Who's who in human recombination: BRCA2 and RAD52

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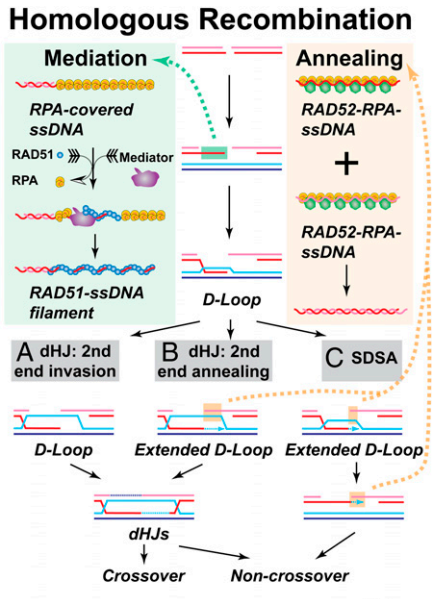
Homologous recombination (HR) is a key pathway to repair complex DNA damage, such as DNA gaps, double-strand breaks, and interstrand cross-links (1) (Fig. 1). Moreover, HR supports the recovery of stalled or broken replication forks and ensures faithful chromosome segregation during meiosis. The *RAD52* gene in the budding yeast *Saccharomyces cerevisiae* encodes the lynchpin of HR in this organism. Hence, the genes of the HR pathway are called the *RAD52* epistasis group. However, much to everybody's surprise, the *RAD52* gene knockout in mice exhibits minimal to no phenotypes in recombination, repair, and meiosis (2). Instead, the breast and ovarian tumor suppressor protein BRCA2 maintains a central HR function, a protein that is missing in budding yeast. Analyzing HR in *BRCA2*-deficient human cells, a study reported in PNAS (3) redefines human RAD52 by showing that it plays a critical role in HR in *BRCA2*-deficient cells. This important result mandates a re-examination of vertebrate RAD52 and its functions, which opens perplexing mechanistic conundrums.

In the study (3), Feng et al. manipulate the BRCA2 and RAD52 status by mutation, complementation, or RNA-mediated knockdown in three different human cell lines expressing either normal or truncated BRCA2 proteins. They show that lower RAD52 protein levels cause decreased levels of RAD51 foci and HR in *BRCA2*-deficient cells. These data greatly expand on a recent observation of a negative genetic interaction between mutants in the *Ustilago maydis BRCA2* and *RAD52* homologs (4). As noted by Feng et al. (3), these results have interesting implications for tumor therapy and suggest a more important role of RAD52 protein in human HR than previously thought.

Analyzing the proliferation rates in their cell lines, Feng et al. (3) show that normal levels of RAD52 expression are required for proliferation in cells with no or low BRCA2 function. This identifies a vulnerability of *BRCA2*-deficient cells besides inhibition of poly(ADP-ribose) polymerase (5), because depletion of RAD52 in such cells causes a strong proliferation defect (synthetic lethality) associated with severe chromosomal fragility. This identifies RAD52 as a potential therapeutic target not only in *BRCA2*-deficient cells (3) but possibly also in *BRCA2* revertants that become treatment resistant (6, 7).



		Mediator	Annealer	HR Phenotype
H. sapiens *M. musculus*	BRCA2	✔	NO	++
	RAD52	NO	✔	(+)
C. elegans	BRC-2	✔	✔	++
	No Rad52			
U. maydis	Brh2	✔	✔	++
	Rad52	low	✔	–
S. cerevisiae	No BRCA2			
	Rad52	✔	✔	++

Fig. 1. Roles of BRCA2 and RAD52 in homologous recombination. *Upper:* Schematic representation of the mediator and annealing functions during recombination involving (*A*) invasion of both ends, (*B*) invasion of the first end and second end capture by annealing, or (*C*) invasion of the first end, D-loop disruption, and annealing of the extended first end to the second end (SDSA, synthesis-dependent strand annealing). *Lower:* BRCA2 and RAD52 functions and phenotypes in selected organisms. Checkmark denotes presence of the biochemical activity, – denotes absence of phenotype, ++ denotes a strong and (+) a weak phenotype.

Why are the results by Feng et al. (3) so interesting from a mechanistic point of view? Biochemical analysis shows that the *S. cerevisiae* Rad52 protein performs two critical reactions in HR: (*i*) it mediates the assembly of Rad51 filament on replication protein A (RPA)-coated ssDNA (8–10), and (*ii*) it performs the annealing step in second end capture and synthesis-dependent strand annealing (11) (Fig. 1). This dual function readily explains why *rad52* mutants display even more extreme phenotypes than defects in the Rad51 protein, which performs the signature reactions of HR: homology search and DNA strand invasion (1). Surprisingly, in organisms containing a BRCA2 homolog, such as *U. maydis*, chicken, and mice, *RAD52* inactivation causes minimal or no HR and DNA repair defects (2, 4, 12). *Caenorhabditis elegans* and *Drosophila melanogaster* seem to lack a *RAD52* homolog entirely (Fig. 1).

