DNA Replication and Recombination, pages 597–607
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ON THE MECHANISM OF RecA PROTEIN-PROMOTED DNA BRANCH MIGRATION[1]

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ABSTRACT RecA nucleoprotein filaments promote a unidirectional branch migration reaction coupled to ATP hydrolysis as the final phase of DNA strand exchange reactions. Movement of the branch could, in principle, be coupled to association or dissociation of recA protein at the ends of these filaments. A variety of evidence, however, now indicates that association and/or dissociation of recA protein plays no role in this reaction. Models in which the recA nucleoprotein filament remains intact throughout DNA strand exchange must therefore be considered.

INTRODUCTION

The recA protein of *E. coli* promotes a strand exchange reaction between circular (+) single strands and linear duplex DNA molecules derived from bacteriophages. This reaction provides a convenient *in vitro* model system in which to examine the recombinase functions of this protein.

Strand exchange occurs in three experimentally distinguishable phases (1-3). The first and second phases involve the assembly of a stoichiometric (1 recA monomer

[1] This work was supported by National Institutes of Health grant GM32335. BCS, JEL, and SWM were supported by Training Grant 5-T32 GM07215 from the National Institutes of Health. MMC is supported by National Institutes of Health Research Career Development Award AI00599.

per 4 nucleotides) recA filament on the ssDNA, followed by a search for homology in which homologous sequences within the ssDNA and dsDNA are aligned. ATP is required for these steps but ATP hydrolysis is not (1,4). The single-stranded DNA binding protein of E. coli (SSB) plays an important but not yet fully understood role in the formation and stability of the recA/ssDNA complex formed in the first phase (2,5).

We are primarily interested in the mechanism of the third phase, a branch migration in which the circular single strand completely replaces the (+) strand within the linear duplex, creating a circular heteroduplex DNA with a nick in one strand (see fig. 1). This phase requires ATP hydrolysis (1,4). RecA protein-promoted branch migration also exhibits a unique polarity, proceeding 5' → 3' relative to the strands being exchanged (6-8). This coupling of chemical energy to a unidirectional process defines a classical biochemical problem, analogous in a sense to muscle contraction and ATP-driven ion pumps.

The most important constraint on considerations of mechanism in this system is imposed by the structure and properties of the active species--the recA nucleoprotein filament formed in the first phase. Whereas recA protein binds preferentially to ssDNA at neutral pH, important structural clues have come from studies of recA complexes with dsDNA. RecA protein binding results in extensive unwinding of dsDNA, with 20-24 base pairs and perhaps 6 recA monomers per turn in the final complex (9,10 and references therein). RecA/ssDNA complexes are similar except for the absence of a DNA strand (10).

Other constraints are imposed by a variety of experimental observations. Among the most difficult to explain are the tolerance of the system for heterology in the DNA molecules involved and the properties of the ATP hydrolytic activity. RecA protein-promoted branch migration proceeds not only past short mismatches and pyrimidine dimers, but also past regions of heterology which can be as large as several hundred base pairs (11). ATP is hydrolyzed ($k_{cat} \cong 30\ min^{-1}$) when recA protein is bound to DNA, whether or not strand exchange is in progress. Once strand exchange begins, the efficiency of the reaction is apparently low, with approximately 100 ATPs hydrolyzed per base pair of heteroduplex produced under optimal conditions (12). Whereas this efficiency may reflect a largely uncoupled reaction, models considered below provide a rationale for this observation.

Much of the speculation which has appeared to date on this reaction (3,13) has drawn on a potential analogy to the treadmilling reaction coupled to ATP or GTP hydrolysis promoted by tubulin and actin filaments (14). In principle, recA protein-promoted branch migration would be coupled to association of recA monomers at the head of a filament, dissociation at the tail, or either one within a treadmilling system (figure 1, models I.A-C). Alternatively, the recA nucleoprotein filament could remain essentially intact throughout the reaction (model II). A detailed description of a dissociation-type model (I.A) has been provided by Howard-Flanders and colleagues (13) and has served as inspiration for a number of the experiments described below.

