RAD51, BRCA2 and DNA repair: a partial resolution

Christopher J Lord & Alan Ashworth

Cooperation between the DNA recombinase RAD51 and the breast cancer susceptibility protein BRCA2 is crucial for the repair of double-strand DNA breaks. Two papers provide new insight into the BRCA2-RAD51 interaction, revealing two different sets of motifs within BRCA2 that bind structurally distinct forms of RAD51. The balance between these interactions seems to be crucial for the function of BRCA2 in DNA repair.

Inheritance of a single mutated allele of *BRCA2* confers a strong predisposition to breast cancer and a number of other malignancies[1]. This gene encodes a 3,418-residue protein that is likely to have multiple cellular functions and is important for the repair of double-strand breaks (DSBs) by homologous recombination. Homologous recombination involves the identification of a DNA sequence homologous to that at the DSB for use as a template in the generation of new, repaired DNA, restoring the original sequence at the site of damage. In the absence of functional BRCA2, cells use alternative, more error-prone forms of DNA repair, and as a consequence, the genome becomes peppered with chromosomal rearrangements and breaks. This genetic instability is thought to foster the development of malignancy[2]. BRCA2 functions in homologous recombination through its RAD51-binding activity: BRCA2 sequesters RAD51, mobilizes it to the site of damage and then facilitates the formation of helical RAD51–single stranded DNA (ssDNA) nucleoprotein filaments that search for a homologous DNA template. The RAD51–ssDNA filament invades the template double-stranded DNA (dsDNA) and initiates DNA polymerization and the strand exchange that characterizes homologous recombination[3].

Christopher J. Lord and Alan Ashworth are at The Breakthrough Breast Cancer Research Centre, The Institute of Cancer Research, Fulham Road, London, SW3 6JB, UK.
e-mail: chris.lord@icr.ac.uk and alan.ashworth@icr.ac.uk

It has been known for some time that BRCA2 has two distinct RAD51-binding domains. The first, termed the BRC repeats, consists of a set of eight 35-residue motifs, located in the central region of the protein and encoded by exon 11 of the *BRCA2* gene. BRCA2 also binds RAD51 via a C-terminal motif that is unrelated to the BRC repeats and encoded within exon 27. The importance of both of these motifs is highlighted by their conservation among vertebrates, whereas the overall sequence of BRCA2 is poorly conserved. What has remained a mystery until now is why BRCA2 has both of these distinct RAD51-binding domains and how they function in RAD51 binding and DNA repair.

Three forms of RAD51 association have been described: the RAD51–ssDNA nucleoprotein filament found at the site of DNA damage, a self-assembled RAD51 oligomer and a BRCA2–RAD51 complex[4]. The characterization of the latter complex demonstrated that BRCA2 BRC repeats disrupt the oligomeric form of RAD51 and facilitate the formation of a BRCA2–RAD51 complex with one RAD51 monomer binding one BRC repeat. The interaction between RAD51 and the BRCA2 C-terminal binding domain (called TR2 by Esashi *et al.*[5]) is less well understood and is the focus of two related studies from the West and Pellegrini groups appearing on pages 468 and 475 of this issue[5,6].

As those in the field are all too well aware, the size of the BRCA2 protein poses significant technical challenges, and to date there have been no reports of the purification of the full-length mammalian protein. Therefore, both groups use peptides derived from BRCA2 to define the differences between the RAD51-binding characteristics of the BRC and TR2 regions. A combination of coprecipitation, electrophoretic mobility shift assays and dynamic light-scattering analysis was used to demonstrate that not only is the TR2 region able to bind oligomeric RAD51, but this interaction is abolished when Ser3291 in the TR2 region of BRCA2 is phosphorylated. This phosphorylation event is known to occur at the G_2-M transition of the cell cycle, abrogating RAD51 binding as cells enter mitosis[7], and is thought to limit the occurrence of homologous recombination. Furthermore, both groups use constitutively monomeric forms of RAD51 to demonstrate that the TR2 region is unable to bind monomeric RAD51, in contrast to the BRC repeats. Therefore, BRCA2 may have two modes of RAD51-binding: the BRC repeats bind monomeric RAD51, disrupting RAD51 oligomers, whereas the TR2 region binds only an oligomeric form of RAD51.

