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CHEMTRACTS

Tailed Duplex DNA Is the Preferred Substrate for Rad51 Protein-Mediated Homologous Pairing

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Condensation of the Research

Purpose of the Study

To elucidate the mechanism of homologous recombination and double-strand break repair mediated by the eukaryotic recombination pin, Rad51

Backgrounds

The stability and integrity of genomes is constantly challenged by a variety of exogenous and endogenous factors causing different types of DNA damage, e.g., formation of thymidine dimers, single-strand gaps, and double-strand breaks. Already early in the history of life several mechanisms evolved to maintain the functionality of the DNA by repairing damaged DNA.

Double-strand breaks (DSBs) arise in living cells mainly as a result of the normal replication activities and, to a lesser extent, in rearrangement reactions like mating-type switches in yeast or the V(D)J joining of immunoglobulin genes in higher animals.1,2 Environmental factors such as ionizing radiation also contribute to DSB formation. Experimentally DSBs can be induced by X-rays or by mobilization of transposable elements. The repair of these potentially lethal events is accomplished by replication linked to homologous recombination (error-free repair) and by error-prone pathways (nonhomologous end-joining). The nonhomologous end-joining mechanism, where any severed DNA ends are simply rejoined, seems to be the preferred repair strategy for vertebrate somatic cells. However, germ line cells, certain specialized cells, and unicellular organisms have to rely on the higher and crucial fidelity of the recombination-linked

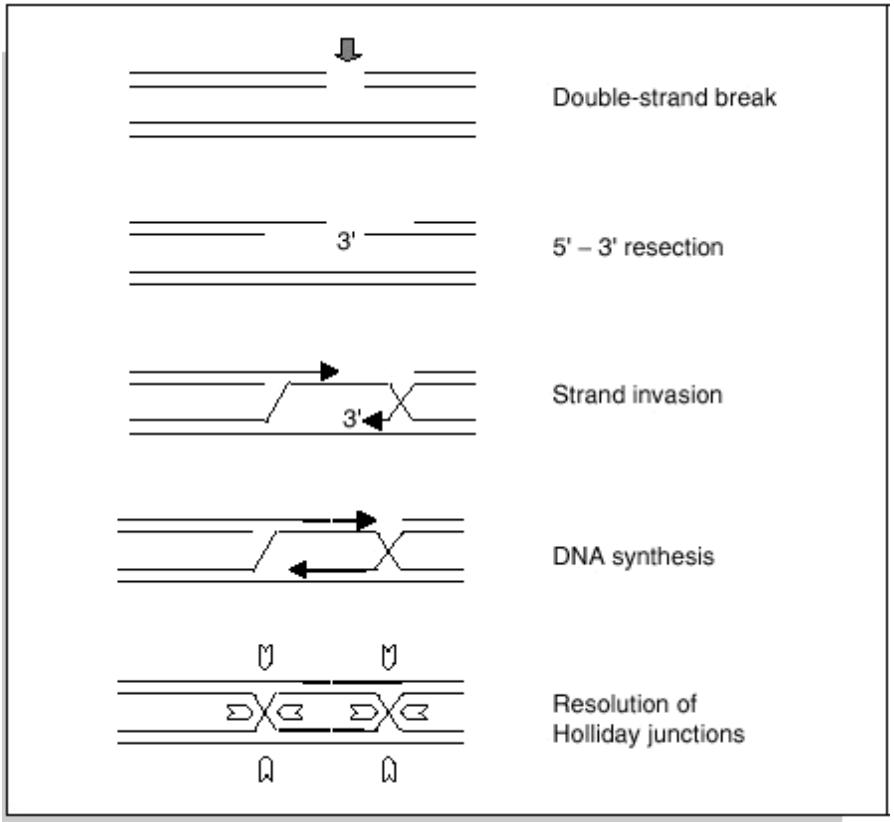


Figure 1. Model of recombination-linked double-strand break repair (based on ref. 4). Double-strand breaks are processed by 50-30 exonucleases resulting in 30-ssDNA tails. After formation of presynaptic complexes, the 30-ssDNA ends invade homologous dsDNA and serve as primers for a subsequent DNA synthesis. Following synthesis and branch migration, the arising symmetric Holliday junctions are resolved. Alternative models for replication-mediated DSB repair exist, but all of them require the invasion of dsDNA by a 30 tail (for a recent review, compare ref. 1).

