Molecular mimicry connects BRCA2 to Rad51 and recombinational DNA repair

Stephen C. Kowalczykowski

The crystal structure of the central region of Rad51 bound to a BRC peptide from BRCA2 shows that the BRC peptide mimics a structural motif within Rad51 and can thereby regulate assembly of the Rad51 nucleoprotein filament needed for DNA repair.

Repair of damaged DNA is essential for the viability of all organisms, and sophisticated pathways exist to repair chromosomal breaks. Recombinational repair, also known as homologous recombination, takes advantage of the surplus genetic content of homologous chromosomes, thereby ensuring the accurate repair of both double-stranded (ds) and single-stranded (ss) DNA breaks. Repair by recombination requires many proteins, most of which are tightly conserved through all domains of life[1]. One highly conserved and essential class of proteins is the DNA strand-exchange family, defined by the prototypic bacterial protein RecA[2]. Counterparts of RecA exist in every organism examined thus far and, in eukaryotes, comprise the Rad51 protein subfamily[3]. The need for functional Rad51 protein in mammalian cells is evident from the embryonic lethality of *RAD51*$^{-/-}$-knockout mice and the conditional inviability of *rad51* chicken DT-40 cells[3].

In addition to the core components of the homologous recombination system that have been defined largely from genetic and biochemical studies in unicellular organisms[1,4], studies in mammals identified additional components that connect the recombinational repair system to a variety of cellular processes. One such component is BRCA2 (ref. 5), which was initially discovered as a human gene in which mutations were associated with a predisposition to breast cancer[6]. As for Rad51, null *brca2* mice are embryonic lethal[7]. This established an intriguing link between defective recombinational repair and a predisposition to malignancy, but the mechanistic basis of this phenomenon has not been well defined.

Rad51 promotes the exchange of DNA strands between homologous sequences, a central step in recombination[1–3]. Rad51 assembles at sites of DNA damage, manifest by the formation of cytological foci; interestingly, BRCA2 protein co-localizes at these foci. To understand the significance of this co-localization, one must appreciate that Rad51 protein function requires many accessory proteins. One such group of proteins are the 'mediators' (ref. 8); each mediator acts somewhat differently, but all lead to the production of a contiguous filament of Rad51 proteins assembled on ssDNA. This filament is a highly ordered structure containing one molecule of Rad51 per three nucleotides of ssDNA, in the form of a right-handed helix that extends DNA to a 5.1 Å rise per base pair and 18.6 base pairs per turn[9]. Thus, one can ask whether BRCA2 co-localizes to Rad51 foci because of a direct interaction or by a simple coincidence.