Modeling these interactions with oligomeric and monomeric forms of RAD51 is informative but tells only half the story: RAD51 also forms a BRCA2-dependent nucleoprotein filament with single-stranded DNA at DSBs. It is this structure that invades homologous dsDNA, initiating homologous recombination. Using synthetic DNA substrates that model the ss-dsDNA hybrids present at a DSB, both groups demonstrate that the TR2 region of BRCA2 binds an oligomeric form of RAD51 that is present in the context of a RAD51-ssDNA helix. As expected, an individual BRC repeat is able to disrupt this oligomeric RAD51 form but seems to be prevented from doing so when TR2 is present in excess. Thus, a further distinction between the RAD51-binding regions becomes apparent: the BRC repeats bind monomeric RAD51 and then possibly 'hand over' these monomers to

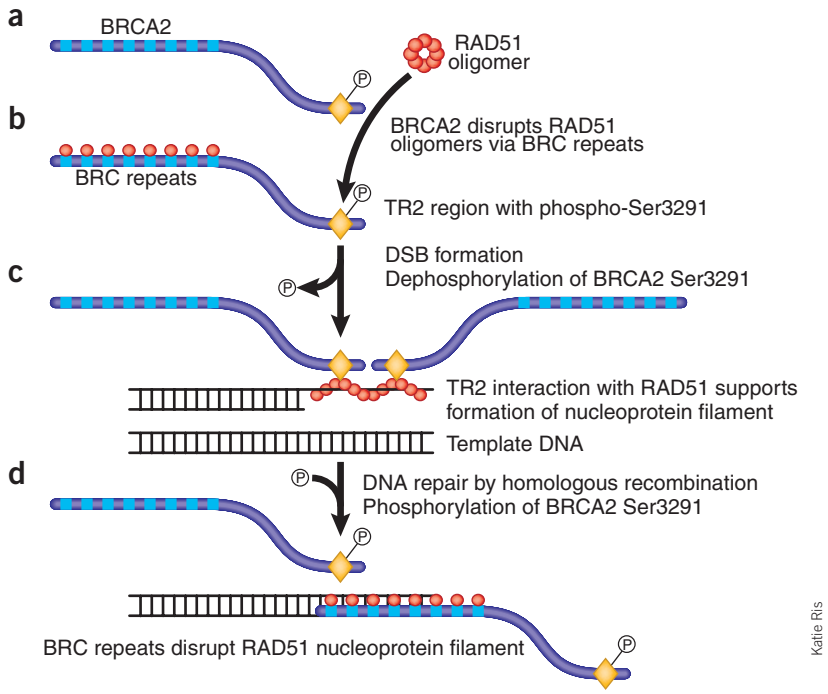

Figure 1 The BRCA2-RAD51 interaction in DSB repair. **(a,b)** There are several distinct pools of RAD51 in the nucleoplasm[4]. Forty percent of RAD51 is either part of self-assembled oligomers **(a)** or bound to the BRC repeats of BRCA2 **(b)**. **(c)** Detection of DSBs in DNA leads to initiation of DNA-repair processes[3]. Phosphorylation of BRCA2 at Ser3291 is lost, enabling RAD51 binding by the TR2 region of BRCA2 and the subsequent loading of RAD51 onto ssDNA at the site of a DSB. It is not known whether RAD51 bound to BRC repeats on one BRCA2 molecule is transferred to the TR2 region on the same molecule or whether RAD51 is transferred from one BRCA2 molecule to another. **(d)** The TR2-RAD51 interaction protects the integrity of RAD51-ssDNA nucleoprotein filaments from BRC-mediated disruption by bridging RAD51 ATPase core subunits on adjacent RAD51 monomers. The nucleoprotein filament invades DNA homologous to the site of damage and uses it as template to restore the DNA sequence at the DSB. Once repair is completed, phosphorylation of BRCA2 at Ser3291 is restored, disabling TR2's protection of the nucleoprotein filament and thus allowing the filament's disruption by the BRC repeats of BRCA2. It is not known whether BRC repeats disrupt the TR2-RAD51 interaction within the same BRCA2 molecule or whether interactions with separate BRCA2 molecules are required (the latter model is shown for clarity). It is also unclear whether RAD51 is returned to a self-assembled oligomer or remains bound to the BRC repeats of BRCA2.