processes, where homologous recombination and replication mechanisms assure restoration of the original connectivity along the DNA. There appears to be a balance between the benefits and costs of those two distinct pathways. Whereas yeast cells employ mainly the error-free, homologous recombination pathway, somatic vertebrate cells use preferentially the apparently less sophisticated, simple and fast, but frequently error-prone pathway of DNA end joining.

The recombination pathway requires that DSBs are processed to yield a 30 single-stranded DNA tail, on which a homologous pairing protein can be loaded, resulting in the formation of a nucleoprotein filament. This filament is competent for the search for homologous dsDNA, strand invasion and initiation of replicational repair.2,3

In the bacterial system, the 30 ends, covered by the homologous pairing protein RecA, serve as invasive ends and can also be used as a primer for replication. Branch migration mediated by RecA proceeds in the 50 to 30 direction. Following the synthesis of new DNA, the Holliday junctions are resolved, which can lead to crossing over (Fig. 1).4

The prokaryotic RecA is involved in general recombination and repair pathways.3,5-7 The protein forms a right-handed extended filament on DNA, and this complex catalyzes the search for homologous regions, the pairing of DNA, and the exchange of strands.8 RecA protein function is crucial not only in recombination, but also for the repair of stalled replication forks (reviewed in ref. 9). Furthermore, RecA is required for the induction of the SOS response in *Escherichia coli*.

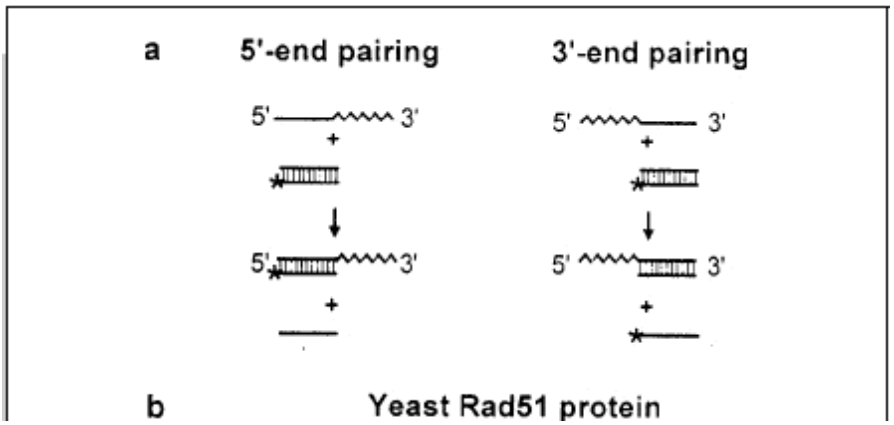
The eukaryotic proteins ScRad51 and hRad51 show both structural and functional homology to RecA.10,11 The yeast protein shows about 30% identity to RecA within a 207-amino-acid core region. Rad51 protein is necessary for homologous recombination and double-strand break (DSB) repair.12 However, in some biochemical activities each of the Rad51 proteins differs from RecA. In contrast to RecA protein, the direction of branch migration for the human protein shows an opposite 30 to 50 preference and is bidirectional in the case of ScRad51.13,14 It is well established that RecA polymerizes on ssDNA in the 50 to 30 direction,15 ensuring coverage of 30 ends, which are activated and protected against exonucle-ases. Rad51 proteins do not exhibit the same bias for binding ssDNA as RecA, but have similar affinities for both ss and dsDNA.11,16,17 The direction of filament formation has not been demonstrated yet. In the context of their role in DSB repair, the direction of branch migration for the eukary-otic proteins is posing the question of how can it be accommodated with a model for DSB repair, which requires the DNA synthesis to start from the invading 30 end of ssDNA.

