Crystal Structure of the Homologous-pairing Domain of the Human Rad52 Protein

A DNA break that remains unrepaired is lethal for any cell. Double strand breaks occur in cells exposed to ionizing radiation and chemotherapeutic agents, or naturally by replication errors and oxygen radicals generated from respiration. To repair such lesions, cells have developed efficient homologous recombinational repair systems. In budding yeast, the RAD52 epistasis group genes (*RAD51*, *RAD52*, *RAD54*, *RAD55*, *RAD57*, *RAD59*) play central roles in homologous recombination and are conserved among eukaryotes. Rad52, a key member of this group, displays two critical activities that have been demonstrated in vitro: homologous pairing and interaction with the Rad51 recombinase. These activities are likely to be essential for the initiation of homologous recombination, although no detailed molecular mechanisms are currently available.

The human Rad52 protein consists of 418 amino acid residues, and we, as well as others, have found that the N- and C-terminal halves of this protein have distinct roles in recombination. The N-terminal half of Rad52 (about 200 amino acid residues) is highly conserved among Rad52 homologs, and this region composes a structurally stable domain as suggested from limited proteolysis experiments [1]. The isolated, N-terminal domain of Rad52 binds to single-stranded DNA (ssDNA), and has homologous-pairing activity equivalent to that of the full-length protein [1]. Interestingly, alternative-splicing variants containing only the N-terminal half of Rad52 were found in humans, indicating that the isolated, N-terminal domain of Rad52 has a role in recombination [2]. The C-terminal half, by contrast, is poorly conserved among Rad52 homologs. This region physically interacts

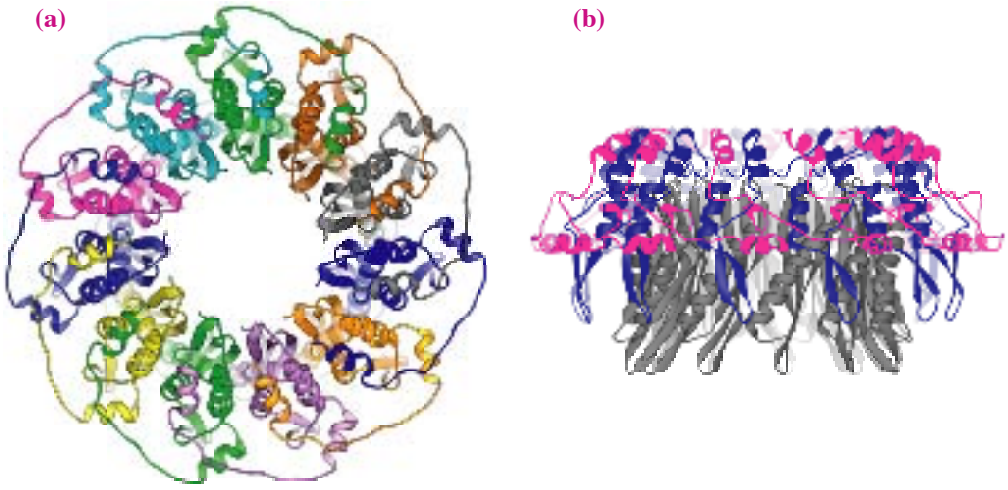

*Fig. 1. The structure of $Rad52_{1-212}$. **(a)** Ribbon diagram of the undecameric ring of $Rad52_{1-212}$, viewed down the central channel from the top of the domed cap. **(b)** The ring viewed from the side. The domed cap region is coloured in blue and magenta, and the stem region is coloured in grey. The diameter of the ring is about 120 Å, and that of the central channel is about 50 Å at the narrowest point.*

with the Rad51 recombinase [1], and may have an important role in the recombination mediated by Rad52 and Rad51. Therefore, the N-terminal half is present in both the longer (full-length) and shorter (alternative splicing variant) forms of Rad52, whereas the C-terminal half is only present in the longer form of Rad52.

To gain insight into the mechanism of homologous-pairing promoted by Rad52, the N-terminal fragment ($Rad52_{1-212}$) containing the homologous-pairing domain was designed, and crystals of this fragment were prepared for structural analyses. Data from both the native and selenomethionine-substituted $Rad52_{1-212}$ crystals were collected at the RIKEN Structural Biology beamline I (**BL45XU**), and the structure was determined by the multi-wavelength anomalous dispersion (MAD) method at 2.85 Å resolution [3]. The crystal structure of $Rad52_{1-212}$ revealed an undecameric ring, indicating that the shorter form of Rad52 is undecameric. The overall structure resembles a mushroom with a stem and a domed cap region (Fig. 1).