the nucleoprotein filament after DNA damage, where they are constructed into an oligomeric form. The TR2 region of BRCA2 then serves to protect this oligomeric RAD51 form from the disruptive effects of BRC repeats, allowing strand invasion to proceed.

Further support for such a model comes from electron microscopic visualization of nucleoprotein filaments containing RAD51 lacking the N-terminal helical domain. The TR2 region of BRCA2 appears to bridge adjacent RAD51 ATPase cores in the context of the nucleoprotein filament. Such adjacent ATPase domains are present only in the oligomeric forms of RAD51, which explains the binding preference of TR2.

Together, these data suggest a new model of BRCA2–RAD51 function (**Fig. 1**). The BRC repeats of BRCA2 are proposed to hold RAD51 in an essentially inactive, monomeric form. DNA damage occurs and RAD51–BRCA2 complexes localize to the DNA break sites. Dephosphorylation of BRCA2 Ser3291 (by an unknown phosphatase) probably activates the TR2 region, which in turn supports the oligomerization of RAD51 on the nucleoprotein filament. Homologous recombination is eventually halted by the phosphorylation of BRCA2 at Ser3291 (by an unknown cyclin-dependent kinase[7]), inactivating the TR2-mediated protection of the RAD51 nucleoprotein filament and allowing the BRC repeats to disrupt the oligomeric form of RAD51. An analogous model has been proposed from similar work on a nematode BRCA2 ortholog[8].

Although these observations resolve the mystery of why BRCA2 has two unrelated RAD51-binding domains, they raise some issues that require further investigation. Both groups used BRCA2 peptides to characterize the BRCA2-RAD51 interaction, but how the BRC and TR2 domains behave in the full-length protein in the cell may be somewhat different. Moreover, how these findings relate to cancer predisposition needs clarification. Most pathogenic BRCA2 mutations result in protein truncation and therefore exclude the C terminus and the TR2 region. However, some mutations also result in the loss of the BRC repeats encoded by exon 11. Is there a difference in repair capacity between these alleles and therefore a difference in disease susceptibility? As families with BRCA2 mutations that cluster within a region of exon 11 show proportionally more cases of ovarian cancer than cancers of the breast[9], it is possible that loss of BRC repeats and the TR2 region, as opposed to the TR2 region alone, affects the mechanism of pathogenesis. How this might occur is unclear. Regardless of these issues, at least one question, the role of TR2, seems to have been answered.

COMPETING INTERESTS STATEMENT
The authors declare no competing financial interest.

1. Wooster, R. & Weber, B.L. *N. Engl. J. Med.* **348**, 2339–2347 (2003).
2. Gudmundsdottir, K. & Ashworth, A. *Oncogene* **25**, 5864–5874 (2006).
3. Hoeijmakers, J.H. *Nature* **411**, 366–374 (2001).
4. Yu, D.S. *et al. Mol. Cell* **12**, 1029–1041 (2003).
5. Esashi, F., Galkin, V., Yu, X., Egelman, E.H. & West, S. *Nat. Struct. Mol. Biol.* **14**, 468–474 (2007).
6. Davies, O.R. & Pellegrini, L. *Nat. Struct. Mol. Biol.* **14**, 475–483 (2007).
7. Esashi, F. *et al. Nature* **434**, 598–604 (2005).
8. Petalcorin, M.I., Galkin, V.E., Yu, X., Egelman, E.H. & Boulton, S.J. *Proc. Natl. Acad. Sci. USA* **104**, 8299–8304 (2007).
9. Gayther, S.A. *et al. Nat. Genet.* **15**, 103–105 (1997).