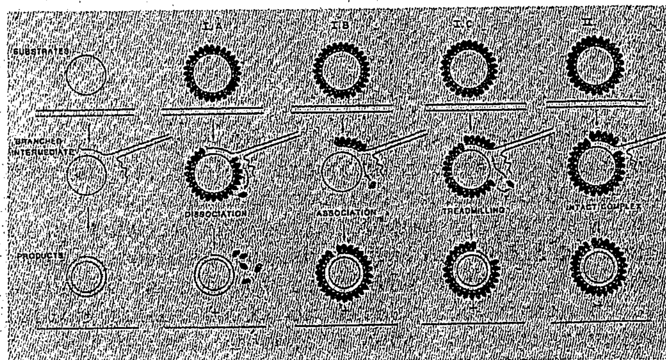

Figure 1. Alternative possibilities for the disposition of recA protein during DNA strand exchange. See text for details.

Our efforts to date have been directed at distinguishing between type I and type II models. Type I models couple branch migration to association or dissociation of recA protein at the ends of filaments. Considerations of microscopic reversibility require that association or dissociation be coupled to ATP hydrolysis if it is to result in unidirectional branch migration. Relevant predictions and results are described below.

MATERIALS AND METHODS

All experiments, reagents, and procedures are described in reports which are, or will be, published elsewhere (5,15-17, B. F. Pugh, unpublished results).

RESULTS AND DISCUSSION

If recA protein-promoted branch migration involved a type I mechanism, we reasoned that the system should exhibit at least some and perhaps all of the following properties: a) ATP hydrolysis should result in observable movement of recA protein between recA/ssDNA complexes and pools of free recA protein, i.e., association and dissociation, b) by analogy to tubulin and actin, it might be expected that ATP hydrolysis would be limited to recA monomers at or near filament ends, and c) branch migration should exhibit some sensitivity to the total concentration of reaction components. In the case of model I.A (dissociation), we additionally expected that d) recA protein would be absent from the heteroduplex DNA product, and e) the rate of ATP hydrolysis would decline over the course of the reaction as a result of this dissociation. All experiments were carried out under conditions previously determined to be optimal for recA protein-promoted DNA strand exchange. In each case the prediction has been contradicted by experiment.

Results related to predictions a, b, and e will be described in detail elsewhere. Very little exchange is observed between free recA protein and recA/ssDNA complexes formed in the presence of ATP and SSB. The hydrolysis of over a thousand ATPs is observed for every measurable exchange of a single recA monomer, indicating that association or complete dissociation is not a required step in the ATP hydrolytic cycle (16). ATP hydrolysis, in addition, is not restricted to filament ends. Instead, all recA monomers in a filament exhibit an ATPase activity which is, to a first approximation, equivalent (17). Finally, ATP hydrolysis during strand exchange was measured in experiments carried out in the presence of SSB. Once strand exchange is initiated, the rate of ATP hydrolysis remains constant, with no decrease either during or 10-30 min. after completion of the reaction which would indicate that recA protein has dissociated from the DNA (B. Schutte, unpublished).

We have often observed an increase in the apparent rate and extent of DNA strand exchange when the concentrations of protein and DNA reaction components were increased (without altering stoichiometries). The experiment in figure 2, in which recA and DNA concentrations are varied 4-fold, indicates that in this case the effect is on initiation of strand exchange rather than the branch migration phase. A rapid dilution of an ongoing reaction

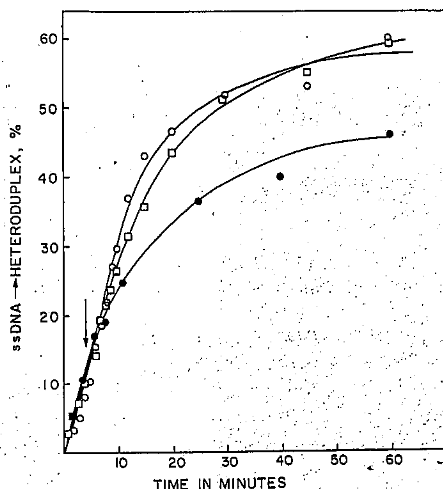

Figure 2. Dilution experiment. Reactions (1X) contained 0.84 μM recA protein, 0.16 μM SSB, 1.65 μM ssDNA, 2.8 μM dsDNA. Standard reaction conditions (ATP, ATP regenerating system, buffer, salt) were maintained at all times and are described elsewhere (15). Symbols: ●-●, 1X reaction; ○-○, 4X reaction; □-□, 4X reaction diluted to 1X at arrow (4 min.). The S_1 nuclease assay for heteroduplex DNA formation has been described (15).

from 4X → 1X has no effect on the reaction. A limited set of experiments of this type indicate that the rate of branch migration is relatively insensitive to the concentration of reaction components (prediction c).

