

RecA-mediated strand exchange traverses substitutional heterologies more easily than deletions or insertions

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ABSTRACT

RecA protein in bacteria and its eukaryotic homolog Rad51 protein are responsible for initiation of strand exchange between homologous DNA molecules. This process is crucial for homologous recombination, the repair of certain types of DNA damage and for the reinitiation of DNA replication on collapsed replication forks. We show here, using two different types of *in vitro* assays, that in the absence of ATP hydrolysis RecA-mediated strand exchange traverses small substitutional heterologies between the interacting DNAs, whereas small deletions or insertions block the ongoing strand exchange. We discuss evolutionary implications of RecA selectivity against insertions and deletions and propose a molecular mechanism by which RecA can exert this selectivity.

INTRODUCTION

Homologous recombination helps to create genetic diversity by reassembling DNA sequences from homologous, but not completely identical, DNA molecules (1). The recombination is usually initiated by homologous pairing between a short single-stranded (ss) region in one molecule with the corresponding double-stranded (ds) region in the partner molecule. This leads to creation of heteroduplex regions composed of strands of different origin and to displacement of one strand of the original duplex. During this ss–ds phase of the reaction the 'decision' is made, whether the engaged molecules are homologous and the reaction should proceed further, or whether the partners are heterologous or homeologous and the reaction should be aborted (2). In the case of a positive decision, the ss–ds phase is followed by a ds–ds phase where the displaced strand also forms a heteroduplex with the complementary strand from the other molecule. In this second phase, RuvAB proteins take over the function of active promotion of strand exchange (3,4). However, it is the RecA protein in Bacteria and its homologs in Eucarya and Archaea that initiate the strand exchange between homologous DNA molecules (5–7).

Creation of new sequence combinations by homologous recombination has to be delicately balanced. New combinations should be rather conservative to give the highest chance

of functional maintenance of coded proteins. Accordingly, strand exchange should only rarely extend into highly heterologous regions. In fact RecA has very limited abilities to promote ds–ds strand exchange through substantial heterology (8,9). However, there should be an efficient strand exchange between homologous DNA regions coding for functional proteins. In individual lines of organisms, their DNA is subject to stochastic mutations wherein base substitutions are the most frequent non-detrimental mutations in protein-coding genes. Thus, for example, individual base substitutions due to the degeneracy of the genetic code can even maintain the same amino acids in coded proteins. In general, base substitutions maintain the same reading frame during translation and, in the case of a single substitution, result in a change of just one codon while the rest of the codons remain unchanged. Except for rare cases of accidental creation of stop codons, the base substitutions are usually much less harmful than even single base insertions or deletions that, by changing the reading frame, will result in coding for completely scrambled polypeptides. From an evolutionary point of view, single amino acid substitutions provide a safe testing ground for the generation of new functional variants of active proteins. Consequently, homologous genes in different individuals, or even on homologous chromosomes in the same diploid organism, should often have the same length and thus, upon pairing and aligning during DNA recombination, can maintain the homologous register upstream and downstream of independent substitutions. This fact can be used by RecA and by its eukaryotic homologs to distinguish substitutions from deletions and insertions. Heteroduplex formation between strands that differ by substitutions may proceed unimpeded. However, the strand exchange between DNA molecules that differ in length would probably require substantial rearrangement of synaptic RecA–DNA filaments. This in turn could trigger antirecombinogenic activity of mismatch repair proteins that inhibit heteroduplex formation between diverged sequences (2,9) or activate proteins such as RecG or RecQ that can reverse the ongoing strand exchange (10–12).

Numerous *in vitro* studies demonstrated that in a ss–ds strand exchange reaction, which normally initiates the recombination process, RecA is able to exchange DNA strands through large heterologies (13–16), but the efficiency of pairing and strand exchange is reduced by the presence of heterologies. So for example it was demonstrated that the presence of six mismatches in 83 nucleotide long substrate decreased 3-fold

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the rate constant for strand displacement (17). Corresponding *in vivo* studies are more difficult to perform, however it was observed that MutS and MutL proteins inhibit RecA-mediated strand exchange over heterologies (2). In addition the RecA ability to traverse heterologies may be downregulated *in vivo* by limited supply of ATP for example. Indeed, the ss-ds reactions performed *in vitro* were shown to be dependent on the presence of ATP to pass through insertions or deletions as small as 6 nucleotides (18), while the strand exchange between completely homologous regions of ss- and dsDNA molecules proceeded efficiently in the absence of ATP hydrolysis (18–22).

