, BRCA1 is a component of the mRNA-synthesizing machine called the RNA polymerase II (Pol II) holoenzyme complex (11, 12) and the chromatin remodeling complex SWI/SNF (13). DNA damage induces the association of BRCA1 into a complex with BARD1 (BRCA1-associated ring domain protein) and other polyadenylation regulatory proteins (14). BRCA1 has also been found associated with the MRN complex (4). Multiple other interactions have been described with Rad51, BRCA2, CREB-binding protein, and p53, to list a few (2, 15–17). It is unclear whether BRCA1 exists in the nucleus unassociated with other proteins. The experiments in Paull *et al.* (1) used pure BRCA1 polypeptide produced in baculovirus-infected cells or a fragment expressed as a fusion protein in bacteria.

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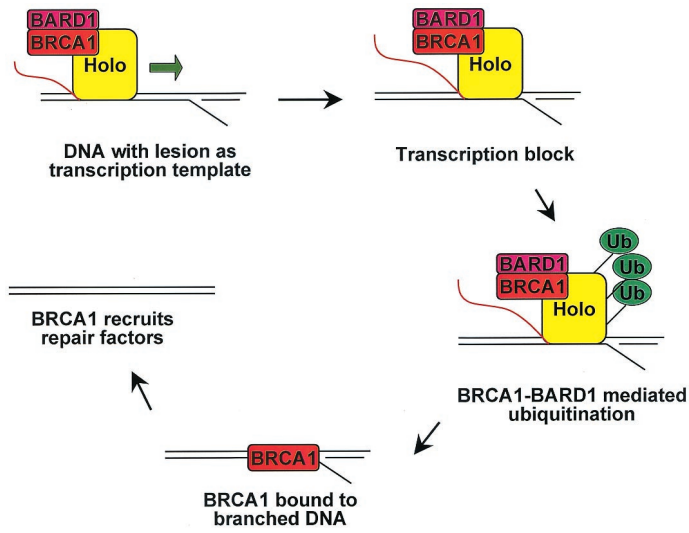

Fig. 2. Model for function of BRCA1 in transcription and repair. DNA damage is depicted as a DNA flap on the template strand. See text for details.

Thus it will be important to determine whether BRCA1, in the various protein complexes with which it has been found, is capable of binding the DNA.

It seems clear that BRCA1 is recruited to the sites of DNA damage. However, the mechanisms by which BRCA1 is recruited are not known. Is BRCA1 free in the nucleus to diffuse to sites of double-strand breaks, or is BRCA1 physically brought to the lesion? One explanation can be inferred from the demonstrated role of BRCA1 in transcription-coupled repair (18, 19) and by the presence of BRCA1 in a key transcriptional regulatory complex called the Pol II holoenzyme (11, 12). A model described in Fig. 2 brings together many of the diverse functions of BRCA1. According to the model, the BRCA1 protein is associated with the mRNA-synthesizing Pol II holoenzyme, which halts at the site of DNA damage. A role for the Pol II holoenzyme in DNA damage repair is supported by the observation in yeast of alleles of genetic suppressors of a hyper-recombination mutant, hpr1, which map to specific subunits found in the Pol II holoenzyme (20). The yeast HPR1 gene is required for both transcription elongation and also genomic stability (21). These findings suggest that this transcription complex has a fundamental role in the recombination pathways. BARD1 was identified by virtue of its binding to BRCA1 (22), and it is associated with BRCA1 in the Pol II holoenzyme as well as in other complexes (N. Chiba and J.D.P., unpublished observations). Recently, BRCA1 and BARD1 have been shown to collaborate in ubiquitin ligation (23), although the target of BRCA1-BARD1-mediated ubiquitination was not identified. It is known that Pol II ubiquitination and degradation occur after DNA damage (24, 25). Thus, drawing all these observations together, we propose that blockage of transcription at the site of DNA damage results in BRCA1-BARD1-mediated ubiquitination of the Pol II holoenzyme, which dissociates from the DNA, leaving the BRCA1 bound to the DNA lesion. BRCA1 then could recruit factors that repair the damaged DNA, such as the MRN complex or Rad51. This model is compatible with the halting of transcriptional elongation directly at the DNA lesion or through the presence of γ-H2AX, which rapidly accumulates at the site of damage (5).

This model accommodates many disparate findings in BRCA1 research. Biochemical purification of BRCA1 from cell extracts has revealed that a major fraction of the total cellular BRCA1 is associated with the transcriptional regulatory complexes of the Pol II holoenzyme and also the chromatin remodeling complex, SWI/SNF (11–13). Transcription function of the BRCA1 has been noted *in vitro* (26, 27), as a coactivator in transiently transfected cells (28–30), or as a chromatin remodeler (31). Cells deficient in BRCA1 are deficient in transcription-coupled repair (18, 19) and in homologous recombination (9, 10). DNA damage is associated with the rapid accumulation of γ-H2AX in foci, followed by the slow accumulation of BRCA1 in these foci (3). Clearly, the timing is consistent with BRCA1 recruitment to these foci by the transcription process.

Recently it has been shown that DNA damage stimulates BRCA1 association with BARD1, and that together these repress the polyadenylation of mRNA transcripts (14). According to the model, BRCA1 and BARD1, along with destroying the polymerase, would prevent the aborted transcript from being processed. It is possible that the suppression of RNA processing is part of the destruction of the polymerase, or alternatively that the capped RNA at the site of the DNA lesion provides a signal in the repair pathway.

One major issue in BRCA1 research is to understand how the mutation of a single component of the generally functioning processes of DNA transcription and repair can result in breast and ovarian cancer and not other common cancers. Are mammary and ovarian cells in an environment that is especially sensitive to DNA damage? Alternatively, it is possible that mammary- and ovary-specific gene activation pathways make these cells especially sensitive to the effects of mutant BRCA1.

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