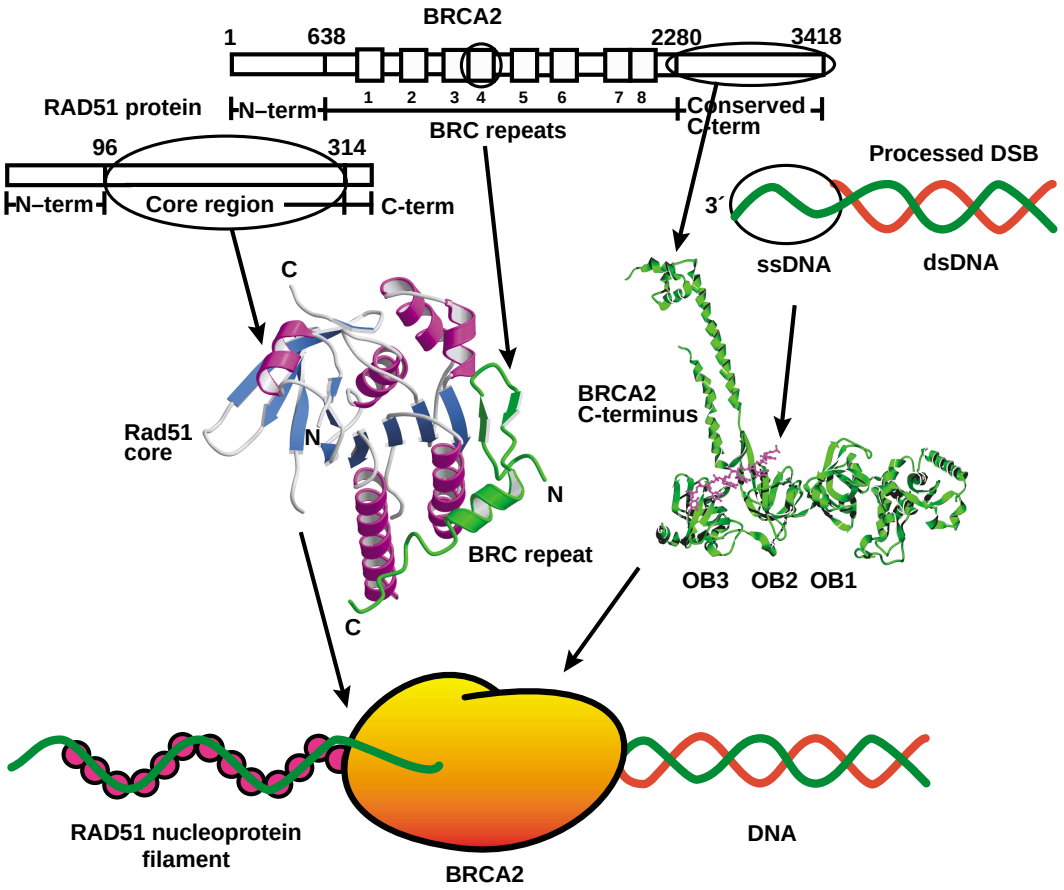
Molecular studies revealed that, in fact, Rad51 does interact directly with BRCA2 *via* the latter's 'BRC' repeats: eight motifs of ~30 amino acids in length sharing high sequence similarity (Fig. 1). Ectopic expression of just one isolated BRC repeat is enough to block co-localization of Rad51 and BRCA2 at cellular sites of DNA damage[10,11]. Therefore, BRC repeats appear to be the sites of direct interaction between BRCA2 and Rad51, and it has been suggested that intact BRCA2 protein serves to deliver Rad51 to damaged DNA. It is proposed that, in response to an appropriate signal, Rad51 protein would be released and filament assembly ensue from BRAC2.

The results of Pellegrini *et al.*[12] define the structural basis for BRCA2's interaction with Rad51. Using the cunning approach of fusing a BRC repeat peptide to the core region of Rad51, the authors were able to determine the crystal structure of this portion of Rad51 with a bound BRC repeat from BRCA2. This represents a major contribution to the rapidly evolving story of recombinational repair.

Since the determination of the crystal structure of the RecA filament (in the absence of DNA) 10 years ago[13], no high resolution structures of related proteins have appeared. The region of Rad51 used in the recent structural work is the 'core' of the RecA/Rad51/RadA family of DNA strand-exchange proteins[14,15]. The core is highly conserved among these proteins, binds ATP, contains the catalytic residues for ATP hydrolysis and defines the 'RecA-fold' that is found within ATP-binding regions of functionally and structurally distinct proteins, such as DNA helicases[16]. The structure of the ATPase domain from human Rad51 is topologically identical to the catalytic domain of RecA; in fact, some three-quarters of the Cα atoms are virtually superimposable. Interestingly, the nucleotide-binding site of the Rad51 protein is more closed than the corresponding region of RecA, which seems to hinder ATP binding, perhaps because of a conformational change induced by its association with the BRC repeat.

The structure of the BRC peptide on its own is unassuming, comprising a β-hairpin (a type I turn), an α-helix and an irregular coil with elements of a 3_{10}-helix. However, the nature of the Rad51–BRC complex is particularly revealing. A stretch of 28 amino acids in the BRC repeat interacts with a region of the Rad51 protein that, in the RecA filament structure, mediates nearest-neighbor interactions with the adjacent monomer in the filament. Even more surprisingly, the BRC structure appears to 'mimic' the immediate structure of the adjacent Rad51 monomer, again extrapolating from intersubunit interactions implied by the related RecA filament. The interaction places two antiparallel β-strands of BRC against the extensive β-sheet structure of Rad51 protein (Fig. 1). Consequently, by interacting with Rad51 at a site needed for self-assembly, BRCA2 may assist and modulate assembly of the Rad51 nucleoprotein filament that is needed for recombinational repair of damaged DNA (Fig. 1).