MATERIALS AND METHODS

RecA, Proteinase K, ATP and ADP were from Roche, additional fine chemicals were supplied by Fluka. Oligonucleotides were purchased from MedProbe. The sequences of oligonucleotides used are available as supplementary data to this article at NAR Online. dsDNA was obtained by annealing two complementary synthetic oligonucleotides. ³²P-end labeling was performed with a DNA 5'-end labeling kit (Fermentas).

Strand exchange assays

Strand exchange assays were carried out in a buffer consisting of 25 mM triethanolamine-HCl pH 7.2, 2 mM Mg-acetate and 2.5 mM ATP or adenosine 5'-(β , γ -imido) triphosphate (AMP-PNP).

The ssDNAs (concentration of nucleotides: 8 μ M) were incubated at 37°C with RecA protein at a concentration of 16 μ M in the presence of ATP or AMP-PNP for 5 or 15 min, respectively. After addition of the dsDNA (concentration of nucleotides: 8 μ M) the concentration of Mg-acetate was raised to 5 mM and the strand exchange reaction was allowed to proceed. The reaction aliquots were stopped by addition of SDS and EDTA (final concentrations: 0.1% SDS, 20 mM EDTA) and were further incubated for 5 min at 37°C. The reaction products were separated by non-denaturing polyacrylamide gel electrophoresis (12% polyacrylamide) in 1 \times TBE, stained with SYBR-Gold (Molecular Probes) and visualized on a UV-transilluminator.

Spontaneous branch migration

Spontaneous branch migration was assayed in the strand exchange buffer, supplemented with 0.1% SDS and 20 mM EDTA, at 37°C for 20 min. The DNA substrates are shown in Figure 2, the concentration of the dsDNA was 0.2 μ M. The strand to be displaced in the reaction was radioactively labeled. A 15-fold excess of unlabeled ssDNA (3 μ M of oligonucleotide) substrate was used to ensure efficient annealing of protruding strands of the labeled partial duplexes with their partners. The reaction was analyzed by non-denaturing polyacrylamide gel electrophoresis (12% polyacrylamide). The gels were then dried and exposed to X-ray films.

Topological unwinding assays

RecA aliquots (30 μ M) were incubated with oligonucleotides (50 μ M) in a buffer consisting of 20 mM triethanolamine-HCl pH 7.2, 1 mM Mg-acetate, 1 mM DTT and 2 mM adenosine-5'-O-3-thiotriphosphate (ATP- γ -S), for 60 min at 37°C. Presynaptic complexes were separated from unbound RecA on gravity flow columns of Sepharose CL-4B, equilibrated with

20 mM triethanolamine-HCl pH 7.2, 1 mM Mg-acetate, 1 mM DTT and 2 mM ATP- γ -S. The fractions containing RecA-oligonucleotide complexes were added in a 5-fold molar excess to relaxed plasmids (pUC18) and the magnesium concentration was raised to 6 mM. The pairing reaction was allowed to proceed for 1 h at 37°C. After additional incubation in the presence of topoisomerase I for 60 min at 37°C, the reactions were deproteinized by phenol/chloroform treatment and chloroform extraction. The samples were separated on 1.3% agarose gels containing 0.5 μ g/ml chloroquine. Electrophoresis was carried out for 24 h at 80 V, followed by staining with ethidium bromide and visualization on a UV-transilluminator.

