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 protein, Rad51

Background

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

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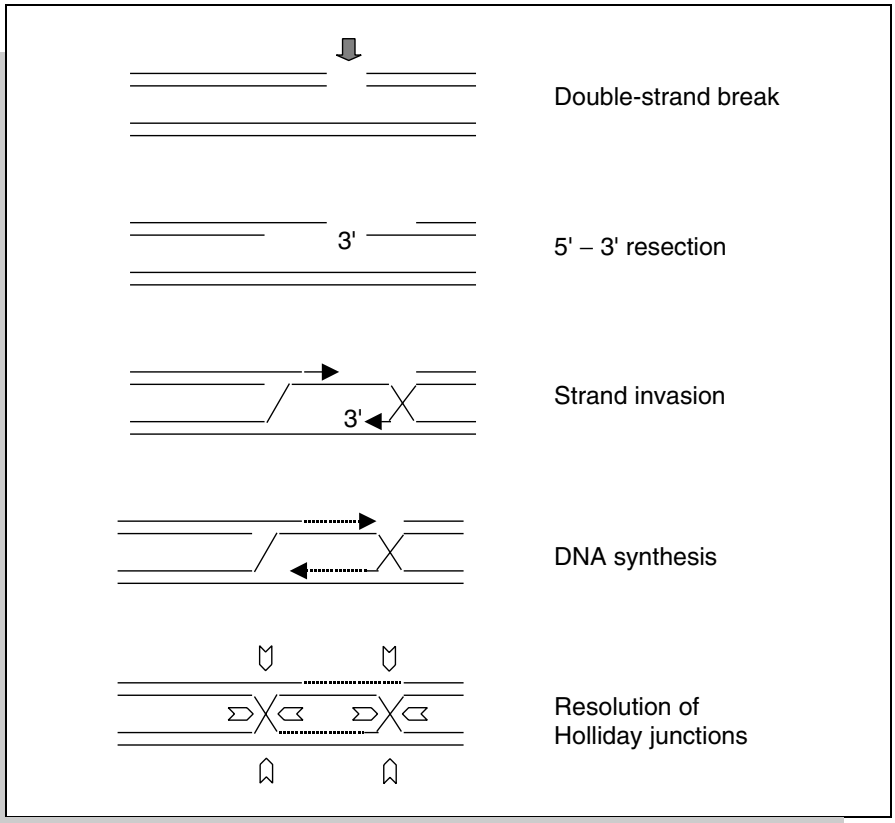

Figure 1. Model of recombination-linked double-strand break repair (based on ref. 4). Double-strand breaks are processed by 5′-3′ exonucleases resulting in 3′-ssDNA tails. After formation of presynaptic complexes, the 3′-ssDNA ends invade homologous dsDNA and serve as primers for a subsequent DNA synthesis. 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 3′ 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 3′ 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 3′ 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 5′ to 3′ 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 3′ to 5′ preference and is bidirectional in the case of ScRad51.[13,14] It is well established that RecA polymerizes on ssDNA in the 5′ to 3′ direction,[15] ensuring coverage of 3′ ends, which are activated and protected against exonucleases. 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 eukaryotic 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 3′ end of ssDNA.

Here the authors investigate the pairing bias of yeast and human Rad51, demonstrating that in both cases the 5′ end of purely ssDNA substrates is more invasive than the 3′ 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 3′-tailed and 5′-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 3′ or 5′ 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 3′ homology, resulting in higher yields of strand exchange. However, both the yeast and the human proteins showed a bias for 5′ 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 3′ or the 5′ end. The eukaryotic Rad51 proteins showed a preference for the 5′ homology resulting in higher yields of joint molecules, whereas RecA was more reactive with oligonucleotides harboring the homology at the 3′ end (Fig. 3). The pairing bias of Rad51 was not changed by the presence