RecA protein remains on the product heteroduplex DNA well after strand exchange is completed in reactions carried out in the presence of SSB (prediction d). This conclusion is supported by DNAse protection studies (B. F. Pugh, unpublished results) and by the experiment in figure

3. RecA protein extensively unwinds duplex DNA to which it is bound (9,18). Ligating the ends of the duplex DNA without removing the recA protein therefore results in a DNA molecule with a superhelical density several-fold greater than that of plasmid DNA isolated from E. coli cells (18). This highly unwound DNA has been referred to as form X, and is diagnostic of recA protein binding. In fig. 3, a large concentration of DNA ligase was added to a strand exchange reaction about 20 min. after the reaction

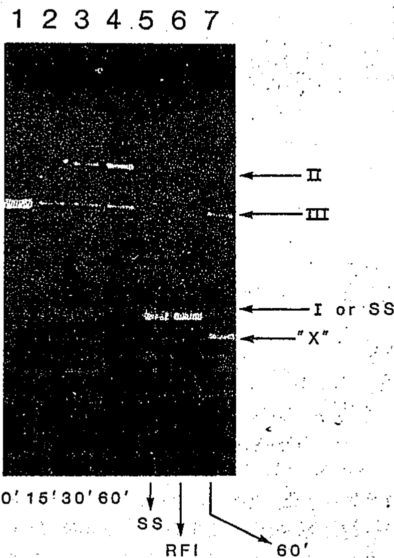

Figure 3. RecA protein binding to heteroduplex DNA. Lanes 1-4, reaction timecourse. Lane 5, M13mp8 ssDNA; lane 6, supercoiled M13mp8 dsDNA; lane 7, DNA from lane 4 after addition of DNA ligase (1 mg/ml final concentration, incubation at 37° for 5 min). Reaction was carried out under standard conditions described elsewhere (15). Experimental details will be described elsewhere.

had reached completion. It can be seen that most of the nicked circular DNA product is converted to form X DNA by this treatment, indicating that recA protein remains bound to it. No ATP[γS] was added to stabilize recA protein binding to duplex DNA in this experiment. Several additional experiments (B. F. Pugh, unpublished) indicate that

the form X band has a superhelical density comparable to that of form X DNA described elsewhere (18, and references therein).

Taken together, these and other results (19) indicate that the recA nucleoprotein filament remains intact throughout the strand exchange reaction, making it necessary to consider type II models. To convince the reader that it is possible to think about this reaction without invoking recA protein association or dissociation, one such model is presented in figure 4.

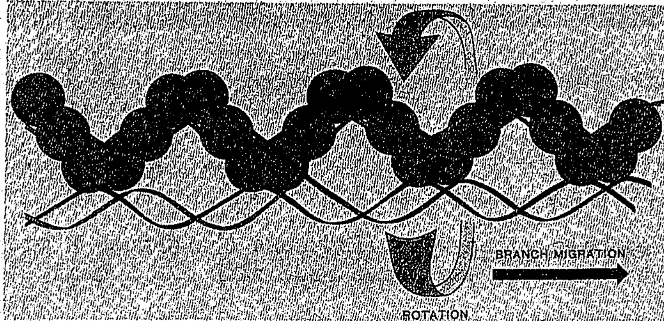

Figure 4. A type II model for recA protein-promoted branch migration.

As drawn, the model depicts a 4 strand exchange rather than the 3 strand exchange described above. RecA protein is bound to one unwound duplex DNA in a right-handed nucleoprotein filament, 24 base pairs and 6 recA monomers per turn. The DNA is exposed in a major groove of the filament. A second DNA molecule, equally unwound, is bound to every sixth recA monomer on the outside of the filament as drawn (many possibilities exist here). A crossover or branch point between the two DNA molecules is depicted within the filament groove. The outer DNA molecule is rotated around the inner one by passing it from a set of recA monomers to their neighbors in a coordinated fashion throughout the filament. Rotation in the direction illustrated results in the indicated branch migration with the branch point remaining within the groove at all times.

