The Mechanism of Homologous DNA Strand Exchange Catalyzed by the Bacteriophage T4 uvsX and Gene 32 Proteins*

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A strand exchange reaction between a single-stranded DNA circle and a homologous linear double-stranded DNA molecule is catalyzed by a mixture of two T4 bacteriophage proteins, the uvsX protein (a DNA-dependent ATPase that resembles the recA protein) and the gene 32 protein (a helix-destabilizing protein). The products are different from those formed in the corresponding recA protein-catalyzed reaction; rather than producing a linear single strand plus a nicked circular double-stranded (form II) DNA molecule as the final products, interlinked DNA networks are rapidly generated. Electron microscopy reveals that these networks form from multiple pairing reactions that involve the recombination intermediates. Since the uvsX protein is present in substoichiometric quantities, it presumably recycles to catalyze these successive pairing events. Recycling of the uvsX protein has been more directly examined in an assay that monitors the rate of uvsX protein-catalyzed branch migration. The branch migration reaction is rapidly inhibited by dilution of the uvsX protein or by the addition of a heterologous competitor DNA, showing that the uvsX protein-DNA filaments that catalyze strand exchange are dynamic structures. The evidence suggests that individual uvsX protein monomers are continuously entering and leaving the cooperatively formed filament in a cycle that is strongly affected by their ATP hydrolysis.

Our understanding of genetic recombination has been greatly enhanced by detailed in vitro studies of the recA gene product of Escherichia coli. This remarkable 40,000-dalton protein (a "recombinase") catalyzes homologous pairing and directional branch migration (1, 2), two reactions central to the recombination process. As a result the recA protein will promote an exchange of strands between a single-stranded DNA circle and a linear double-stranded DNA molecule of the same sequence. The first step in this process is the formation of long nucleoprotein filaments on the single-stranded DNA circle, which are generated by the ATP-dependent, 5' to 3' polymerization of the recA protein along a DNA strand (3, 4). A helix-destabilizing protein from E. coli, the single strand-binding protein, facilitates this process, in part by removing the short helical regions in the DNA strand (5, 6). The nucleoprotein filament then finds a DNA double helix and displaces a short portion of one of the helical strands, enabling complementary base pair interactions to form between the two DNA molecules. This pairing requires nucleotide sequence homology and leads to the formation of a "D-loop" (7, 8). After pairing directional branch migration ensues in the 5' to 3' direction with respect to the two competing strands, producing a good yield of nicked double-stranded circular (form II) DNA molecules and releasing one strand of the original linear DNA double helix as a linear single strand (2).

recA-like proteins have been isolated from several other organisms, including the recI protein from Ustilago maydis (9) and the uvsX protein from phage T4 (10-12). A partially purified activity from human cells has also been reported (13). The sequence of the uvsX protein reveals 23% identity to the recA protein (14), and we and others have been interested in understanding its chemistry and its interactions with DNA (10-12, 14-18). While it catalyzes the same reactions, this T4 protein is sufficiently different from the recA protein that detailed comparisons should help to distinguish the essential features of a recombinate from these particular to a single protein. Moreover, genetic and biochemical evidence has suggested that other identified T4 gene products interact specifically with the uvsX protein during homologous recombination in vivo (19, 20). In order to reconstruct the complete recombination reaction, we need to understand the function of all of these interacting protein components, whose analogues may be less accessible in other recombination systems.

In this report we characterize the strand exchange reaction catalyzed by the uvsX protein and find that it differs from that catalyzed by recA protein, being driven by protein-DNA filaments that are dynamic in nature, constantly collapsing, and reforming in a cycle coupled to ATP hydrolysis. Molecular models for the strand exchange process that incorporate this finding are discussed.