RESULTS

Strand displacement assays with oligonucleotide substrates

We first decided to check if RecA in ss-ds reactions performed in the absence of ATP hydrolysis distinguishes base substitutions from deletions or insertions. To this aim we tested strand exchange reactions between ss- and dsDNA oligonucleotides containing short heterologies. The strand exchange reaction between ss- and dsDNA molecules is the most frequently used model system to study the RecA-mediated formation of heteroduplex regions. RecA protein binds co-operatively to ssDNA forming helical presynaptic filaments. These filaments then bind the homologous duplex DNA and form synaptic complexes in which ss- and dsDNA are kept in the homologous register (23,24). The actual strand switch seems to occur within the synaptic complexes. The DNA substrates used by us for RecA-assisted strand exchange reactions are schematically presented in Figure 1. To ensure that a productive RecA-ssDNA complex can be formed along the whole ss region homologous to the duplex partner we used ss oligonucleotides (72 nt) that were longer than their ds partners. Therefore, even if the 5'-ends of ssDNAs did not get covered with RecA (25) this should not affect the reaction. The substrate duplex DNA had the strand that is displaced in the reaction slightly longer than the strand that switches the partners during the reaction. This experimental design decreased the possibility of initiation of strand exchange by spontaneous annealing between a few terminal bases of the exchanged strand with the substrate ssDNA. The chosen sequences were of a natural origin and did not contain palindromic regions or other apparent sequence motifs that could induce formation of non-canonical DNA structures. Short heterologies between the used duplex and ssDNA were located in the centre of the oligonucleotides (Fig. 1A). Deletions and insertions consisted of elimination or addition, respectively, of a given number of bases in the substrate ssDNA. Substitutions consisted of replacements in the substrate ssDNA of short runs of bases by different sequences of the same length (the actual sequences are listed in the Supplementary Material). The reaction conditions are described in Materials and Methods. The yield of the reaction was evaluated on the basis of gel electrophoresis of deproteinized DNA molecules. Thorough deproteinization was needed to properly identify substrates and products of the reaction.

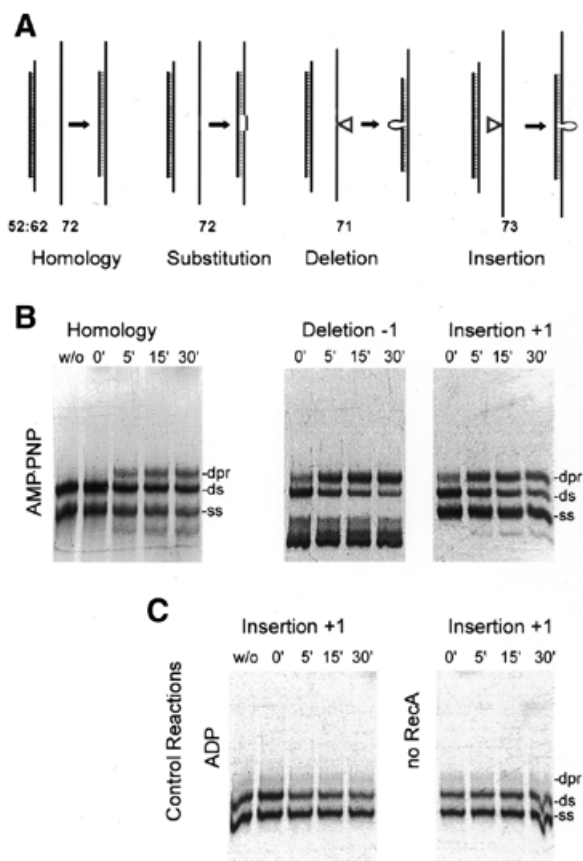


Figure 1. Strand exchange through 1-nt insertions or deletions seems to proceed as efficiently as through a complete homology. (A) Schematic presentation of substrates and products of RecA-assisted strand exchange reactions. The sequences of the oligonucleotides are available as supplementary material, the sizes are indicated. (B) Comparison of the reaction efficiency through a homology with those through a deletion or insertion of 1 nt. All reactions were performed in the presence of AMP-PNP (for details see Materials and Methods). (C) Control reactions. In the reaction in the left panel, AMP-PNP was substituted by ADP, whereas the right panel shows the reaction in the absence of RecA. Positions of single- and double-stranded substrates are indicated with ss and ds respectively. The position of double-stranded products of the reaction is denoted with dpr; weak single-stranded product bands can be seen below these of single-stranded substrates; w/o denotes control reactions performed without RecA and incubated for 5 min. The native, 12% acrylamide gels were stained with SYBR-Gold before visualization in UV.

Strand exchange passage through small insertions or deletions is a possible artifact of standard methodology

Since even one base heterology consisting of an insertion or deletion distorts the homologous register between ss- and dsDNA we decided to compare the efficiency of RecA-mediated strand exchange reactions between completely homologous partners and those differing by one base deletion or insertion. We performed the reactions in the presence of AMP-PNP, a non-hydrolyzable analog of ATP. As shown in Figure 1B, the strand exchange reaction seemed to proceed at least as efficiently through deletions and insertions as through a complete homology. Control reactions demonstrated that the observed strand exchange did depend on the presence of RecA (Fig. 1B, first lane and Fig. 1C) and when AMP-PNP was replaced by ADP the reaction was practically blocked, although a low level of RecA-independent reactions could also be observed (Fig. 1C).