The reaction is rendered unidirectional by the investment of chemical energy. ATP hydrolysis would be coupled to a series of conformation changes leading to the passing of the outer DNA between recA monomers in a direction defined by the inherent polarity of the filament (20). A 3 strand exchange reaction would be similar, with the filament bound to ssDNA ahead of the branch point and to dsDNA behind it. The interaction between each recA monomer and the outer DNA molecule could be analogous to the interaction of actin and myosin in muscle contraction. We make no assumptions about the pairing or synapsis steps which initiate this process. In the cell, a topoisomerase would be required to resolve topological problems derived from this reaction, as must be the case for recA protein-promoted branch migration regardless of its mechanism.

The model is consistent with a variety of experimental results. No association or dissociation of recA protein is required. Since all of the recA protein in the filament is involved in the reaction, a rationale is provided for the observed efficiency of ATP hydrolysis. If it is assumed that every recA monomer must hydrolyze one ATP to move the outer DNA 360° around the inner one, and the filament contains 1500 recA monomers (for a 6000 bp DNA molecule), 1500 ATPs will be hydrolyzed for each rotation. This will move the branch point by 24 base pairs, for an efficiency of 63 ATPs per base pair of heteroduplex produced. The model also implies that a large number of binding sites for dsDNA should exist on the outside of the nucleoprotein filament, consistent with the coaggregation of recA/ssDNA complexes by heterologous dsDNA observed by Radding and colleagues (21).

Several other observations suggest that this model is not correct in detail. Branch migration through an extended region of heterology (11) would probably require some dissociation of recA protein. Also, we have observed that whereas recA protein does not exchange between free and bound forms, a relatively rapid exchange occurs between adjacent recA/ssDNA complexes (16). We have not yet determined the significance of this exchange reaction.

As drawn, the model has several other features of potential interest. RecA protein need not be present at the branch point to drive branch migration. The potential for driving branch migration beyond the end of the filament may permit the bypass of regions of heterology. It also implies that more efficient branch migration could be

carried out by shorter filaments. This could provide an explanation for improvements in reaction efficiency observed under some conditions (12). The longer filaments, however, may be advantageous in the cell. Maintaining the branch point within a filament groove may prevent its resolution or degradation. This may insure the extensive branch migration required to bypass DNA damage in recombinational postreplication repair (22).

We offer one final caveat. The extensive literature on recA protein provides information which in some cases appears contradictory. An example is the fact that dissociation of recA protein from recA/DNA complexes can be readily observed under a variety of conditions (12,23-25). In the case of recA/dsDNA complexes, where binding is relatively weak, an apparent dissociation in electron microscopic or centrifugation experiments may reflect the selective disruption of these complexes as a result of the procedures employed. In other cases, additional research is required to determine the functional significance of exchange or dissociation reactions. The result obtained in a strand exchange experiment can also be affected in crucial ways by the presence of SSB. We have observed that the dissociation of recA protein from dsDNA is expedited by ssDNA (B. F. Pugh, unpublished). In the absence of SSB, a rapid and possibly fortuitous transfer of recA protein from the heteroduplex DNA to the displaced single strand might be expected as the branch point passes. In the presence of SSB, evidence to date indicates that the SSB is transferred to the displaced single strand (5,19), where it would block or inhibit the recA protein transfer postulated above. The detection of recA protein bound to heteroduplex DNA may therefore be, to a large degree, a function of the presence or absence of SSB. At present, we argue that if efficient strand exchange can occur without dissociation, then dissociation is not an integral part of the reaction mechanism. Refinement or elimination of this or any other model will require more detailed information about recA/DNA complexes and the reactions they promote.

ACKNOWLEDGEMENTS

The authors acknowledge helpful and stimulating discussions with SL Brenner and IR Lehman. We also thank IR Lehman, SL Brenner, CM Radding, and J. Griffith for communicating results prior to publication.

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