Here the authors investigate the pairing bias of yeast and human Rad51, demonstrating that in both cases the 50 >end of purely ssDNA substrates is more invasive than the 30 end; however, these DNA substrates do not resemble those used during DSB repair. Instead, when a more natural substrate, ssDNA-tailed dsDNA, is used, both proteins used 30-tailed and 50-tailed DNA almost equally well to initiate pairing with homologous dsDNA.

What Researchers Accomplished

An in vitro system was designed to establish which end of single-stranded DNA substrates (oligonucleotides) was more efficient in DNA strand exchange reactions, using short dsDNAs as the second substrate. The oligonucleotides, carrying a homologous region to the dsDNA at either the 30 or 50 end, were incubated with scRad51, hRad51, or RecA, respectively; after strand exchange with the dsDNA substrates, the products were analyzed by gel electrophoresis and quantified (Fig. 2). As expected, RecA showed a strong preference for 30 homology, resulting in higher yields of strand exchange. However, both the yeast and the human proteins showed a bias for 50 homology.

To verify these results, the authors used a D-loop assay that measured the invasion of supercoiled dsDNA by single-stranded DNA, and the subsequent formation of a joint molecule. The 90-mer oligonucleotides used in this assay carried a homologous region of 63 bases either at the 30 or the 50 end. The eukaryotic Rad51 proteins showed a preference for the 50 homology resulting in higher yields of joint molecules, whereas RecA was more reactive with oligonucleotides harboring the homology at the 30 end (Fig. 3). The pairing bias of Rad51 was not changed by the presence



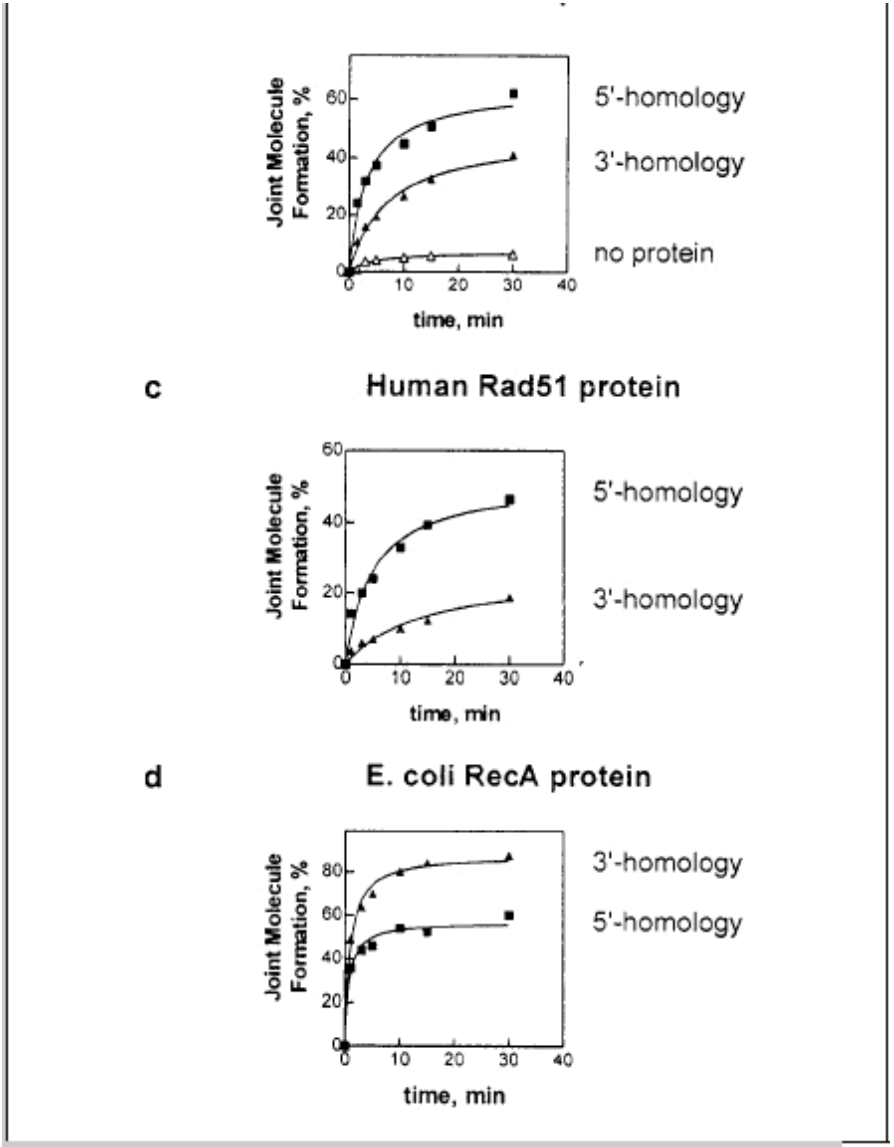
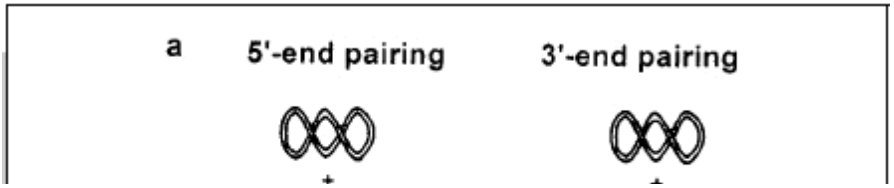