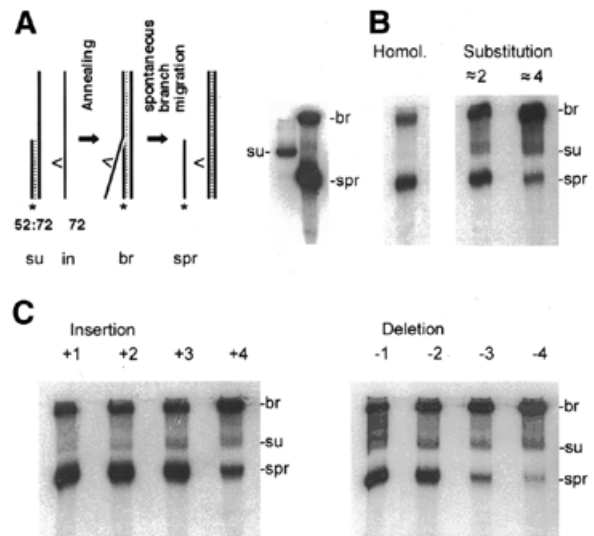


Figure 2. Spontaneous strand exchange easily traverses 1 or 2 nt long heterologies, but is attenuated by heterologies of 4 nt. (A) Reaction scheme presenting substrates and products of the spontaneous strand exchange and showing the migration of the labeled species. Notice that only the short strand of the substrate duplex (su) was radiolabeled (*) as this permits us to trace the formation of the branched intermediates (br) and of displaced single-stranded products (spr) while the single-stranded substrates present in excess do not interfere with the visualization of the relevant bands. < indicates the position of the heterology in the unlabeled, incoming oligonucleotide (in), which is in 15-fold molar excess. The size of the oligonucleotides is indicated. (B) and (C) Autoradiograms of the gels (native 12% polyacrylamide) demonstrating that, as the size of heterology increases up to 4 nt, the formation of labeled products of the reactions (displaced ssDNA) becomes strongly attenuated. Reaction conditions are detailed in Materials and Methods.

The fact that in the absence of ATP hydrolysis we observed an efficient strand passage through a heterology was surprising since earlier studies using a similar system showed that 6-nt insertions or deletions completely blocked the strand exchange reaction (18). We decided, therefore, to investigate the possibility that RecA-promoted strand exchange advances only to the point of heterology but then upon deproteinization (required for the gel analysis) it can proceed further. In fact, earlier studies by Panyutin and Hsieh (26) demonstrated that spontaneous branch migration involving ss- and dsDNA molecules efficiently traverses small heterologies. To check whether under the applied deproteinization conditions small heterologies can be efficiently traversed by the RecA-independent, spontaneous branch migration we used a set of substrates schematically presented in Figure 2. Partial duplex molecules with one strand having a long protruding end were incubated with ssDNA complementary to the long strand of the partial duplex. Upon spontaneous annealing between complementary ss regions, a joint molecule is formed that can undergo a branch migration resulting in a displacement of the shorter strand of the original duplex (Fig. 2). A similar experimental setup was used by Panyutin and Hsieh (26) in their studies of spontaneous branch migration. To characterize the effects of different types of heterology on spontaneous branch migration we introduced substitutions, deletions and insertions of different lengths into the portion of ssDNA that was to be exchanged with the short strand of partial duplex DNA

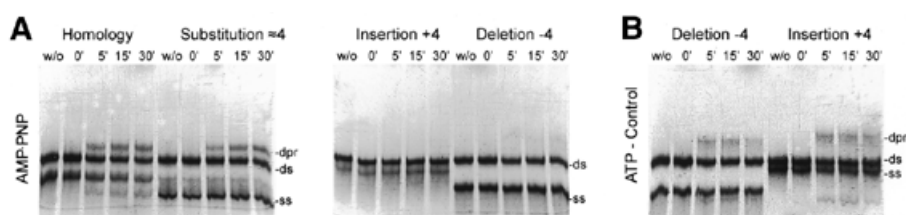


Figure 3. (A) RecA-mediated strand exchange reactions performed in the absence of ATP hydrolysis easily traverse substitutions of 4 nt but not insertions or deletions of this size. Native, 12% polyacrylamide gels of the respective reactions in the presence of AMP-PNP were stained with SYBR-Gold. Positions of single- and double-stranded substrates are indicated with ss and ds, respectively. The position of double-stranded products of the reaction is denoted with dpr (see Fig. 1 for schematic presentation of the substrates and products of the reaction). Lanes w/o, control reactions performed without RecA and incubated for 5 min. (B) Control reactions in the presence of ATP.