MATERIALS AND METHODS

Strand Exchange Reactions—The reactions contained, in 100 µl, 2.5 µM (100 µg/ml) uvsX protein, 1.6 µM (50 µg/ml) 32 protein, 15.4 µM (5 µg/ml) single-stranded circular M13 viral DNA, 9.2 µM (3 µg/ml) double-stranded linear M13 DNA (3' end-labeled to a specific activity of 80 cpm/pmol), 100 µM bovine serum albumin, and 29 µM/ml creatine phosphokinase. (The DNA concentrations, given as molarity of nucleotides, represent somewhat more than a 3-fold molar excess of single-stranded over double-stranded molecules.) These ingredients were mixed in 90 mM potassium acetate, 10 mM Tris acetate (pH 7.4), 10 mM magnesium acetate, 10 mM creatine phosphate, and 1 mM 2-mercaptoethanol. After this solution was incubated at 37 °C for 2 min, 2 mM ATP was added to initiate the reaction. Aliquots of 10 µl were withdrawn at the indicated times, quenched by
adding Na₂EDTA to 20 mM and sodium dodecyl sulfate (SDS)¹ to 0.5%, and electrophoresed through a 0.8% agarose gel at 6.5 V/cm for approximately 5 h. The gels were dried onto DE81 paper (Whatman) and the DNA bands visualized by autoradiography. Radioactive M13 form II and form III DNAs were prepared by nicking M13 form I DNA with S1 nuclease and cutting this ssDNA with Ball restriction endonuclease, respectively; the 3' end of these DNAs was then labeled by the "chech back-fill in" method of O'Farrell (22) using T4 DNA polymerase and [γ³²P]ATP.

Proteins and DNAs—M13 single-stranded DNA was prepared by the method of Yamamoto et al. (23), and M13 double-stranded DNA was purified as described by Messing (24). The uvsX and gene 32 proteins were purified from T4-infected E. coli cells by published methods, and each was at least 99% homogenous and free of detectable nucleases (11, 25).

Electron Microscopy—The strand exchange reactions were conducted as described above, except that bovine serum albumin was omitted from the reaction mixture and the double-stranded DNA was not radioactive. Aliquots removed from the reaction at various times were quenched by adding Na₂EDTA to 20 mM. Proteins were removed by a high salt (2 M) treatment just prior to spreading in formamide using modifications of standard techniques (26). After spreading samples were platinum shadowed and examined in a Phillips EM400 microscope.

Branch Migration Assays—Reactions were conducted as described above. However, 10-μl aliquots were removed at intervals and added to a 10-μl solution containing 300 mM NaCl, 50 mM Tris-Cl (pH 7.4), 25 μg/ml poly(dT) (length 25–30), and 24 units of the indicated restriction endonuclease. The high concentration of chloride and restriction endonuclease, respectively; the 3' end of these DNAs was then labeled by the "chech back-fill in" method of O'Farrell (22) using T4 DNA polymerase and [γ³²P]ATP.

RESULTS

The uvsX Protein Catalyzes the Formation of Recombination Intermediates That Have Undergone Multiple Synaptic Exchanges—The bacteriophage T4 uvsX protein, in the presence of the gene 32 helix-stabilizing protein and ATP, catalyzes pairing reactions between two DNA molecules that share the same nucleotide sequence. When a 3’-end labeled double-stranded DNA is mixed with an unlabeled single-stranded DNA circle, autoradiography after gel electrophoresis of de-proteinized samples reveals a time-dependent disappearance of the labeled double helix and the concomitant production of a diffuse, slower migrating DNA band (Fig. 1). As the reaction proceeds further, all of the label is eventually incorporated into very large aggregates that barely enter the gel. These DNA networks are the major product regardless of the ratio of the single- and double-stranded DNA molecules used as substrates or the amount of the proteins added (see also Ref. 11). They are apparently connected via direct DNA-DNA interactions, since treatment with high concentrations of SDS, ADP, phenol, or other protein-releasing agents has no effect on the observed gel pattern (data not shown). As judged by the same criteria, similar DNA-linked multimers are not produced in the recA/single strand binding system (although protein-connected aggregates of DNA form as intermediates (27, 28)).

In order to characterize the products of the above uvsX protein-mediated reaction further, we have used electron microscopy to examine the structures that form shortly after pairing is initiated. Fig. 2A presents a micrograph of the expected initial product (molecule A, Fig. 3) that forms when the double-stranded DNA molecule pairs at one end with a single-stranded DNA circle. In addition various multimeric DNA species are present. Fig. 2B shows a double-stranded DNA molecule that has reacted with two DNA circles; one circle has migrated into the middle of the double-stranded DNA, while a second circle has invaded the free end (molecule C, Fig. 3). Fig. 2C shows a molecule in which the heteroduplex region of the product in Fig. 2A has been invaded by a second single-stranded circle (molecule E, Fig. 3). Finally, highly interlinked multimers consisting of many single- and double-stranded DNA molecules are also observed (Fig. 2D). The exact nature of these very large species is difficult to discern, but they presumably arise from further pairings of the dimeric and trimeric intermediates shown in Fig. 2.

