BOX 4 - BRCA1 and BRCA2 -

Mutations in the breast cancer susceptibility genes *BRCA1* and *BRCA2* are responsible for the majority of the familial breast and ovarian cancers (for reviews see (63, 176, 310, 469)). Mutants are deficient in homologous recombination, transcription repair and cell cycle checkpoint control, leading to increased genetic instability and sensitivity to DNA damaging agents. Despite the many similar symptoms in different mammalian species there is little sequence conservation, and homologues of the breast cancer genes have not been found in yeast or flies (but a homologue has been found in the fungus *Ustilago maydis* (212)). Even though the genes are evolutionary relatively new, they are essential for mammals: knock out mutations are embryonic lethal.

BRCA1 and 2 proteins interact with each other. They also share some more properties as the proteins form partially co-localizing foci in somatic cells and co-localize on the axial elements of developing synaptonemal complexes.

BRCA1 is a 220 kD protein with a N-terminal RING domain and a C-terminal BRCT domain. The protein binds strongly to DNA, with a preference for branched structures. It can bind multiple DNA molecules simultaneously and forms DNA loops. It is reported to inhibit MRE11-RAD50-NBS1 exonuclease activity by binding to the DNA (334), thereby influencing the length of resected DNA ends. BRCA1 forms foci, which co-localize with RAD50 foci. *BRCA1* mutants display reduced amounts of RAD50 foci (501).

Foci form in S-phase cells and during meiosis (386) like RAD51, with which it co-localizes. BRCA1 can be phosphorylated by kinases, such as ATM, ATR and CDS1 (76, 235) after DNA damage (by HU, IR, MMC, UV), which is important for mediating cell cycle arrest. Initially, foci disperse after phosphorylation, but then redistribute and accumulate, most of them co-localizing with PCNA foci (235, 385).
BRCA1 foci also co-localize with BLM foci and co-localization is greatly increased after treatment with HU (475). This, together with RAD51 co-localization after HU treatment, and its preference for binding 4-way DNA structures suggests a role for BRCA1 in stalled replication forks.

BRCA1 is involved in transcription, transcription coupled repair and in altering the DNA topology via its association with the RNA polymerase II holo-enzyme complex (384), the BASC complex (475) and the SWI/SNF chromatin remodeling complex (34, 384). BRCA1 is recruited from the holo-enzyme complex to the HUIC complex (HU inducible complex) upon treatment with HU (65). The latter complex also contains BARD, with which BRCA1 forms an active E3 ubiquitin ligase. BASC on the other hand, is a super complex, which also includes the MRE11-RAD50-NBS1 complex, the mismatch repair proteins MLH1, MSH2 and MSH3 and the BLM helicase. This complex recognizes aberrant DNA structures and may provide the link for BRCA1 influence on transcription coupled repair as does the RNA polymeraseII holo-enzyme complex (1, 136, 475).

BARD binds BRCA1 via its RING domain, the MRE11-RAD50-NBS1 complex binds the middle part and both RNA polymerase II and the BACH helicase interact with BRCA1 by binding its BRCT domain.

BRCA1 can also act as a transcriptional activator (e.g. for p21 (412) or XPC (151)) with maximal activity in presence of co-activators, such as PC-4, and under-wound DNA (145).

BRCA2 is a 460 kD protein with as main characteristic its eight BRC repeats localized in exon 11. Results of crystallization studies (341, 396) suggest that these repeats mimic the RAD51 polymerization motif. A 10 to 20% of intracellular Rad51 is associated with BRCA2 via these repeats (274, 483) and many cancer associated BRCA2 mutations are in exon 11, affecting RAD51 sub-cellular location. BRCA2 mutations abolish the damage induced RAD51 foci formation, but not the S-phase associated RAD51 foci (87, 440). BRC peptides bind to RAD51, thereby blocking nucleoprotein filament formation and damage induced RAD51 foci formation (87, 341). It also results in diminished BRCA2-RAD51 interaction, hypersensitivity to IR and failure of the G2/M checkpoint control (60).

Crystallization studies on the C-terminal domain (in combination with the DSS protein) of BRCA2 reveals similarities with the DNA binding domain of recombinases. It indeed has a strong preference for binding ssDNA and biochemical experiments show that the C-terminal part stimulates RAD51 mediated DNA pairing and strand exchange in presence of RPA (493).

Since RAD51 has no preference for either ss or dsDNA binding, the BRCA2 function may be to bring RAD51 to the resected ssDNA ends. Upon delivery to the site of damage it may release RAD51 and disappear due to phosphorylation. After the recombination processes BRCA2, may come back to remove RAD51 from the DNA as was suggested by Venkitaraman (469). Cellular observations support this hypothesis, as BRCA2 forms big dots in normal cells, which disperse upon damage (IR, DNA cross-linking agents) to reappear later on as smaller sized foci, co-localizing with both RAD51 and PCNA.