molecules. As shown in Figure 2, the spontaneous branch migration proceeded with a similar efficiency for strands that are completely homologous and for strands containing one base heterology in the form of substitution, deletion or insertion. This result is consistent with the notion that the efficient strand exchange detected on the gels shown in the Figure 1 can be attributed to spontaneous RecA-independent branch migration that occurred only after deproteinization of joint molecules and, therefore, is not a result of RecA action. Panyutin and Hsieh (26) reported that, as the size of heterology increases, the spontaneous branch migration through a heterology becomes attenuated. Indeed, that was also what we observed in our reactions. As the size of substitutions, insertions and deletions was stepwise increased up to 4 nt we observed a progressive attenuation of the spontaneous strand displacement (Fig. 2). Interestingly, the progression of attenuation was similar for substitutions, insertions and deletions. For heterologies of 4 nt, the attenuation of spontaneous branch migration reached 80–90%. Therefore, we decided to look at RecA-mediated strand exchange reaction over heterologies of this size since the background level due to spontaneous branch migration should no longer dominate the reaction outcome. In addition, in the substrates used for RecA-mediated strand exchange, the spontaneous branch migration had less chance of bypassing the heterology since, upon deproteinization, the joint molecules blocked at heterology would rather reverse their branch migration over a homologous part. This would then lead to irreversible separation of joint molecules (Fig. 1).

RecA distinguishes substitutions from insertions or deletions when the deproteinization artifact is eliminated

Figure 3 shows the results of RecA-mediated strand exchange proceeding over 4-nt substitutions, deletions and insertions. It is clearly visible that while the substitution of 4 nt can be efficiently traversed in a RecA-promoted reaction this is not the case for 4-nt long deletions and insertions. This difference in the efficiency of passage can be clearly attributed to the RecA-dependent reaction, since in the spontaneous reaction the efficiency of passage did not depend on the type of heterology (Fig. 2). Thus, even if RecA-mediated reactions would indiscriminately stop at each type of heterology, the deproteinization should not favor a passage through substitutions.

To eliminate the possibility that the observed strand displacement could happen after deproteinization, we decided to use an assay in which the consequences of strand exchange are topologically fixed before removal of RecA from the DNA.

We employed a topological unwinding assay (27,28), in order to determine the degree of unwinding of a ds circular plasmid as a result of pairing and strand exchange with a RecA-covered oligonucleotide. The pairing and strand exchange in the region of the synaptic complexes results in the local unwinding of DNA and formation of positive supercoils in the plasmid. Addition of topoisomerase I relaxes the positive supercoils and changes the linking number to another value. Upon deproteinization, which rapidly deactivates topoisomerase and eliminates RecA, the plasmid DNA rewinds, resulting in negative supercoils. The amount of unwinding, which is dependent on the length of the pairing region, can be determined by observing the change in the linking number of the plasmid (for a thorough exposé on DNA topology, see ref. 29). We decided to compare the unwinding caused by an oligonucleotide that is completely homologous to plasmid DNA with the unwinding caused by oligonucleotides containing base substitutions or insertions. Figure 4A and B shows the principle of the assay and the unwinding obtained by pairing with completely homologous oligonucleotides of different size. It can be seen that progressive increase of size of the oligonucleotides (72, 94, 116 bases) results in increased level of unwinding as compared to plasmids that were relaxed in the absence of RecA-covered oligonucleotides. Notice that, to improve the resolution of topoisomers, the agarose gels were run in the presence of chloroquine which changes the DNA twist and induces positive supercoiling. Therefore, DNA molecules that were negatively supercoiled before loading on the gel become weakly positively supercoiled, while the molecules that were relaxed become strongly positively supercoiled. Changes of DNA twist induced by chloroquine do not change the linking number of covalently closed DNA molecules and, therefore, the effective unwinding caused by the RecA-mediated pairing and strand exchange can be easily calculated by counting the topoisomers separating the centres of topoisomer distribution in different reactions.