The products shown in Fig. 2 are common ones, as seen from Table I which lists the distribution of products observed after a 1-min incubation. Thus, secondary synaptic reactions, both intramolecular and intermolecular, must occur readily. Likely pathways are illustrated in Fig. 3. For example intramolecular invasion of the newly formed heteroduplex region by the initially displaced 5’ end, followed by branch migration, will produce molecules in which the single-stranded circle has migrated toward the middle of its double-stranded partner (molecule B). Various permutations of such secondary pairing reactions can produce the other observed products (molecules C–E and larger aggregates).

As observed for the recA protein (27), the uvsX protein-covered DNA molecules tend to adhere to each other rapidly by protein-protein interactions before forming base pairing interactions that are stable to deproteinization. One expects "presynaptic assemblies" of this kind to be heterogeneous in size, inasmuch as the first assemblies that form should preferentially recruit new members. This heterogeneity may explain why the larger aggregates are abundant in the Table I experiment, even though many double-stranded molecules have not yet found a single-stranded partner.

Under our standard conditions, the products expected for a complete exchange of strands, a nicked double-stranded circle and a linear single strand, are not observed even after very long incubations (see Fig. 1). If the newly formed single-stranded regions present in reaction intermediates can rapidly pair with any homologous double helix, the initial products should react further and thereby prevent such simple products from accumulating. To explain why the same reaction me-

¹ The abbreviations used are: SDS, sodium dodecyl sulfate; ATP·S, riboadenosine 5’-O-3’-thiotriphosphate.

² T. Kodadek and M. L. Wong, unpublished results.
diated by the recA protein results in a high yield of nicked double-stranded circles with relatively few larger complexes, it has been proposed that binding of the recA protein to the double-stranded products created by the strand exchange reaction persists well after completion of branch migration, rendering the nicked circular DNA product and all of the earlier reaction intermediates inert to further recombination events (2, 28). In this view molecules of the type shown in Fig. 2, B and C are not produced in recA protein-catalyzed reactions because the double-stranded DNA that has been involved in pairing remains coated with the recA protein, making it refractory to further reaction.

The total amount of single-stranded DNA remains constant throughout the course of our reactions, and a subsaturating amount of the uvsX protein is present (assuming a stoichiometry of one uvsX protein monomer for every four nucleotides of DNA (15), there is sufficient protein to cover only 75% of the single-stranded DNA). The continued pairing of the reaction intermediates, therefore, suggests that the uvsX protein can be recycled through a number of pairing reactions and that, unlike the recA protein, it does not remain bound to the new heteroduplex regions that form.

It is noteworthy that multimeric DNA of extremely high molecular weight is observed in T4-infected E. coli cells; these species are thought to be resolved by the action of the gene 49 nuclease immediately prior to packaging into phage heads (29). Since these networks are not formed upon infection with uvsX- or certain other recombination-deficient phage mutants (30), they would seem to be highly intertwined branched intermediates formed by genetic recombination.

**Rapid Branch Migration Is Catalyzed by the uvsX Protein—** Like the recA protein, the uvsX protein catalyzes branch migration in the 5’ to 3’ direction with respect to the invading strand (10). In order to measure the rate of this reaction, as well as to probe its mechanism, we employed the assay depicted in Fig. 4 (17). The same substrates (single-stranded circular M13 DNA and double-stranded linear M13 DNA labeled at its 3’ ends) and reaction conditions were employed as in the previous reactions, but instead of quenching the reaction with a mixture of SDS and EDTA and loading the sample onto a neutral agarose gel (as in Fig. 1), aliquots removed at various times were treated with a large excess of 