Figure 2. Human and yeast Rad51 proteins show a pairing preference for 50-ssDNA ends. **a**, Experimental design (straight lines indicate homologous regions, zig-zag lines heterology ; asterisks represent 32P-labeled nucleotides). **b**, **c**, and **d**, Strand exchange kinetics for the reaction catalyzed by yeast Rad51, human Rad51, and RecA, respectively. (Reproduced with permission, from Mazin, A. V., et al. *EMBO J.* ***2000****, 19, 1148. Copyrightã 2000 by Oxford University Press.)*



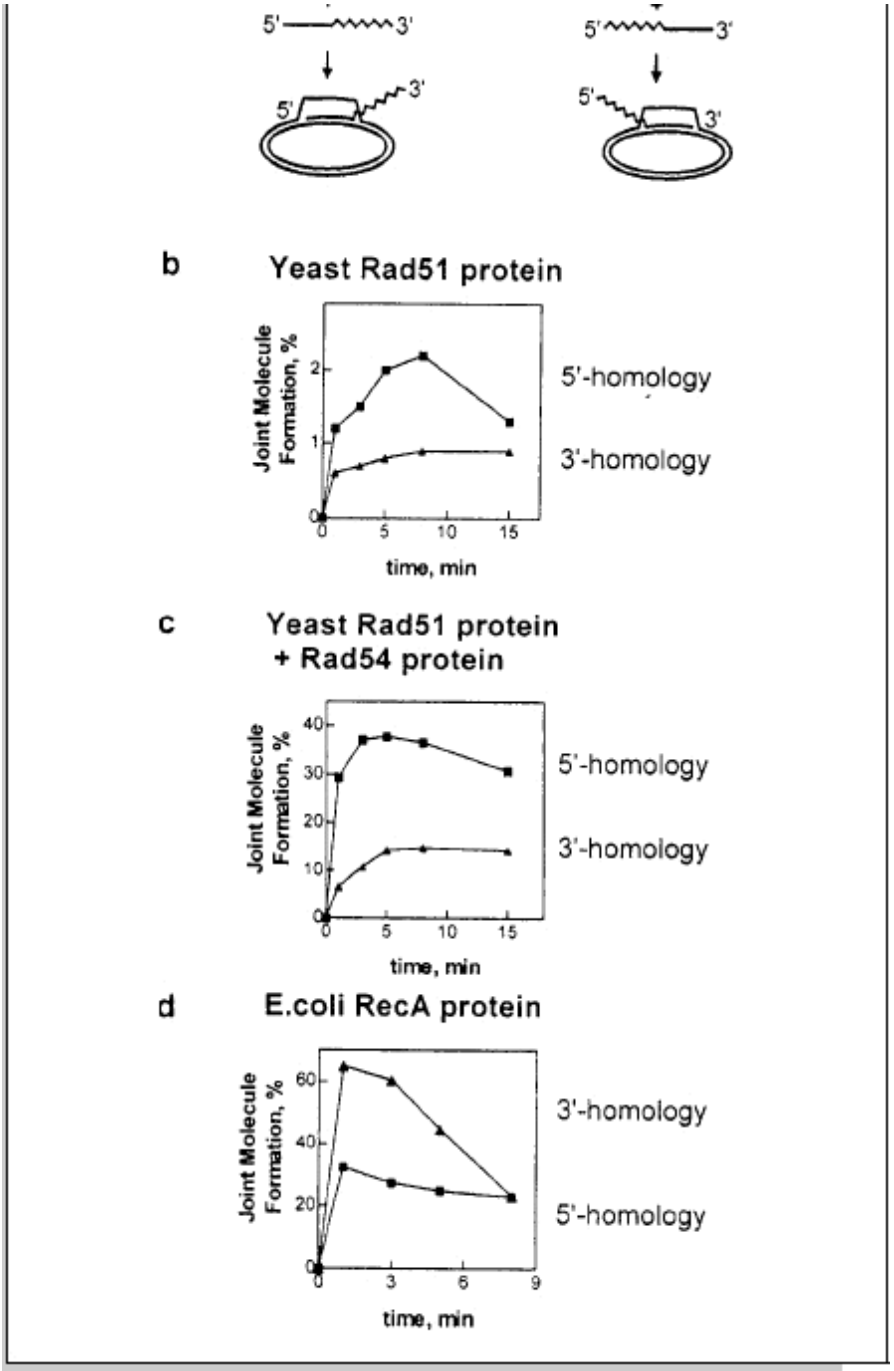


Figure 3. D-loop assays testing the invasion of supercoiled dsDNA by ssDNA. **a**, Experimental design (the homology is either limited to the 30 or 50 end of the ssDNA). **b**, Reaction mediated by yeast Rad51. **c**, Kinetics of the reaction catalyzed by yeast Rad51 in the presence of Rad54, which leads to a much higher product yield. **d**, RecA protein of *E. coli* has opposite substrate preferences and is more efficient with both substrates. (Reproduced with permission, from Mazin, A.V., et al. *EMBO J.* **2000**,

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findings of the authors partly resolve the paradox, which results from the fact that 30 ssDNA tails are established as the intermediates in DSB repair rather than 50 ssDNA-tailed dsDNA.

Rad51 shows a strong binding bias for tailed DNA substrates, and this increase in affinity is higher for 30 ends than for 50 ends, which counteracts the preference for 50 ends. Also both the displacement of RPA and the loading of Rad51 onto the DNA is faster with ssDNA-tailed dsDNA, i.e., naturally occurring substrates.

Therefore, 30-tailed dsDNA, produced in vivo after processing of DNA ends, can be very efficiently used by Rad51 to initiate the homologous recombination needed for DSB repair (compare Fig. 1). It seems that for Rad51, and also for RecA, the choice of the polarity of pairing required for DSB repair is left to the enzymes processing the break. These enzymes, nucleases and helicases, prepare 30-tailed ends which, upon pairing, can serve as primers for DNA polymerases.