Figure 4C illustrates the effect of the pairing with oligonucleotides harboring different type of heterologies. The oligonucleotides 72_4Sub and 72_4Ins contain four single base substitutions and insertions, respectively. These substitutions and insertions are regularly spaced at 10-nt intervals within the 5'-portion of the oligonucleotide. The 3'-portion of these oligonucleotides therefore contains a 36-nt long non-interrupted homology to the plasmid DNA. It can be seen that pairing with the 72_4Sub oligonucleotide causes unwinding to the same extent as pairing with a completely homologous 72mer (72),

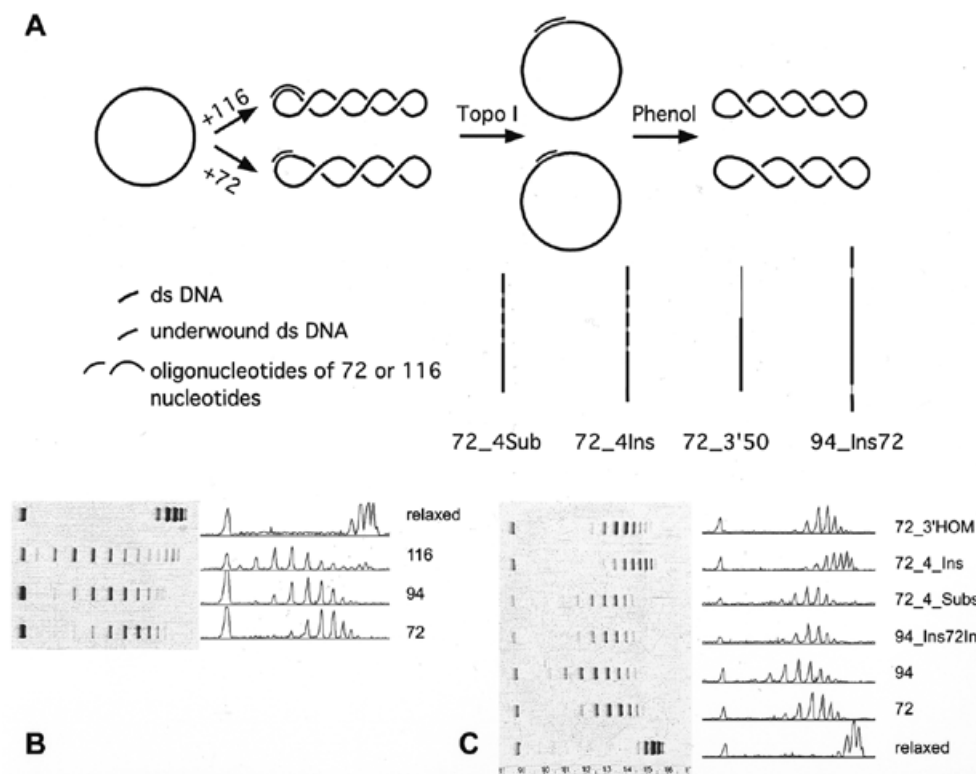


Figure 4. Topological unwinding assays. (A) Schematic presentation of the experimental procedure, shown for pairing with ssDNA of 72 and 116 bases, respectively (not to scale). Heterologous regions are shown in gray. The protocol is described in more detail in Materials and Methods. (B) Unwinding assay with homologous ssDNA of 72, 94 and 116 nt, respectively. (C) Topological unwinding experiment using ssDNA with various heterologies. Note that the pairing of pUC18 (2686 bp) with the substituted ssDNA leads to the same degree of unwinding as the homologous oligonucleotide, whereas the four insertions in 72_4Ins oligonucleotide (76 nt) effectively prevent such a high degree of unwinding. In oligonucleotide 94_Ins72 (96 nt) the two insertions of two bases each, bordering the central homologous region of 72 bases, allow unwinding only of the continuous region of homology, but not into the flanking homologous regions of 10 bases on each end.

showing that individual substitutions are easily traversed by pairing and strand exchange. However, the 72_4Ins oligonucleotide greatly diminishes the unwinding as if only the 36-nt non-interrupted homology participated in pairing and strand exchange and demonstrates that pairing and strand exchange cannot traverse individual insertions that distort the homologous register between paired molecules. Notice that the reaction with oligonucleotide 72_3'50, which has 50 nt of homology located at the 3'-end of a 72-nt long oligonucleotide unwinds significantly stronger than the reaction with 72_4Ins. We then measured the unwinding due to pairing and strand exchange with the oligonucleotide 94_Ins72, which is homologous to the plasmid, except for two insertions of two bases, which flank a central homologous region of 72 bases and bring terminal 10-nt long stretches out of homologous register. As expected, these two insertions cause the observed unwinding to correspond only to a pairing over a region of 72 bases, and is the same as that caused by a completely homologous 72mer. This experiment demonstrated once more that pairing and strand exchange performed in the absence of ATP hydrolysis cannot pass over small heterologies distorting the homologous register. Therefore, the unwinding assay showed unambiguously that small substitutional heterologies do not interfere

with RecA-mediated strand exchange reactions but that this is not the case for small insertions that distort the pairing register.