$Rad52_{1-212}$ has an exposed groove around the entire ring structure. This groove encircles the stem region, and is highly positive in charge, as determined from the surface potential calculations (Fig. 2). The groove has an approximate width of 10 Å and an appropriate size to accommodate ssDNA. To identify the precise DNA binding site, the basic and aromatic amino acid residues on the positively charged surface, which have the potential to interact directly with ssDNA, were replaced with alanine by site-directed mutagenesis (Fig. 3(a)). These mutants were then tested for ssDNA binding. The mutations of Arg55, Tyr65, Lys152, Arg153, and Arg156, with side chains inside the groove, clearly decreased the ssDNA binding activity (Fig. 3(b)). Therefore, the shorter form o Rad52 fits ssDNA inside the exposed groove, and wraps ssDNA around the stem region.

In contrast to the shorter form, our sedimentation equilibrium studies on the longer form of Rad52 demonstrated that the protein is heptameric in solution. Previous electron microscopic studies also concluded that Rad52 forms a heptameric ring [4]. Interestingly, the two ring forms have similar

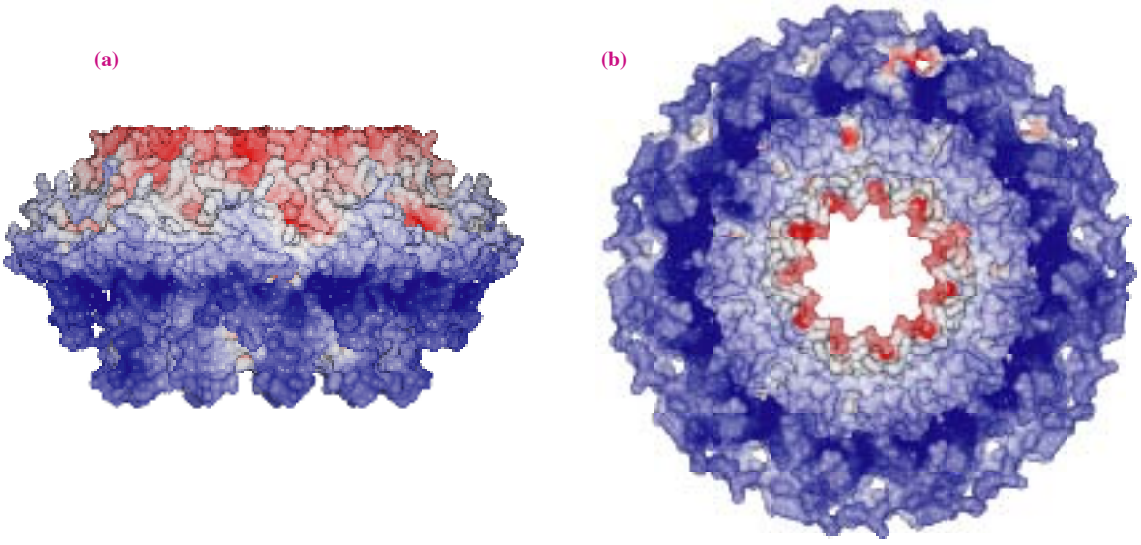

*Fig. 2. Side (**a**) and bottom (**b**) view of the surface of $Rad52_{1-212}$. The surface is coloured according to the electrostatic potential. (red) $-12\ k_BT^{-1}$ to (blue) $12\ k_BT^{-1}$.*