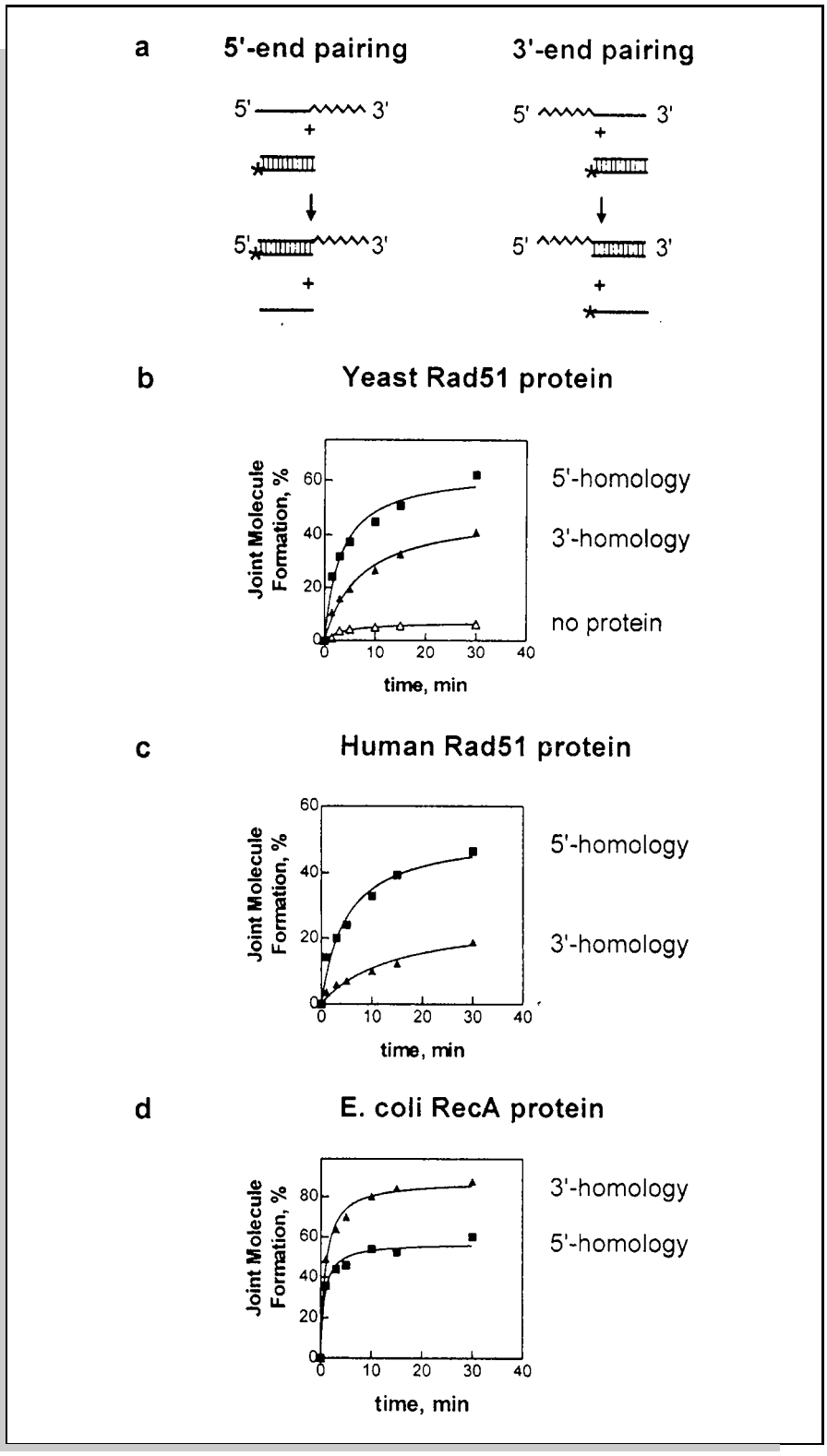

Figure 2. Human and yeast Rad51 proteins show a pairing preference for 5′-ssDNA ends. **a**, Experimental design (straight lines indicate homologous regions, zig-zag lines heterology ; asterisks represent ^{32}P-labeled nucleotides). **b**, **c**, and **d**, Strand exchange kinetics for the reaction catalyzed by yeast Rad51, human Rad51, and RecA, respectively. (Reproduced with permission, from Mazin, A.V., et al. *EMBO J.* **2000**, *19*, 1148. Copyright © 2000 by Oxford University Press.)