**FIG. 1. A time course for the uvsX and gene 32 protein-catalyzed reaction between a circular single-stranded DNA and a linear double-stranded DNA from bacteriophage M13.** The double-stranded DNA had been converted from its natural circular form (form I) to a linear molecule (form II) by cutting at the HpaII site; this molecule was then labeled at its 3’ end with 32P. The experiment was conducted and the results analyzed by gel electrophoresis as described under “Materials and Methods.” A diffuse DNA band representing the initial heteroduplex intermediate is produced early in the reaction. However, at later times the simple products expected for a complete strand exchange are not observed. Instead of being converted to form II DNA plus a linear single strand, all of the labeled DNA is eventually incorporated into very large aggregates that remain at the origin of the gel. The leftmost lane contains, as standards, form II (upper band) and form III (lower band) M13 DNA. Under these conditions, the reaction shown proceeds about 20-fold more slowly if the 32 protein is omitted (11).
FIG. 2. Visualization by electron microscopy of selected intermediates produced during the pairing of single-stranded circular and double-stranded linear M13 DNAs. Standard reactions were carried out in the presence of the uvsX and gene 32 proteins and the DNA then deproteinized by treatment with high salt prior to spreading (see “Materials and Methods”). A, the initial heteroduplex intermediate formed early in the reaction (molecule A in Fig. 3). B, a trimer composed of two single-stranded molecules and one double-stranded molecule (molecule C in Fig. 3). C, another type of trimer composed of two single-stranded molecules and one double-stranded molecule (molecule E in Fig. 3). D, a multimeric aggregate. The intermediates shown here are common 1 min after the initiation of pairing (see Table I). ssDNA, single-stranded DNA; dsDNA, double-stranded DNA.

single-stranded circles off the end, completely separating the two DNA molecules that have paired.

Branch Migration Is Accompanied by a Rapid Recycling of the uvsX Protein—In order to assess whether the uvsX protein remains associated for a prolonged period with the same DNA molecule during branch migration, we performed exper-
FIG. 3. A scheme for production of the multimeric products observed in the Fig. 2 experiment. The letter designations for each intermediate correspond to those in Table I. Interstrand hydrogen bonds (shown as lines) are drawn only for the heteroduplex regions in order to highlight them.

DNA Strand Exchange Catalyzed by T4 Proteins

TABLE I
Classification by electron microscopy of the DNA structures formed as intermediates in the uvsX protein-catalyzed homologous pairing reaction

A pairing reaction between M13 single- and double-stranded DNAs (3:1 molar ratio of ssDNA/dsDNA) was carried out in the presence of the uvsX and 32 proteins as described under “Materials and Methods.” After a 1-min incubation at 37 °C, Na3EDTA was added to 20 mM to quench the reaction. The sample was held at 0 °C and processed rapidly for electron microscopy (see “Materials and Methods”) in order to minimize spontaneous branch migration. All molecules within a grid square were examined, and a total of 485 double-stranded DNA-containing species were scored. Of these, 238 (48.7%) were unreacted double-stranded molecules. The molecules labeled A–E have the structures depicted in Fig. 3. The products classified as aggregates contained at least four DNA molecules and usually more; the detailed structure of these complex species could not be discerned. Note that all of the intermediates listed are scored as one molecule even though they can contain many molecules of the starting species. Thus, the true percent of unreacted double-stranded molecules is much less than the value directly observed in this experiment.

| Type of intermediate | No. of molecules | % of total species observed |
|----------------------|------------------|----------------------------|
| A                    | 38               | 7.8                        |
| B                    | 43               | 8.9                        |
| C                    | 17               | 3.5                        |
| D + E                | 61               | 12.6                       |
| Aggregates           | 85               | 17.5                       |

FIG. 4. An assay used to monitor the rate of protein-catalyzed branch migration. Aliquots are withdrawn from a standard strand exchange reaction at various times, cut with a large excess of BamHI restriction endonuclease, and then electrophoresed through a denaturing agarose gel. The appearance of an uncut, radioactive full-length strand (6407 nucleotides) heralds the passage of a branch beyond the unique BamHI restriction site. The inset shows a typical result using BalI-linearized double-stranded M13 DNA. See text for details.
fig. 5. The results of branch migration assays of the type shown in fig. 4, employing 3' end-labeled, double-stranded M13 DNA that had been cut with either BsmI, Hpal, or Ball, placing the unique BamHI site, respectively, 474, 2220, or 3547 base pairs away from the end at which pairing begins. Autoradiograms of the denaturing gels were scanned with a densitometer and the percent of molecules in which one strand is protected and the percent of molecules in which both labeled strands are protected from nuclease digestion plotted as a function of time. As the BamHI site is moved farther from the “left end” (as drawn) of the DNA, a greater length of time is required to observe significant protection, confirming the 5' to 3' polarity of the uvsX protein-catalyzed branch migration. A branch migration rate of about 15 nucleotides/s can be calculated from this data. Note that the restriction sites never become 100% protected. The remaining molecules in which both labeled strands are cut presumably reflect those where the original displaced 5' end has reinvaded and re-established the original double helix at the site being tested (see fig. 3).