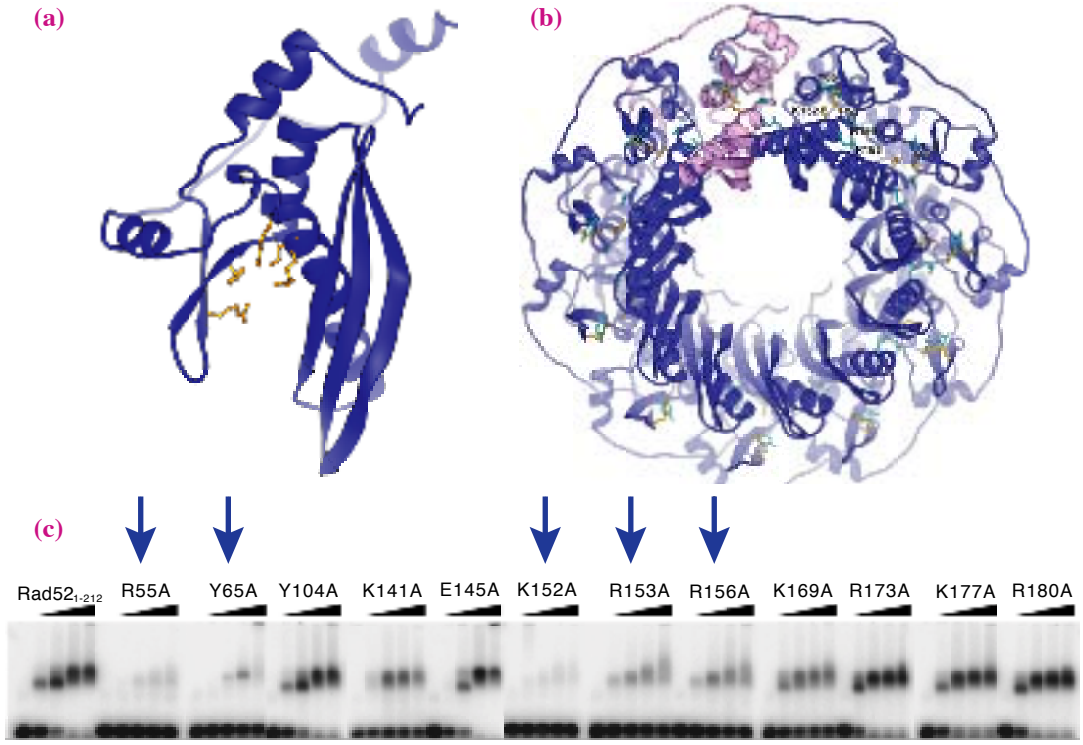

*Fig. 3. Mutagenesis and ssDNA binding. **(a)** Amino acid residues (coloured in yellow) essential for ssDNA binding mapped on the Rad52$_{1-212}$ monomer. **(b)** The residues essential for ssDNA binding are located inside the groove. **(c)** Gel shift assay of the Rad52$_{1-212}$-ssDNA complexes. The ssDNA binding was analyzed by 1% agarose gel electrophoresis.*

diameters, which allowed us to model the heptameric ring using the crystal structure of Rad52$_{1-212}$ (Fig. 4). In the resulting heptamer model, a positively charged DNA groove is present, suggesting that the groove for DNA binding is conserved in both ring forms. This is consistent with the similar ssDNA binding and homologous-pairing activities of the longer and shorter forms of Rad52 [1].

In conclusion, the crystal structure of Rad52$_{1-212}$ revealed that the homologous-pairing domain of Rad52 is organized into a ring with a circular groove essential for ssDNA binding. Mutagenesis studies demonstrated that ssDNA binds to Rad52 by wrapping around the ring structure. Both ring forms are capable of promoting homologous pairing, suggesting that ring formation is essential for the activity. On the other hand, only the heptameric form of Rad52 can interact with the Rad51 recombinase through the C-terminal half, which was predicted to be located between the monomers in the heptamer model. The two ring forms of Rad52 could be involved in different recombination pathways. The shorter form, which lacks the Rad51-interacting region, may function in recombination pathways that are independent of Rad51. By contrast, the longer form could function in Rad51-dependent recombination pathways. In both cases, our results suggest that the N-terminal half of Rad52 promotes homologous pairing in the DNA binding groove around the ring structure.

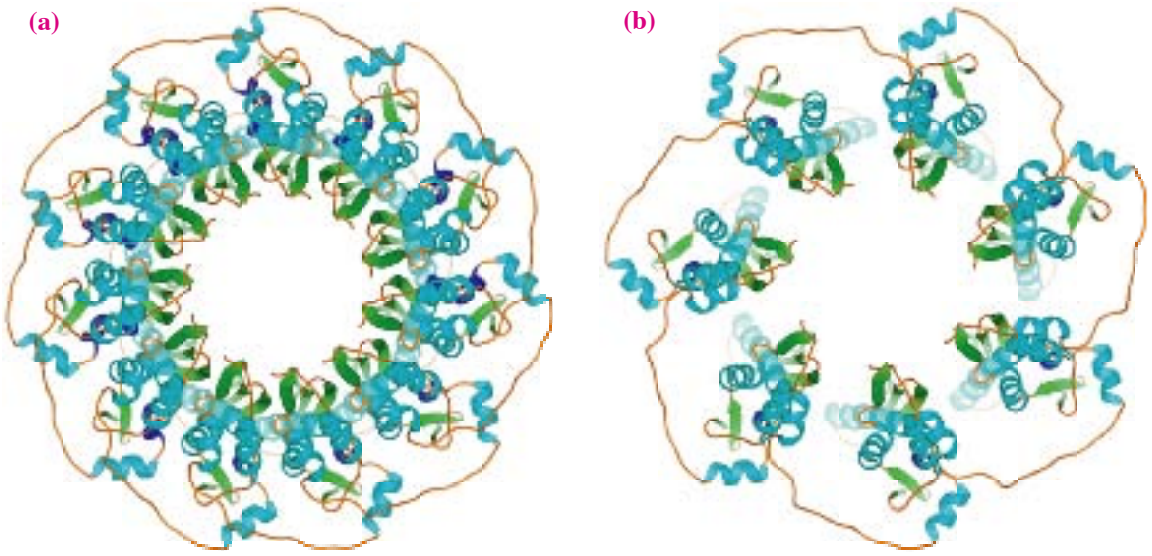

*Fig. 4. Heptamer model of Rad52. The heptameric ring of the longer form was modeled by simply removing four monomers from the undecameric ring (**a**), and spacing the remaining seven monomers evenly, without changing the diameter of the ring (**b**).*

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