DISCUSSION

What is the likely mechanistic explanation of the observation that RecA in the absence of ATP hydrolysis can promote strand exchange over short substitutions but is unable to bypass short insertions or deletions? The presently accepted model of RecA-mediated strand exchange assumes that RecA helical filaments with stretched ssDNA bound by the primary DNA-binding site of RecA interact with protein-free duplex (23,24,30). The duplex DNA then binds to the secondary DNA-binding site of RecA. This binding causes duplex DNA to adopt a stretched and partially unwound structure that matches the stretching and the helicity of the ssDNA already present in the filament (23,24,27,28). The energetic cost of these structural deformations of duplex DNA is approximately equal to the binding energy of duplex DNA to the secondary binding site within RecA filaments. The alignment of duplex and ssDNA is therefore unstable unless there is a strand switch between the molecules (22) (Fig. 5A). Upon strand switch, the rearranged strands are more strongly bound by RecA (18,19). The strong binding of strand exchange products to RecA

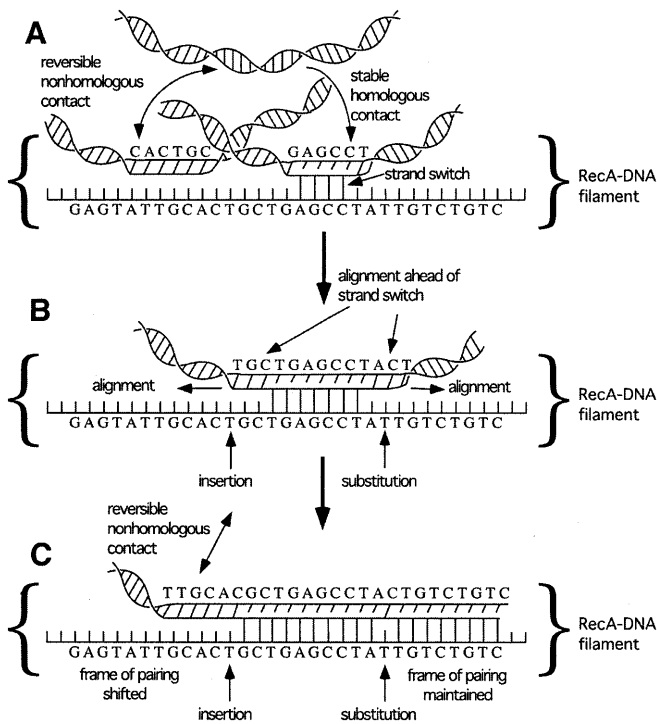


Figure 5. A mechanistic model of RecA selectivity against frameshift mutations. For simplicity of presentation, the helicity of the DNA within RecA–DNA filaments is completely removed instead of being only strongly reduced (36). However, the interbase distances in the DNA within the filament are kept at 150% of the corresponding distance in protein-free DNA (37). (A) Phase of homologous recognition. Protein-free duplex DNA interacts with the secondary DNA-binding site in RecA–ssDNA presynaptic filaments. This interaction induces a profound structural change of the duplex DNA. The energetic cost of this structural change is approximately equal to the RecA–dsDNA binding energy. Therefore, dsDNA binding by presynaptic filaments is unstable unless it is stabilized by the strand switch in the case of accidental finding of homology with the ssDNA resident in the RecA filament. (B) Propagation of alignment. Upon finding the homologous register, the duplex DNA is progressively drawn into the synaptic filament in such a way that the original register is strictly kept. In the case of a perfect homology between the interacting DNAs, propagation of heteroduplex is observed. (C) Strand exchange traverses a substitution but is stopped by an insertion. Small substitutions can be bypassed as they do not change the homologous register (right hand side); however, an insertion that changes the frame of pairing blocks progression of the strand exchange within RecA filaments (left hand side).

