PNAS COMMENTARY

RAD52's active form is not a ring

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A longtime point of scientific disagreement has been the role of RAD52 ring structures versus monomeric RAD52 in DNA repair mechanisms. In the purified, concentrated state, the RAD52 protein forms a ring that varies in size. A long-standing question is what the form of the protein is when actively performing DNA repair in cells. In PNAS, Kharlamova et al. (1) answer this question using state-of-the-art experiments to determine the oligomeric state (monomers and short oligomers) of RAD52 at physiological concentrations that actively perform single-strand annealing (SSA) (Fig. 1).

A better understanding of RAD52 structure is needed because of the importance of RAD52 in DNA repair and cancer therapy. For example, RAD52 provides a backup repair pathway for the major homologous recombination (HR) pathway that accurately repairs double-strand breaks—the most serious form of DNA damage. In fact, HR-deficient cancers become addicted to the RAD52 pathway. The inhibition of RAD52 in these cases is synthetically lethal, meaning the cancer cells will die, but the normal cells will remain. This observation has created great interest in finding RAD52 inhibitors for personalized cancer therapies (3). To effectively target RAD52, we need to understand its structure and the atomic mechanisms of its activities in cells.

Previously, structural biology and molecular biophysics experiments studied the structure and oligomeric state of RAD52 particles and complexes with Replication protein A (RPA) were measured using dynamic light scattering (4), size exclusion chromatography-multiangle light scattering (SEC-MALS) (5), small angle X-ray scattering (SAXS) (2) and single-crystal X-ray crystallography (6, 7). These studies all used concentrated samples of RAD52 and usually measured an oligomeric ring when RAD52 was in isolation. The RAD52 ring has a history of varying in size. For example, fractions off SEC frequently vary in aggregate size (2). The first transmission electron microscopy images assigned a seven-membered ring to RAD52 (8). Interestingly, the presence of an undecameric ring in the crystal structures of RAD52 led to many scientists assuming that this ring was the active physiological form and designed their experiments and models accordingly. This is not surprising since structural biologists usually try to justify their structures as the biological form. A complex with RPA with monomeric RAD52 was measured by SEC-MALS (5), indicating that the active form is likely a monomer. Especially since, in cells, the RPA heterotrimer greatly outnumbers RAD52 monomers. The

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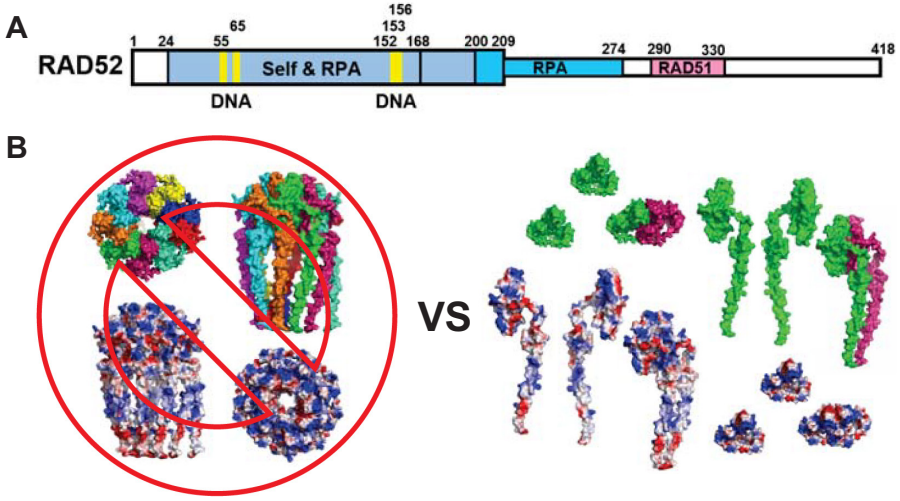

Fig. 1. Monomers and short oligomers of human RAD52 promote SSA. (*A*) Structural and biophysical data updated the domain diagram for human RAD52 where thick rectangles are part of the crystal structure (PDB ID 1KN0). (*B*) The 7 to 13 member rings seen at μM concentrations and higher are inactive for SSA (*Left*). The *Top* view shows the single-stranded DNA binding site. The side view shows the C-terminal residues 213 to 303 hanging down from the ring like the tentacles of a jellyfish. Kharlamova et al. (1) showed that RAD52 is primarily monomeric (*Right*) and active at low nM concentrations. This figure was prepared using a RAD52(1 to 303) model that was created using SAXS data in combination with RAD52(1 to 212) (PDB ID 1KN0) and I-TASSER modeled RAD52(213 to 303) (2). The surfaces that interact with DNA and other proteins were made in Pymol, colored by chain or by electrostatics, and shown as top or side views. Monomers and dimers are shown in a 2:1 ratio. Two side views for the monomer (*Right*) are shown rotated about the vertical by 90°.

work by Kharlamova et al. agrees with this observation that the monomer is the active form of RAD52.

