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TOWARDS AN UNDERSTANDING OF RECA PROTEIN-MEDIATED DNA STRAND EXCHANGE[1]

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ABSTRACT The DNA strand exchange reaction promoted by recA protein has a structural and mechanistic complexity that can be easily rationalized only if the primary function of the system is DNA repair. The recA nucleoprotein filament plays an important role in each step of the reaction. The initial complex in which the two homologous DNAs are paired (the paranemic joint) is formed much more readily between a single strand and a duplex DNA than between two duplexes. Homologous contacts between the two DNAs may involve regions with a right-handed triple-helical structure. In the interdomainal regions separating the triple helices, we suggest that the two DNAs are unpaired and twisted about each other in left-handed turns that compensate topologically for the triple helices. Strand exchange itself requires both a switch in DNA strand pairing patterns and a rotation of both DNAs. These reaction requirements are illustrated and accommodated by two models. Data are also summarized that argue strongly against models that couple association or dissociation of recA protein (treadmilling) to strand exchange.

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INTRODUCTION

Two key molecular events lie at the heart of a homologous genetic recombination event. The first is the alignment of homologous sequences in two DNA molecules. This process precedes any actual exchange of genetic information and may involve the formation of a DNA structure for which no model yet exists. This structure has been referred to as a paranemic joint. The second step is an exchange of DNA strands that leads to the formation of heteroduplex DNA and, when four strands are involved, a Holliday-type structure. In Escherichia coli, these two steps are promoted by recA protein. These processes are mimicked in vitro by the recA-mediated strand exchange reaction, often involving single-stranded circular and homologous linear duplex DNA substrates (1-4).

In the in vitro system, the pairing and strand exchange are preceded by another important step that governs the entire reaction: the formation of a recA nucleoprotein filament that represents the active species (1-3). This filament completely covers the single-stranded DNA with one recA monomer every 3-4 nucleotides. This DNA is bound along the phosphate backbone, extended so that the axial separation of bases is 5.1 Å, and arrayed with the bases exposed in the major right-handed helical groove evident in image reconstructions derived from electron microscopy (5,6).

Strand exchange begins with the binding and homologous alignment of the duplex DNA with the single strand in the filament. The actual exchange of strands is coupled to ATP hydrolysis. It is also unidirectional, proceeding 5'→3' relative to the single-stranded DNA bound within the original nucleoprotein filament. Strand exchange is relatively slow, with maximum measured rates on the order of 20 bp s^{-1}. ATP is hydrolyzed by recA protein with a turnover number on the order of 25 min^{-1}. ATP is hydrolyzed uniformly throughout the filament, with the result that during strand exchange about 100 ATPs are typically hydrolyzed for every base pair of heteroduplex DNA formed (2,7,8).

A decade of research has not yet provided satisfactory solutions for three key problems: a) the structure of the paranemic joint, b) the mechanism of strand exchange with respect to the relative dispositions and motions of the two DNAs involved, and c) the molecular function of ATP hydrolysis. This chapter will focus on some observations

and speculations relevant to these problems. Some of the ideas presented here are described in much greater detail in two recent reviews (8,9).

MATERIALS AND METHODS

All experiments, reagents, and procedures are described in reports that are, or will be, published elsewhere (7,10,11, J.E. Lindsley, S.W. Umlauf, R.B. Inman, and M.M. Cox, unpublished results).

RESULTS AND DISCUSSION

Rationalizing the System as a Whole.

As pointed out elsewhere (9), strand exchange between two homologous and undamaged DNA molecules is a thermodynamically neutral reaction, and enzymatic systems can be envisioned that might promote this reaction without expenditure of ATP. In this context, the investment of large amounts of ATP and the use of a long protein filament seems to refute the general notion of cellular efficiency. The system begins to make sense, however, if the recombinational repair functions of recA protein represent its primary mission. In this case, damaged DNA is the normal substrate. The use of protein and energy can be rationalized as a means to set up and promote strand exchange efficiently over long DNA distances and past one or more DNA lesions (8,9). The filament functions to exclude other DNA binding proteins from the region and to protect the branch point from nuclease digestion. ATP hydrolysis provides the energy required to get the branch past most types of structural barriers (DNA lesions).

The Structure of Paranemic Joints.