Careful scrutiny of BRC repeats from seven different organisms provided a possible rationale for the loose sequence conservation observed among BRC repeats. The authors compare the interaction



Nicole D. Fournier

Fig. 1 Schematic showing the relationship of the Rad51 and BRCA2 structures to recombinational repair of DNA. Diagrams of the Rad51 and BRCA2 protein sequences and of a processed dsDNA break (DSB) are shown at the top. Rad51 comprises a conserved N-terminal region, a core domain and a short C-terminus. BRCA2 comprises an N-terminal region, the central region with eight BRC repeats and a conserved C-terminus. The processed DSB comprises 3′-terminated ssDNA, which can be up to 1 kb in length, linked to intact dsDNA. The structure of the Rad51 core (blue and magenta) in complex with BRC4 (green) was determined[12]. The conserved C-terminus of BRCA2 (green) was shown as a complex with ssDNA (oligo $(dT)_9$, magenta)[17]. The regions for which structures have been determined experimentally are circled on the top diagrams. The hypothetical complex consisting of the Rad51 nucleoprotein filament assembled on ssDNA, interacting with BRCA2 bound to the processed DSB, is shown at the bottom. Repair of the processed DSB would commence by Rad51-initiated recombinational repair (not shown; see ref. 1).

between Rad51 and the BRC repeat as being akin to a molecular 'Velcro' (a hook and loop fastener) in that the contacts with the peptide seemed to be relatively independent of one another, thereby allowing variants of the BRC motif to bind with substantial affinity. Moreover, the authors were able to predict, and subsequently establish, a conserved motif in the Rad51 protein (85-GFTTATE-91) that is the complementary site in the adjoining monomer needed for *in vivo* filament self-assembly. This oligomerization motif is common to all Rad51 homologs, and its importance was established by both biological and biochemical experiments. Thus, it is now clear why the binding of an ectopically expressed BRC repeat to Rad51 blocks filament formation and prevents co-localization of Rad51 and BRCA2 in the cell.

This study comes close on the heels of a paper reporting the crystal structure of a different region of BRCA2, the conserved C-terminal region[17]. The C-terminal region of BRCA2 contains three domains with the oligonucleotide-binding (OB) fold, previously unrecognized from sequence analysis alone, and an unusual 'tower' structure composed of two long helices emerging from one of the OB-fold domains (Fig. 1). In accord with the predicted function of the OB fold, this region of BRCA2 binds ssDNA, and the tip of the tower contains a helix-turn-helix motif that is inferred to bind dsDNA. Furthermore, the C-terminal portion of BRCA2 was shown to stimulate the DNA strand-exchange reaction promoted by Rad51. Although the mechanism underlying this effect is unknown, it is most likely that this stimulation involves the ssDNA-binding ability of the BRCA2 C-terminal domain because the C-terminal BRCA2 fragment does not contain the BRC repeats that interact with Rad51. Given that the BRC repeats are nearby in sequence (Fig. 1), it is easy to envision how the complete BRCA2 protein could both bind DNA and target the bound Rad51 to ssDNA (Fig. 1). Together, these findings offer a physical link between ssDNA and dsDNA at the site of DNA damage, Rad51 recruitment, BRCA2 and Rad51 co-localization and, ultimately, DNA repair.

A final contribution to this story is the discovery that BRCA2 proteins are not, in fact, unique to mammals: a relative named BRH2 has recently been defined genetically in the fungus *Ustilago maydis*[18]. Cells lacking BRH2 are defective in mitotic and meiotic recombination and suffer from chromosomal instability. Furthermore, searches of genome databases reveal the existence of potential BRCA2-related proteins in organisms as diverse as plants (*Arabidopsis thaliana*[18]) and nematode worms (*Caenorhabditis elegans*[10]). Thus, a more universal role for these BRCA2 relatives in recombinational repair is begin-

ning to emerge, whereby BRCA2 sequesters Rad51 protein in an inactive state from which it can be activated in response to DNA damage. The correlation with disease-associated mutations in the BRC repeats is provocative, and the structures invite new models regarding BRAC2 function. The structural determination of the Rad51- and ssDNA-interacting domains from the mammalian BRCA2 protein is providing unprecedented insight.