For at least one other eukaryotic homolog of RecA, the human DMC1 protein, in vitro DNA strand exchange assays using linear dsDNA and circular ssDNA demonstrated a 30 to 50 directed branch migration.18 The invasiveness of ssDNA ends was not tested in that work; however, additional experiments showed very efficient binding to tailed DNA.18 Therefore it is likely that DMC1, like Rad51, can also use 30-tailed DNA to initiate homologous recombination. The fact that knockout mice without Rad51 are not viable.19,20 suggests that the other eukaryotic RecA homologs can not substitute for Rad51 in vivo. On the other hand, yeast mutants lacking a functional Rad51 are viable, although they are highly susceptible to DNA damaging agents.21 This could implicate additional functions of vertebrate Rad51 in cell proliferation and embryonic development. Perhaps some of these functions will utilize pairing of 50-tailed DNAs.

Summary

In in vitro assays using single- and double-stranded DNA, the eukaryotic homologous pairing proteins, yeast Rad51 and human Rad51, show a pairing bias for the 50 end of ssDNA. This is in contrast to the bias displayed by RecA. This fact was regarded as inconsistent with the role of Rad51 during DSB repair where 30-tailed ssDNA ends are believed to invade homologous dsDNA. However, when 30-tailed and 50-tailed dsDNAs are used, both substrates pair efficiently with homologous dsDNA. Therefore, Rad51 proteins can use naturally occurring 30-tailed ends for efficient DSB repair.

References

1. Haber, J.E. *Trends Biochem. Sci.* **1999**, *24*, 271.
2. Kanaar, R., Hoeijmakers, J.H., van Gent, D.C. *Trends Cell Biol.* **1998**, *8*, 483.
3. Cox, M.M. *Prog. Nucleic Acid Res. Mol. Biol.* **1999**, *63*, 311.
4. Szostak, J.W., Orr-Weaver, T.L., Rothstein, R.J., Stahl, F.W. *Cell.* **1983**, *33*, 25.
5. Roca, A.I., Cox, M.M. *Prog. Nucleic Acid Res. Mol. Biol.* **1997**, *56*, 129.
6. Bianco, P.R., Tracy, R.B., Kowalczykowski, S.C. *Front. Biosci.* **1998**, *17*, D570.
7. Kowalczykowski, S.C. *Trends Biochem. Sci.* **2000**, *25*, 156.
8. Howard-Flanders, P., West, S.C., Stasiak, A. *Nature* **1984**, *309*, 215.
9. Michel, B. *Trends Biochem. Sci.* **2000**, *25*, 173.
10. Ogawa, T., Yu, X., Shinohara, A., Egelman, E.H. *Science* **1993**, *259*, 1896. **11.** Benson, F.E., Stasiak, A., West, S.C. *EMBO J.* **1994**, *13*, 5764.
12. Baumann, P., West, S.C. *Trends Biochem Sci.* **1998**, *23*, 247. **13.** Sung, P., Robberson, D.L. *Cell*. **1995**, *82*, 453.
14. Namsaraev, E.A., Berg, P. *Proc. Natl. Acad. Sci. USA* **1998**, *95*, 10477. **15.** Register, J.C. 3d, Griffith, J. *J. Biol. Chem.* **1985**, *260*, 12308.
16. Baumann, P., Benson, F.E., West, S.C. *Cell* **1996**, *87*, 757.
17. Zaitseva, E.M., Zaitsev, E.N., Kowalczykowski, S.C. *J. Biol. Chem.* **1999**, *274*, 2907. **18.** Masson, J.Y., Davies, A.A., Hajibagheri, N., Van Dyck, E., Benson, F.E., Stasiak, A.Z., Stasiak, A., West, S.C.

EMBO J. **1999**, *18*, 6552.
19. Tsuzuki, T., Fujii, Y., Sakumi, K., Tominaga, Y., Nakao, K., Sekiguchi, M., Matsu-shiro, A., Yoshimura, Y., Morita, T. *Proc. Natl. Acad. Sci. USA.* **1996**, *93*, 6236. **20.** Lim, D.S., Hasty, P. *Mol. Cell. Biol.* **1996**, *16*, 7133.
21. Shinohara, A., Ogawa, H., Ogawa, T. *Cell.* **1992**, *69*, 457.