explains why the reaction can reach close to 100% efficiency even in the absence of ATP hydrolysis (18). Of course, ATP hydrolysis is then needed to dissociate RecA from DNA products of strand exchange (18,19). According to this scenario the first, local strand switch between the resident ssDNA and the duplex DNA entering into the RecA–DNA filament can quickly propagate and lead to the progressive uptake of duplex DNA into helical synaptic complexes (30–33). Clearly, this propagation requires that ss- and dsDNA molecules are homologous to each other. It is also required that at least a short ‘leading edge’ of entering dsDNA adopts a stretched and unwound configuration, matching the stretching and helicity of the ssDNA resident in the synaptic RecA filament (Fig. 5B). In addition, it is necessary that a perfect pairing register is kept between resident and incoming strands so that each entering base pair of duplex DNA is placed in a ‘slot’ opposite the corresponding base of the resident single strand. This perfect

matching is necessary for the recognition of homology leading to the strand switch between the two DNA molecules. To achieve such a match, RecA has to be able to ‘count’ every base pair in the incoming duplex DNA. Skipping just one base pair or one slot would make strand switch impossible. Let us now consider what happens when the incoming DNA contains a short insertion or deletion. The same mechanism that ensures perfect matching will now make the bypass of the insertion or deletion impossible. The homologous register is lost from the first base of insertion or deletion (Fig. 5C, left). How then, can the substitution be bypassed? If the region of alignment ahead of the strand switch is longer than the mismatch, the homologous register, and hence the switch of the strands, can be re-established after the mismatch and then continue (Fig. 5C, right). The fact that 4-nt long substitutions were efficiently traversed (Fig. 3) tells us that the region of alignment ahead of strand switch is longer than 4 bp. In additional experiments (not shown), we observed that 10-nt long substitutions were not traversed by RecA. This latter result suggests that the region of alignment ahead of strand switch is shorter than 10 bp.

The presented mechanism concerns reactions not necessitating ATP hydrolysis. In *in vitro* reactions in the presence of ATP RecA is able to traverse insertions and deletions. Since ATP hydrolysis promotes dissociation of synaptic RecA–DNA complexes, it is likely that the region of exchange can be released from the perfect matching mechanism, upon which another mode of RecA action can be activated. Since passage of long heterologies requires separation of the corresponding portion of the incoming duplex, it is possible that under such conditions RecA can act as a helicase. It should be remembered here that RecA, in addition to forming helical filaments with DNA, also forms hexameric rings with a high structural homology to a group of established helicases (34). In contrast to *in vitro* reactions, *in vivo* experiments have demonstrated that, despite the presence of ATP, strand exchange rarely passes heterologous regions (2) due to the action of anti-recombination proteins that then abort the strand exchange. Earlier studies demonstrated that there are at least two modes of RecA action during strand exchange; one is independent of ATP hydrolysis and the second requires the hydrolysis (18–20,35). Passage through heterology was shown to depend on ATP hydrolysis and probably required substantial rearrangement of synaptic filaments that in turn could signal the presence of heterologies to antirecombination proteins. We observed here that small substitutional heterologies can be bypassed by RecA in an ATP hydrolysis-independent way. Therefore, synaptic filaments do not need to be rearranged to pass over substitutions, and this does not trigger the anti-recombination activity of other proteins ‘controlling’ the fidelity of DNA recombination.

CONCLUSIONS

We have shown here that RecA is able to guard the DNA against possible frameshift changes while permitting efficient strand exchange between DNA strands containing short substitutions. We have also shown that spontaneous (RecA-independent) branch migration can be more efficient in passage of insertions and deletions than the RecA-promoted reaction performed in the absence of ATP hydrolysis (although the assays were not directly comparable). These observations

led us to propose that the sophisticated mechanism of RecA-mediated strand exchange was selected during evolution as it helped to keep frameshift mutations in check, while permitting recombination between 'safe' base substitutions. We propose that, under normal physiological conditions, RecA together with anti-recombination proteins such as MutS and MutL (2,9), maintains a guarding role against frameshifts. However, under some stress situations (like inability of the cell to repair DNA damage), anti-recombination proteins may be 'switched off', allowing RecA to change its mode of action and exchange DNA strands even through large insertions and deletions.

SUPPLEMENTARY MATERIAL

A table listing sequences of oligonucleotides used in this study is available as supplementary material at NAR Online.

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