Another major function of the filament is to facilitate pairing of two DNA molecules. Spontaneous and stable homologous alignment of two DNAs has not been observed in vitro under physiological conditions, except when the DNAs are complementary single strands or when the homologous regions have extensive homopurine or homopyrimidine sequences. At least one of the DNAs in a paranemic joint is duplex, and pairing of homologous DNAs is

independent of the actual sequence of the homologous region. It is therefore likely that the recA filament is also required to stabilize the paired intermediate.

Paranemic joints are transient intermediates and to an extent they are defined by their inherent instability when recA protein is removed (2,12). We have concentrated on joints formed between a single-stranded DNA within the filament and a homologous second DNA that is double-stranded. A 3-stranded joint of this type is formed much more readily than a 4-stranded joint (J.E. Lindsley, J. Waterbury, and M.M. Cox, unpublished results). Several lines of evidence indicate that these joints can extend over thousands of base pairs (13-15). The two DNAs are bound asymmetrically in the joint; the single-stranded DNA is better protected from DNAse digestion than either strand of the incoming duplex (11,16). This duplex is underwound as a result of pairing, but the degree of underwinding indicates that not all of the homologous DNA is simultaneously paired. However, other results indicate that the entire region of available homology is sampled, suggesting that the joints are discontinuous and/or mobile (15).

Models for paranemic joints have been proposed in which the DNAs are either arranged side-by-side (2,8,17) or interwound in a 3- (or 4-) stranded helix (18). We have been unable to develop a satisfactory model for a side-by-side arrangement, and this has led us to consider more alternatives. The triple-helix could be envisioned as a derivative of the 4-stranded helix modeled by Wilson (19) and McGavin (20). This structure has never been observed, but the proposed base interactions in the major groove provide the most plausible mechanism for sequence specificity in homologous alignment.

To resolve the topological problems imposed by the right-handed interwinding of the two DNAs, Wilson (19) proposed that the two DNAs might be impaired and wrapped in left-handed intercoils outside the interwound domains. We have recently suggested a similar model for paranemic joints as illustrated in Figure 1 (9). In this model, domains of right-hand triple helix are interspersed with regions in which the two DNAs are unpaired. By twisting the two DNAs in a left-handed spiral in the interdomainal regions, the right-handed turns in the triple helix can be compensated for and no net interwinding of the DNAs need occur. This structure would most likely be dynamic, with the triple helix and unpaired regions interchanging rapidly.

FIGURE 1. Model for a paranemic joint. A triple helix domain is shown at left. A possible interdomainal conformation is shown at right. This structure is similar to a general model for pairing proposed by Wilson (19).

Note that the left-handed turns do not involve any left-handed (or Z) DNA conformations. The duplex DNA remains right-handed, and it is the superhelical wrapping of this right-handed duplex around the single strand that has the left-handed sense.

The triple helix itself could have several configurations. One we find particularly intriguing is one in which the two strands of the duplex are separated and the - strand of the duplex is paired in Watson-Crick fashion with the single strand in the filament (Figure 2). In effect, this structure would represent a de facto strand switch within the triple helical regions (a plectonemic joint), leading directly to a net strand exchange if a free DNA end is available.

Recent experiments in which DNA in paranemic joints is cross-linked with 4'-amino,4,5',8-trimethyl psoralen (AMT) are consistent with this model. After the removal of recA protein, about 85-90% of the duplex DNAs are cross-linked to single-stranded circles. The cross-linking is restricted to homologous regions of the duplex and in most cases the DNAs are linked over several hundred to several thousand base pairs (Figure 3). Many of these joints exhibit a striking pattern in which the - strand is often cross-linked alternately to the single-stranded circle and its complement in the original linear duplex (Figure 3). Very short regions where all three strands coincide occur in some cases, possibly the remnants of larger triple helices that collapsed upon protein removal. No homologous cross-linking of the two DNAs was observed in control experiments in which the two DNAs were completely heterologous or when recA protein was omitted. When AMT was omitted, <5% of the DNAs were paired and the joints were very short. These experiments will be described in detail elsewhere

(S.W. Umlauf, R.B. Inman, and M.M. Cox, in preparation).

Coupling ATP Hydrolysis to Unidirectional Branch Migration in the RecA System.