So is *RAD52* irrelevant for HR in *BRCA2*-containing organisms? The expectation horizon from budding yeast *rad52* mutants was so high that the absence of strong phenotypes in the mouse *RAD52* mutants left this gene without much interest. However, the findings by Feng et al. (3) in *BRCA2*-deficient cells force another look at *RAD52* and its role in HR in vertebrates. Already the initial reports in *RAD52*-deficient mouse or chicken DT40 cells demonstrate a small but significant reduction in gene targeting (2, 12). Now Feng et al. (3) show that RAD52 depletion in *BRCA2*-complemented EUFA423 cells causes an increase in damage-induced chromosomal abnormalities, such as telomere end associations and radials. The reduction in the development of T-cell lymphomas in *ATM*-deficient mice by the *RAD52* single knockout also indicates a significant in vivo function of mouse RAD52 (13). In conclusion, *RAD52* mutants in vertebrates do have HR-related phenotypes, although they are much more subtle than expected from the yeast paradigm.

How does mammalian RAD52 act: as a mediator or annealer (Fig. 1)? Several genetic results favor the model proposed by Feng et al. (3) that RAD52 defines an alternative mediator pathway to BRCA2. They show that *BRCA2* status does not affect RAD52–RAD51 focus formation and that restoring RAD52 expression enhances RAD51 focus formation in *BRCA2*-deficient cells. Although it is formally possible that RAD52 acts upstream of BRCA2, the alternative mediator model is more consistent with additional synthetic phenotypes involving *RAD52*. In chicken DT40 cells, *RAD52* disruption is lethal with a defect in XRCC3, a RAD51 paralog that forms a complex with RAD51C required for RAD51 filament

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formation/stability (14). In addition in *U. maydis*, *rad52* mutants show a synthetic enhancement of UV and IR sensitivity with a mutant in the only *RAD51* paralog gene, *REC2* (4). However, human RAD52 protein lacks mediator activity in reconstituted biochemical reactions (15). Possibly, RAD52 performs its mediator function in conjunction with other proteins, perhaps the RAD51 paralog complex of RAD51B/C/D-XRCC2, whose inactivation also shows a synthetic defect with an *XRCC3* mutation in chicken DT40 cells (16) just like a RAD52 defect (14).

Last, the analysis of HR in humans poses another interesting conundrum: which protein is responsible for the annealing steps in mammalian HR [(b) and (c) in Fig. 1]? RAD52 is capable of performing this reaction in vitro (15) and in vivo (17), but the single mutant or RAD52 depletion lacks the expected phenotypes (IR sensitivity, strong HR phenotype) (2, 3, 17). *BRCA2* mutants or depletion exhibits strong HR and DNA repair phenotypes (3, 17). However, unlike its *U. maydis* and *C. elegans* homologs (18, 19), the human BRCA2 protein is incapable of annealing RPA-coated ssDNA, although it displays strong mediator activity (15, 20). Several possible solutions can be entertained. The most radical would be that HR in vertebrates does not involve an annealing step [i.e., no synthesis-dependent strand annealing pathway; (c) in Fig. 1] but involves second end strand invasion [(a) in Fig. 1]. The resulting double Holliday junctions (dHJs) will put a considerable burden on the dHJ dissolution pathway involving BLM-TOPO3α-RMI1-RMI2 (21). The high incidence of anaphase bridges in mammalian cells may be an indication of failure of dissolving the frequent dHJs (22). Alternatively, there might be a cofactor that endows BRCA2 with the ability to anneal RPA-coated ssDNA. Finally, an entirely novel protein might be involved. Interestingly, all five human RecQ-like proteins have been reported to anneal ssDNA, but this activity is inhibited by RPA, and the biological significance of these activities remains unclear (23–27).

Lower RAD52 protein levels cause decreased levels of RAD51 foci and HR in BRCA2-deficient cells.

In sum, human RAD52 functions to anneal RPA-coated ssDNA, and the important in vivo role of human RAD52 in *BRCA2*-deficient cells uncovered by Feng et al. (3) may entail RAD51 stimulation not involving classical mediation but possibly the RPA-independent effects seen in earlier biochemical studies (28, 29). The study by Feng et al. (3) forces renewed attention to RAD52 in mammalian HR, with interesting implications not only for the fundamental HR mechanism but also for anticancer therapy.