In PNAS, Kharlamova et al. (1) answer this question using state-of-the-art experiments to determine the oligomeric state (monomers and short oligomers) of RAD52 at physiological concentrations that actively perform single-strand annealing (Fig. 1).

RAD52 has many activities besides its role in HR and can perform SSA (9). Kharlamova and others cleverly measured RAD52's SSA activity over a concentration range from 4 μM down to 10 nM. Interestingly and unexpectedly, the more dilute reactions had the most SSA product. Then, to understand the quaternary structure of RAD52 that anneals DNA, they measured the molecular weight (MW) of full-length, human RAD52 over the same concentration range using a very sensitive method called mass photometry. Mass photometry is a single-molecule technique that uses the basic principle that the intensity of light scattered from the macromolecule is proportional to its MW. For this method, the single molecule is in contact with a measurement surface, such as a coverslip, and the interference between the light scattered by the molecule and the light reflected by the surface is measured. This mass photometry contrast is directly correlated with MW (10). Comparing the mass photometry data with SSA activity data shows that the active form of human RAD52 is primarily a monomer and short oligomers such as dimers. The ring structure at μM concentrations inhibits SSA.

When RAD52 forms a ring structure, the C-terminus of one monomer wraps around the adjacent monomer to form a thermostable ring and then hangs down below the ring (11) (Fig. 1 *B*, *Left*). The protein surface that makes this self-association contact is also necessary for RAD52's protein–protein interaction with RPA (Fig. 1*A*). Thus, when in complex with other purified proteins such as RPA, RAD52 is a monomer, and the sticky surface of the self-association region is satisfied. It makes sense that the monomer is the active form (Fig. 1 *B*, *Right*) since all the needed surfaces are easily available for binding DNA or binding protein partners. When the protein is purified and concentrated beyond physiological levels, these relatively flat interaction surfaces stick to each other and form an artifactual ring.

What does all this mean? RAD52 probably should not be drawn as a ring in schematics that propose RAD52's mechanism of action.

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1. M. S. K. M. A. Kharlamova *et al.*, Monomers and short oligomers of human RAD52 promote single-strand annealing. *Proc. Natl. Acad. Sci. U.S.A.* **122**, e2420771122 (2025).
2. L. R. Struble, J. J. Lovelace, G. E. O. Borgstahl, A glimpse into the hidden world of the flexible C-terminal protein binding domains of human RAD52. *J. Struct. Biol.* **216**, 108115 (2024).
3. D. S. Bhat, M. A. Spies, M. Spies, A moving target for drug discovery: Structure activity relationship and many genome (de)stabilizing functions of the RAD52 protein. *DNA Repair (Amst)* **120**, 103421 (2022).
4. W. Ranatunga *et al.*, Human RAD52 exhibits two modes of self-association. *J. Biol. Chem.* **276**, 15876–15880 (2001).
5. X. Deng *et al.*, Human replication protein A-Rad52-single-stranded DNA complex: Stoichiometry and evidence for strand transfer regulation by phosphorylation. *Biochemistry* **48**, 6633–6643 (2009).
6. M. R. Singleton, L. M. Wentzell, Y. Liu, S. C. West, D. B. Wigley, Structure of the single-strand annealing domain of human RAD52 protein. *Proc. Natl. Acad. Sci. U.S.A.* **99**, 13492–13497 (2002).
7. W. Kagawa *et al.*, Crystal structure of the homologous-pairing domain from the human Rad52 recombinase in the undecameric form. *Mol. Cell* **10**, 359–371 (2002).
8. A. Z. Stasiak *et al.*, The human Rad52 protein exists as a heptameric ring. *Curr. Biol.* **10**, 337–340 (2000).
9. K. Hanamshet, O. M. Mazina, A. V. Mazin, Reappearance from obscurity: Mammalian Rad52 in homologous recombination. *Genes (Basel)* **7**, 63 (2016).
10. G. Young *et al.*, Quantitative mass imaging of single biological macromolecules. *Science* **360**, 423–427 (2018).
11. W. Ranatunga, D. Jackson, I. R. Flowers II, G. E. Borgstahl, Human RAD52 protein has extreme thermal stability. *Biochemistry* **40**, 8557–8562 (2001).