The first model we considered for this reaction

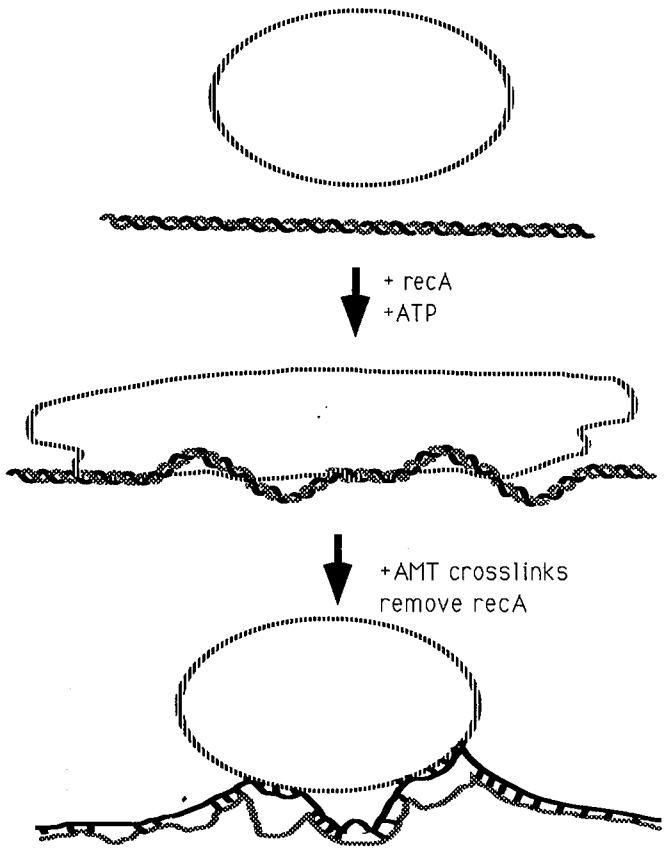

FIGURE 2. Experiment designed to trap paranemic joints. Only the DNA is shown. The central structure is that postulated to form when recA protein is present. This structure is heavily cross-linked with AMT. After recA protein is removed, the - strand of the duplex is cross-linked alternately to the single-stranded circle and its original complementary strand in the duplex. See Figure 3.

involved filament treadmilling (21). Drawing upon a potential analogy to cytoskeletal filaments such as tubulin and actin (22), one can build a model in which recA monomers associate at one end of the filament and dissociate at the other in a reaction coupled to ATP hydrolysis. Movement of the branch point could be coupled to the molecular events occurring at either end.

RecA filaments do, in fact, treadmill. The filament exhibits a clear structural asymmetry (6) and assembly occurs primarily at one end (6,11,23). When dissociation is observed, recent experiments have shown that it occurs primarily at the end opposite to that at which assembly occurs (J.E. Lindsley and M.M. Cox, unpublished results).

Is association or dissociation of recA protein coupled

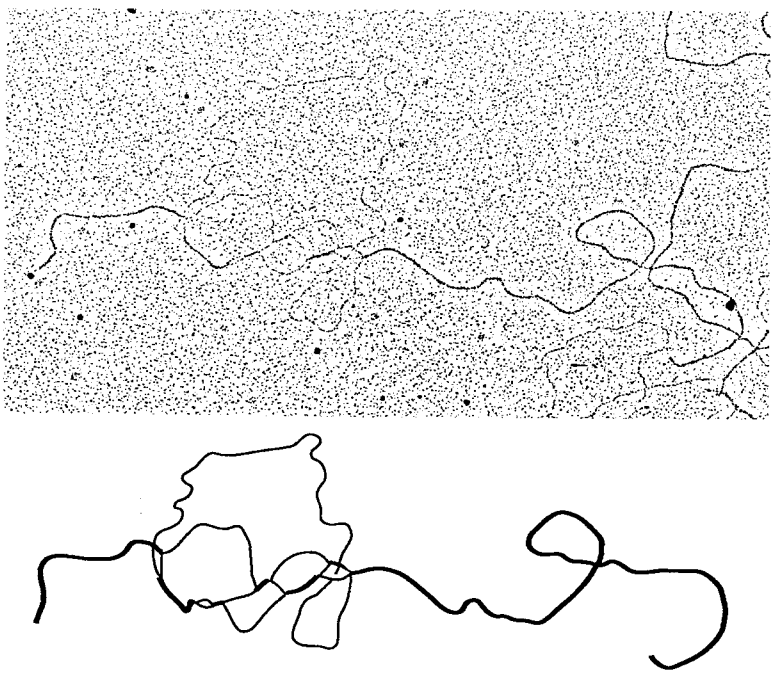

FIGURE 3. Cross-linking of DNA strands in a paranemic joint by AMT. In the interpretive drawing, thin and thick lines are single-strands and cross-linked double strands, respectively. The linear duplex DNA in this experiment has long heterologous sequences at either end, precluding a complete strand exchange reaction.