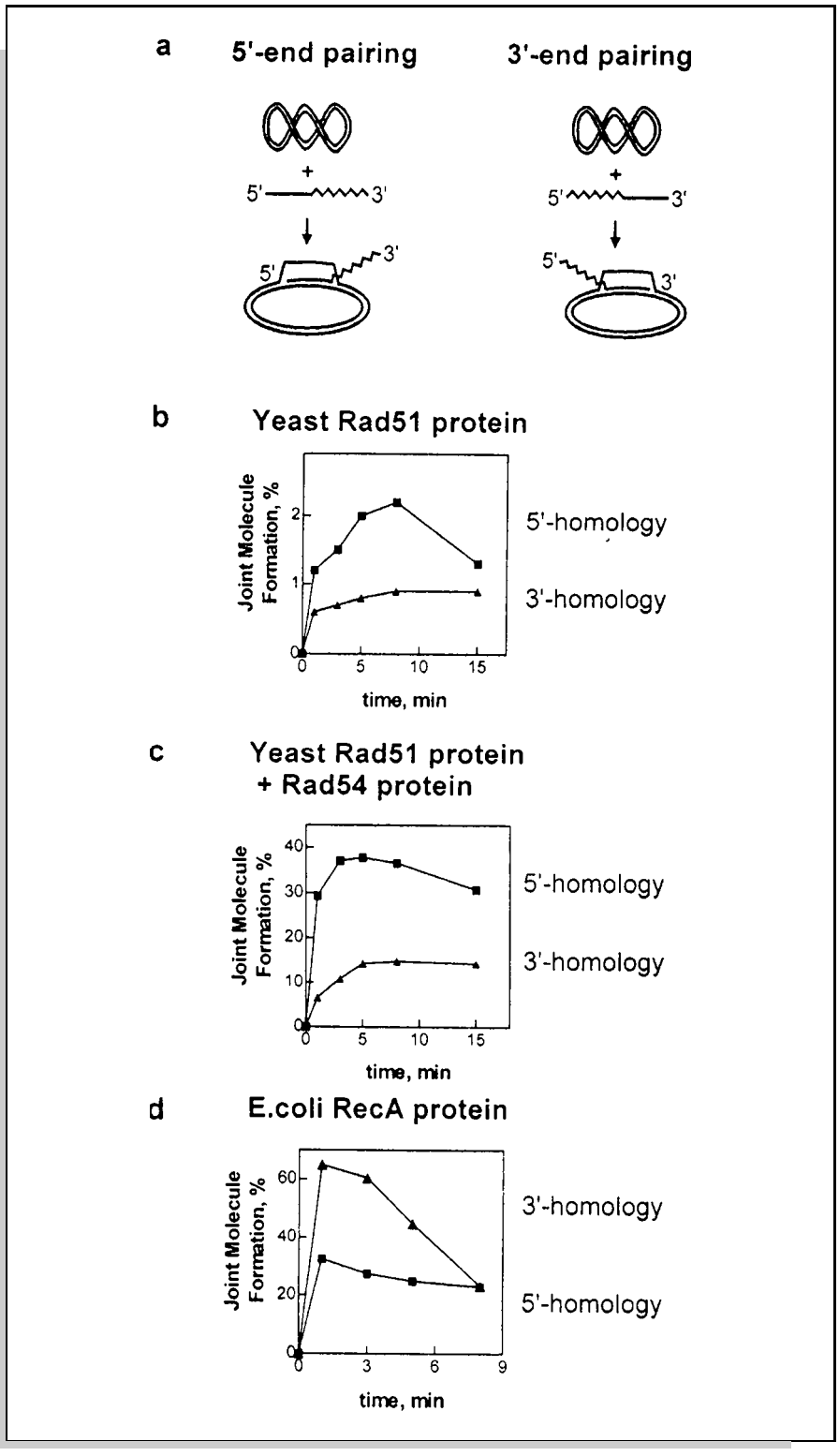

Figure 3. D-loop assays testing the invasion of supercoiled dsDNA by ssDNA. **a**, Experimental design (the homology is either limited to the 3′ or 5′ end of the ssDNA). **b**, Reaction mediated by yeast Rad51. **c**, Kinetics of the reaction catalyzed by yeast Rad51 in the presence of Rad54, which leads 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**, *19*, 1148. Copyright © 2000 by Oxford University Press.)

of other proteins of the Rad52 epistasis group (Rad52, Rad54, Rad55-57), although the addition of Rad54 resulted in higher yields of joint molecule formation.