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1. Heyer WD (2007) Biochemistry of eukaryotic homologous recombination. *Molecular Genetics of Recombination*, eds Aguilera A, Rothstein R (Springer-Verlag, Berlin), pp 95–133.
2. Rijkers T, et al. (1998) Targeted inactivation of mouse *RAD52* reduces homologous recombination but not resistance to ionizing radiation. *Mol Cell Biol* 18: 6423–6429.
3. Feng Z, et al. (2011) Rad52 inactivation is synthetically lethal with BRCA2 deficiency. *Proc Natl Acad Sci USA* 108:686–691.
4. Kojic M, Mao NH, Zhou QW, Lisby M, Holloman WK (2008) Compensatory role for Rad52 during recombinational repair in *Ustilago maydis*. *Mol Microbiol* 67: 1156–1168.
5. Martin SA, Lord CJ, Ashworth A (2008) DNA repair deficiency as a therapeutic target in cancer. *Curr Opin Genet Dev* 18:80–86.
6. Edwards SL, et al. (2008) Resistance to therapy caused by intragenic deletion in BRCA2. *Nature* 451:1111–1115.
7. Sakai W, et al. (2008) Secondary mutations as a mechanism of cisplatin resistance in BRCA2-mutated cancers. *Nature* 451:1116–1120.
8. Sung P (1997) Function of yeast Rad52 protein as a mediator between replication protein A and the Rad51 recombinase. *J Biol Chem* 272:28194–28197.
9. Shinohara A, Ogawa T (1998) Stimulation by Rad52 of yeast Rad51-mediated recombination. *Nature* 391: 404–407.
10. New JH, Sugiyama T, Zaitseva E, Kowalczykowski SC (1998) Rad52 protein stimulates DNA strand exchange by Rad51 and replication protein A. *Nature* 391: 407–410.
11. Sugiyama T, New JH, Kowalczykowski SC (1998) DNA annealing by RAD52 protein is stimulated by specific interaction with the complex of replication protein A and single-stranded DNA. *Proc Natl Acad Sci USA* 95: 6049–6054.
12. Yamaguchi-Iwai Y, et al. (1998) Homologous recombination, but not DNA repair, is reduced in vertebrate cells deficient in RAD52. *Mol Cell Biol* 18:6430–6435.
13. Treuner K, Helton R, Barlow C (2004) Loss of Rad52 partially rescues tumorigenesis and T-cell maturation in Atm-deficient mice. *Oncogene* 23:4655–4661.
14. Fujimori A, et al. (2001) Rad52 partially substitutes for the Rad51 paralog XRCC3 in maintaining chromosomal integrity in vertebrate cells. *EMBO J* 20:5513–5520.
15. Jensen RB, Carreira A, Kowalczykowski SC (2010) Purified human BRCA2 stimulates RAD51-mediated recombination. *Nature* 467:678–683.
16. Yonetani Y, et al. (2005) Differential and collaborative actions of Rad51 paralog proteins in cellular response to DNA damage. *Nucleic Acids Res* 33:4544–4552.
17. Stark JM, Pierce AJ, Oh J, Pastink A, Jasin M (2004) Genetic steps of mammalian homologous repair with distinct mutagenic consequences. *Mol Cell Biol* 24: 9305–9316.
18. Petalcorin MIR, Sandall J, Wigley DB, Boulton SJ (2006) CeBRC-2 stimulates D-loop formation by RAD-51 and promotes DNA single-strand annealing. *J Mol Biol* 361: 231–242.
19. Mazloum N, Zhou QW, Holloman WK (2007) DNA binding, annealing, and strand exchange activities of Brh2 protein from Ustilago maydis. *Biochemistry* 46: 7163–7173.
20. Liu J, Doty T, Gibson B, Heyer WD (2010) Human BRCA2 protein promotes RAD51 filament formation on RPA-covered single-stranded DNA. *Nat Struct Mol Biol* 17: 1260–1262.
21. Wu LJ, Hickson ID (2003) The Bloom's syndrome helicase suppresses crossing over during homologous recombination. *Nature* 426:870–874.
22. Chan KL, Palmai-Pallag T, Ying SM, Hickson ID (2009) Replication stress induces sister-chromatid bridging at fragile site loci in mitosis. *Nat Cell Biol* 11:753–760.
23. Sharma S, et al. (2005) Biochemical analysis of the DNA unwinding and strand annealing activities catalyzed by human RECQ1. *J Biol Chem* 280:28072–28084.
24. Garcia PL, Liu YL, Jiricny J, West SC, Janscak P (2004) Human RECQ5beta, a protein with DNA helicase and strand-annealing activities in a single polypeptide. *EMBO J* 23:2882–2891.
25. Cheok CF, Wu L, Garcia PL, Janscak P, Hickson ID (2005) The Bloom's syndrome helicase promotes the annealing of complementary single-stranded DNA. *Nucleic Acids Res* 33:3932–3941.
26. Macris MA, Krejci L, Bussen W, Shimamoto A, Sung P (2006) Biochemical characterization of the RECQ4 protein, mutated in Rothmund-Thomson syndrome. *DNA Repair (Amst)* 5:172–180.
27. Machwe A, Lozada EM, Xiao L, Orren DK (2006) Competition between the DNA unwinding and strand pairing activities of the Werner and Bloom syndrome proteins. *BMC Mol Biol* 7:1.
28. Benson FE, Baumann P, West SC (1998) Synergistic actions of Rad51 and Rad52 in recombination and DNA repair. *Nature* 391:401–404.
29. New JH, Kowalczykowski SC (2002) Rad52 protein has a second stimulatory role in DNA strand exchange that complements replication protein-A function. *J Biol Chem* 277:26171–26176.