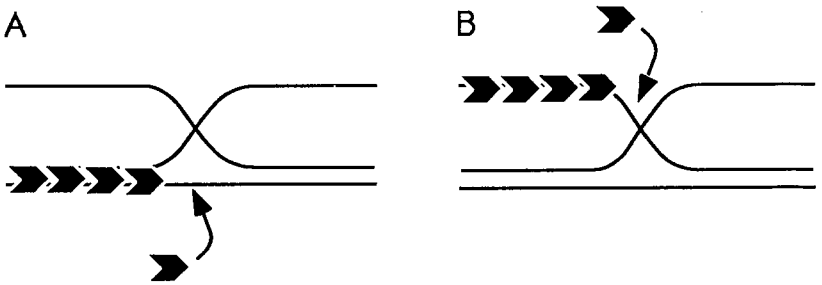

FIGURE 4. Two ways to couple branch movement to association of recA monomers at a filament end. A. Filament growth on the heteroduplex DNA. B. Filament growth on the displaced single strand.

to strand exchange? The evidence gathered to date argues strongly against this hypothesis. A classical treadmilling model is eliminated by the observations that a) ATP hydrolysis occurs uniformly throughout the filament (7), and b) there is no strict coupling of association/dissociation and ATP hydrolysis (2,11). Potential variations on the treadmilling theme are addressed by the data below.

First, consider the association reaction. In principle, branch movement could be coupled to the growth of a filament along either the heteroduplex or the displaced single strand (Figure 4). Several sets of data are relevant here. a) The lower limits for the rate of recA filament assembly are 2-10 fold higher than the reported rates of recA-mediated branch migration (2, J.E. Lindsley and M.M. Cox, unpublished results). b) An intact filament is required to facilitate the pairing steps that precede branch migration. This filament would have to dissociate entirely or in part to initiate a treadmilling process at the site of strand exchange. Pairing is not accompanied by detectable dissociation of the filament, however (13). c) Under optimal conditions for strand exchange (which include SSB), the recA protein is bound almost exclusively to the heteroduplex DNA product after the reaction is complete (10). The displaced single strand is bound by SSB (13), providing a strong argument that recA filament assembly on the displaced DNA strand (Figure 4B) is not required for

strand exchange. d) Finally, the recA filament assembly process exhibits a dependence on recA protein concentration up to at least 1.2 μM recA protein (24, S.W. Morrical and M.M. Cox, unpublished results). Once strand exchange is initiated, however, complexes formed under optimal conditions can be diluted to 0.16 μM total recA protein with no effect on the rate or extent of subsequent branch migration (17, J.E. Lindsley and M.M. Cox, unpublished results). The dilution factor can be up to 50 fold.

A better case can be made for a coupling of branch movement to dissociation of recA monomers (18), but a careful comparison of strand exchange and the dissociation reaction again reveals no significant correlation. Dissociation requires a free filament end and the rate increases sharply with pH over the range pH 6-8 (11). Rates of recA-mediated DNA strand exchange are constant over the same pH range (J.E. Lindsley and M.M. Cox, unpublished results). Low concentrations of ATP[γS] (1-2 μM) effectively halt dissociation but have a minimal effect on ATP hydrolysis and strand exchange (11, J.E. Lindsley and M.M. Cox, unpublished results).

Several lines of evidence indicate that an intact filament extends through the branch point during strand exchange (7,14,16,25). The presence of intact recA filaments on the heteroduplex products long after strand exchange is complete (10) also suggests that filaments can remain intact throughout the reaction.

Taken together, these results argue strongly against treadmilling in any form as a mechanism for recA-mediated DNA strand exchange. Instead, the dissociation reactions observed under some conditions are best viewed as a mechanism for recycling the filaments after strand exchange, while the assembly process is clearly a prerequisite for strand exchange.

As pointed out elsewhere (9), there is no mechanistic advantage to be derived from an association or dissociation of recA protein at the branch point during strand exchange. The reaction requirements are readily accommodated by an intact filament as seen in two models, one proposed by Howard-Flanders and coworkers (18), and another by this laboratory (2,8,9,17). Versions of both models that illustrate the mechanistic possibilities and are consistent with available data have recently been presented and reviewed in detail (9).