One of the possible resolutions of the paradox posed by the opposite pairing bias of RecA and the Rad51 proteins is that the eukaryotic pairing proteins require a special substrate. To investigate this possibility, the authors employed ssDNA-tailed dsDNA substrates, shown to be the intermediates in DSB repair, in assays for presynaptic complex formation and in strand exchange reactions. Presynaptic complexes are formed by assembly of RecA or RecA-like proteins on ssDNA, thereby activating the ssDNA for homology search and DNA strand exchange. The initial rates of DNA strand exchange turned out to be higher for the ssDNA-tailed duplex DNA substrates than for the purely single-stranded DNA substrates. In agreement with the results obtained for ssDNA substrates, tailed dsDNA with 5′ homology was more reactive in the case of both the human and yeast Rad51. Surprisingly, the reaction rate for homologous 3′ ends was increased to a higher extent than the rate for 5′ ends, thereby reducing the difference between these two substrates (Fig. 4). The rate of DNA strand exchange was the highest at a molar ratio of one Rad51 to three nucleotides, irrespective of the nature the DNA substrate.

Interestingly, RecA was also equally efficient with either ssDNA or tailed dsDNA substrates. An additional property of ssDNA-tailed dsDNA, which makes it a favored substrate in in vitro strand exchange reactions, is the fact that the ssDNA binding protein RPA can be more easily displaced by Rad51 from tailed substrates than from ssDNA. This behavior was indicated by a 10- to 15-minute lag phase in the DNA strand exchange reactions using ssDNA, which was not observed with tailed dsDNA. RPA helps to unravel secondary structure in single-stranded regions of DNA, but then needs to be replaced by Rad51 during formation of presynaptic filaments.

Using gel mobility-shift assays, the authors investigated the binding of Rad51 to different DNA substrates in the presence of ATP-γ-S. dsDNA of 63 bp and tailed dsDNA (32 bp plus 31 bases) were the preferred substrates in those assays, whereas ssDNA was not bound sufficiently to be stable and shorter dsDNA (32 bp) was bound efficiently only at higher Rad51 concentrations. Competition experiments, in which Rad51 bound to either dsDNA or tailed dsDNA and then was challenged with heterologous dsDNA revealed that in the presence of either ATP and ATP-γ-S the binding of Rad51 protein to the ssDNA-tailed dsDNA substrate was stronger than the binding to the dsDNA.

Commentary on the Research

The work reviewed here is a valuable contribution to the biochemical characterization of yeast and human Rad51, eukaryotic homologues of the well characterized RecA of *E. coli*. Despite structural and functional similarities, there are some remarkable differences. The authors provide strong in vitro evidence that the filaments formed by Rad51 on 5′ ends of ssDNA are more invasive than filaments formed on 3′ ends, whereas for the prokaryotic protein, 3′ ends are more reactive. However, further