Stephen C. Kowalczykowski is in the Division of Biological Sciences, Sections of Microbiology and of Molecular and Cellular Biology, Center for Genetics and Development, University of California, Davis, California 95616-8665, USA. email: sckowalczykowski@ucdavis.edu

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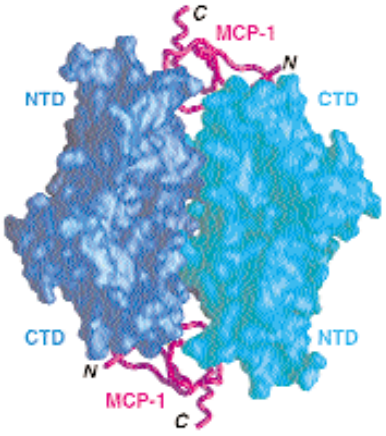
picture story

Sabotage through structural mimicry

If a virus is to prosper inside a living host, it needs to be able to neutralize the immune response that the host organism mounts against foreign invaders. It turns out that mammalian DNA viruses have developed a range of ingenious strategies to evade immune responses that are mediated by chemokines, a group of ~50 proteins present in the mammalian circulatory system that activate adaptive immunity and control leukocyte migration. For example, their viral genomes may encode specialized G-protein coupled receptors (the targets of chemokine function) or specify the production of proteins that stimulate or antagonize the host's chemokine receptors. In essence, viruses encoding their own set of ligands and receptors can manipulate cellular signaling at will. If this alone would not suffice, yet other viruses show that an alternate strategy is to bind endogenous chemokines and thereby throw a cloak of confusion over the immune response. But how can a viral protein hijack an entire chemokine response pathway?

In a recent paper, Alexander *et al.* (*Cell* **111**, 343–356; 2002) report the crystal structures of a chemokine scavenger with broad chemokine specificity from the murine γherpesvirus68 (γHV68) and its complex with the CC class chemokine MCP-1. M3, as the 42 kDa scavenger protein is known, appears to bind a wide range of murine and human chemokines, and is required *in vivo* both for virulence and for inflammatory responses in the host. The structure of the M3–MCP-1 complex reveals a 2:2 complex formed by



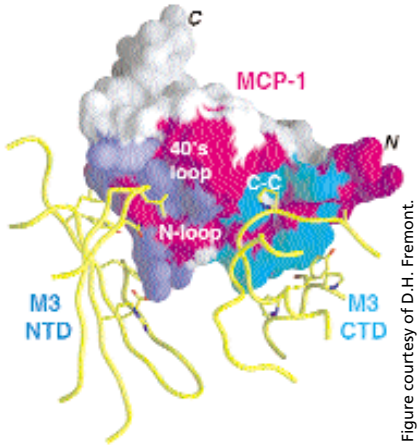


Figure courtesy of D.H. Fremont.

a M3 dimer and two independently associating MCP-1 chemokines (left, molecular surface of M3 is shown in blue and cyan). MCP-1 is recognized by the N-terminal and C-terminal domains of M3 (NTD and CTD, respectively). The NTD recognizes residues in the so-called chemokine 'N-loop' and '40's loop' of MCP-1, whereas the CTD binds the N-terminal region including the CC disulfide bond (right, molecular surface of MCP-1; M3-interacting residues shown in blue and cyan). The ability of M3 to bind a broad range of chemokines with significant but differing affinities seems to stem from structural features of the M3 protein: the use of adaptive, flexible loops as primary binding determinants, a dimeric architecture of the binding site and, perhaps, a very high electrostatic complementarity between the molecular surfaces of M3 and many of its chemokine substrates, exemplified by MCP-1.

The M3–MCP-1 structure also reveals that the herpesvirus M3 protein sequesters chemokines through structural mimicry. The MCP-1 residues bound by M3 are identical to those that are required for MCP-1 interaction with its endogenous CCR2 G-protein coupled receptor, even though there is no sequence homology between M3 and CCR2. The M3 protein thus mimics CCR2 and, by doing so, can lure MCP-1 into binding the viral bait. This viral chicanery severely compromises the host's immune system by removing a necessary chemokine ligand from circulation. The structure by Alexander *et al.* thus reveals how herpesvirus has come up with its own solution to fooling chemokines and their receptors.

Andreas G. Ladurner