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REFERENCES

1. Radding CM (1982). Homologous pairing and strand exchange in genetic recombination. Annu Rev Genet 16: 405.
2. Cox MM, Lehman IR (1987). Enzymes of general recombination. Annu Rev Biochem 56:229.
3. Griffith JD, Harris LD (1988). DNA strand exchanges. CRC Crit Rev Biochem (Supplement) 23:543.
4. Cox MM, Lehman IR (1981). RecA protein of Escherichia coli promotes branch migration, a kinetically distinct phase of DNA strand exchange. Proc Natl Acad Sci USA 78:3433.
5. Leahy MC, Radding CM (1986). Topography of the interaction of RecA protein with single-stranded deoxyoligonucleotides. J Biol Chem 261:6954.
6. Stasiak A, Egelman EH, Howard-Flanders P (1988). Structure of helical recA-DNA complexes. J Mol Biol 202:659.
7. Brenner SL, Mitchell RS, Morrical SW, Neuendorf SK, Schutte BC, Cox MM (1987). RecA protein-promoted ATP hydrolysis occurs throughout recA nucleoprotein filaments. J Biol Chem 262:4011.
8. Cox MM (1989). The role of recA protein in homologous genetic recombination. In Adolph KW (ed): "Molecular Biology of Chromosome Function," New York: Springer-Verlag, in press.
9. Cox MM (1989). Binding two DNA molecules at once: the recA protein. In Revzin A (ed): "The Biology of Nonspecific DNA-Protein Interactions," Boca Raton, Florida: CRC Press, Inc., in press.
10. Pugh BF, Cox MM (1987). RecA protein binding to the heteroduplex product of DNA strand exchange. J Biol Chem 262:1337.
11. Lindsley JE, Cox MM (1989). A dissociation pathway for recA nucleoprotein filaments formed on linear duplex DNA. J Mol Biol 205:695.

12. Riddles PW, Lehman IR (1985). The formation of plectonemic joints by the RecA protein of Escherichia coli: requirements for ATP hydrolysis. J Biol Chem 260:170.
13. Schutte BC, Cox MM (1987). Homology-dependent changes in adenosine 5'-triphosphate hydrolysis during recA protein promoted DNA strand exchange: evidence for long paranemic complexes. Biochemistry 26:5616.
14. Register JC III, Christiansen G, Griffith J (1987). Electron microscopic visualization of the recA protein-mediated pairing and branch migration phases of DNA strand exchange. J Biol Chem 262:12812.
15. Schutte BC, Cox MM (1988). Homology-dependent underwinding of duplex DNA in recA protein generated paranemic complexes. Biochemistry 27:7886.
16. Chow SA, Honigberg SM, Radding CM (1988). DNase protection by RecA protein during strand exchange: asymmetric protection of the Holliday structure. J Biol Chem 263:3335.
17. Cox MM, Pugh, BF, Schutte BC, Lindsley JE, Morrical SW (1987). On the mechanism of recA protein-promoted DNA branch migration. In McMacken R, Kelly TJ (eds), "DNA Replication and Recombination," New York: Alan R. Liss, Inc., p 597.
18. Howard-Flanders P, West SC, Stasiak A (1984). Role of RecA protein spiral filaments in genetic recombination. Nature (London) 309:215.
19. Wilson JH (1979). Nick-free formation of reciprocal heteroduplexes: a simple solution to the topological problem. Proc Natl Acad Sci USA 76:3641.
20. McGavin SJ (1971). Models of specifically paired like (homologous) nucleic acid structures. J Mol Biol 55: 293.
21. Cox MM, Morrical SW, Neuendorf SK (1984). Unidirectional branch migration promoted by nucleoprotein filaments of RecA protein and DNA. Cold Spring Harbor Symp Quant Biol 49:525.
22. Cleveland DW (1982). Treadmilling of tubulin and actin. Cell 28:689.
23. Register JC III, Griffith J (1985). The direction of RecA protein assembly onto single strand DNA is the same as the direction of strand assimilation during strand exchange. J Biol Chem 260:12308.

24. Morrical SW, Lee J, Cox MM (1986). Continuous association of Escherichia coli single-stranded DNA binding protein with stable complexes of recA protein and single-stranded DNA. Biochemistry 25:1482.
25. Chow SA, Honigberg SM, Bainton RJ, Radding CM (1985). Patterns of nuclease protection during strand exchange. J Biol Chem 261:6961.