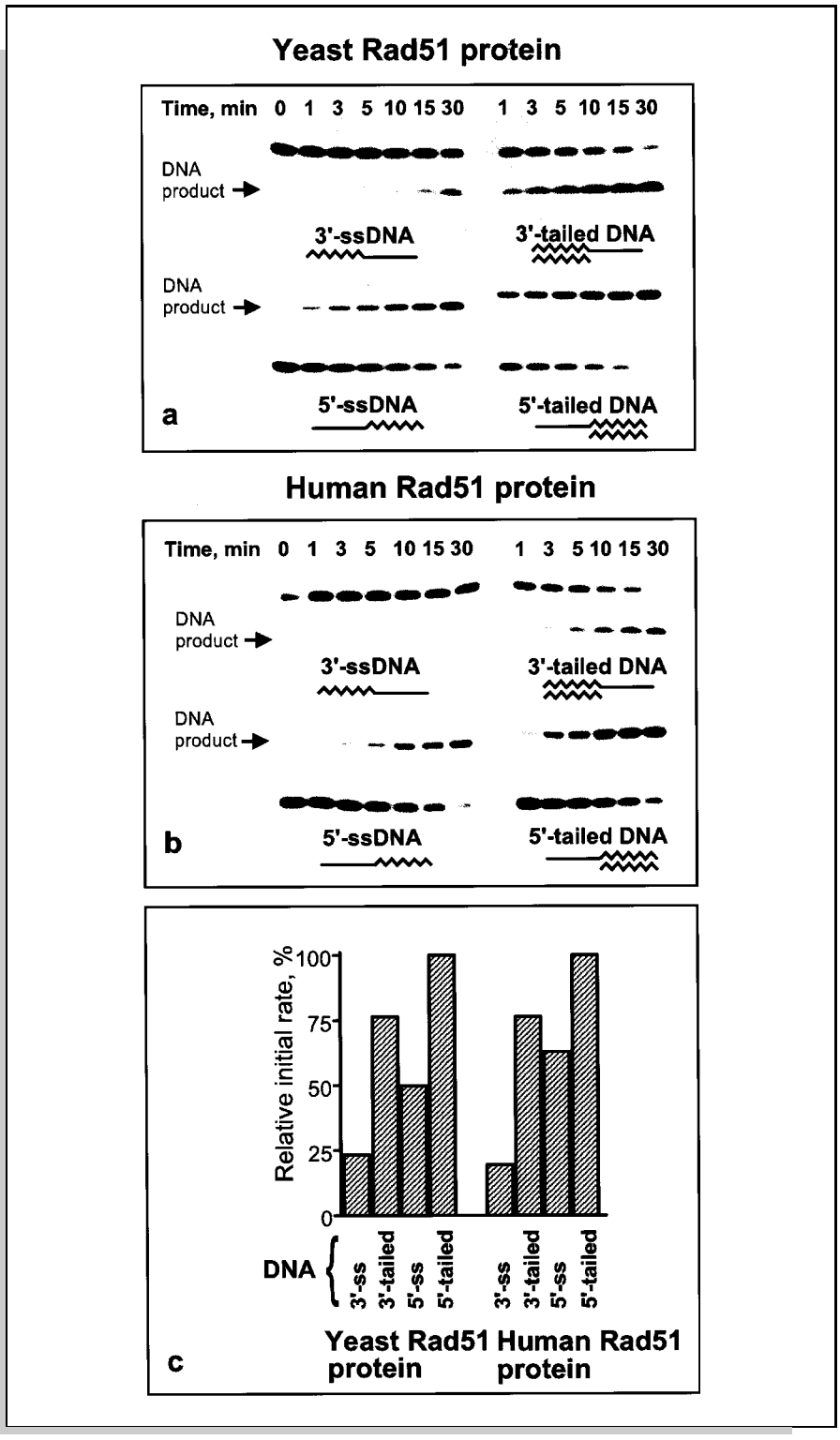

Figure 4. DNA strand exchange reactions utilizing ssDNA-tailed duplex DNA. **a**, The kinetics of DNA strand exchange catalyzed by yeast Rad51. **b**, Human Rad51-mediated reactions. **c**, Comparison of the relative initial rates of strand exchange for the ssDNA and ssDNA-tailed dsDNA substrates, respectively. The value for the 5′-tailed dsDNA was set to 100%. (Reproduced with permission, from Mazin, A.V., et al. *EMBO J.* **2000**, *19*, 1148. Copyright © 2000 by Oxford University Press.)

findings of the authors partly resolve the paradox, which results from the fact that 3′ ssDNA tails are established as the intermediates in DSB repair rather than 5′ ssDNA-tailed dsDNA.

Rad51 shows a strong binding bias for tailed DNA substrates, and this increase in affinity is higher for 3′ ends than for 5′ ends, which counteracts the preference for 5′ 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, 3′-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 3′-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 3′ to 5′ 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 3′-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 5′-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 5′ 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 3′-tailed ssDNA ends are believed to invade homologous dsDNA. However, when 3′-tailed and 5′-tailed dsDNAs are used, both substrates pair efficiently with homologous dsDNA. Therefore, Rad51 proteins can use naturally occurring 3′-tailed ends for efficient DSB repair.

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