fig. 6. Dilution of the uvsX protein slows the rate of branch migration. After a 1-min incubation to permit complete pairing of all the double-stranded DNA, reactions employing Hpal-linearized DNA were diluted 10-fold into identical solutions containing various amounts of the uvsX protein. At the indicated times, the rate of branch migration was assayed by BamHI protection and quantitated as in fig. 5.

has been proposed for recA protein-catalyzed reactions (32), then a dilution following the preincubation period should have no effect. However, when reactions that initially contained 100 pg/ml of uvsX protein were diluted into identical solutions (except for the absence of DNA) containing less of this protein, the rate of branch migration was found to decrease as the concentration of the uvsX protein was reduced (fig. 6). Single-stranded DNA is present in a constant excess compared to the uvsX protein in these reactions and binds rapidly to this protein (11, 15). But, for the concentration of the uvsX protein in the free protein pool to be important, it must be constantly entering the protein-DNA filament throughout a branch migration reaction. The DNA-uvsX protein complex must therefore be a dynamic one that is continuously undergoing a rapid protein dissociation and association.

It is interesting that the decrease in the branch migration rate is not linear below a certain recombinase concentration. Below about 20 pg/ml no reaction occurs, as seen by the almost complete lack of BamHI protection when the reaction is diluted to 15 pg/ml of uvsX protein. Since 25 pg/ml uvsX protein is sufficient to drive most of the branches past the BamHI site, there seems to be a critical concentration below which the protein is inactive.

Further evidence for the dynamic nature of the DNA-uvsX protein complex was obtained by adding a 10-fold excess of heterologous φX174 single-stranded DNA soon after the initial pairing reaction is complete. In this experiment branch migration was shut down as soon as the φX174 DNA was added (fig. 7). Such an inhibition by heterologous DNA added subsequent to initial pairing is only expected if the uvsX protein-DNA complexes are in rapid equilibrium. Thus, if the uvsX protein is continually dissociating and reassociating with the DNA during branch migration, the addition of heterologous DNA will rapidly trap the uvsX protein in nonproductive DNA-protein complexes. This will reduce its effective concentration below that attained at 25 pg/ml under standard conditions, causing all further branch migration to cease (see fig. 6).

ATPγS Binding Is Sufficient to Drive a Low Level of Branch Migration—A central but still poorly understood facet of strand exchange concerns the requirement for the binding and hydrolysis of ATP by the recombinase. What role does this play in the overall reaction? It is known that the ATP-bound form of both the recA and uvsX proteins has a much higher affinity for DNA than the ADP-bound conformer (11, 33). It seems likely, therefore, that ATP binding and hydrolysis results in a series of conformational changes that may direct protein-DNA association and dissociation and that these energy-driven events are somehow intimately related to synapsis and branch migration.

In our attempt to analyze the mechanisms involved, the effect of the tight-binding, nonhydrolyzable ATP analogue ATPγS (11) on the uvsX protein-mediated branch migration reaction was examined by adding an excess of this nucleotide to a standard branch migration reaction after completion of pairing. The rate of subsequent branch migration was then

fig. 7. The effect of addition of ATPγS and heterologous single-stranded DNA on the branch migration reaction. Reactions containing Hpal linearized, 3' end-labeled DNA were incubated under our standard conditions for 1 min; then an excess of either ATPγS (0.5 mM) or φX174 single-stranded DNA (50 pg/ml) was added in 2 μl. In the reaction labeled control, 2 μl of H2O was added. The effect of these perturbants on the branch migration rate was then monitored by the BamHI protection assay. The addition of heterologous single-stranded DNA stops branch migration completely. The addition of the nonhydrolyzable nucleotide ATPγS results in an initial acceleration of the migration of some of the branches, but the overall extent of migration attained is lower than in the control.
measured by the nuclease protection assay described previously. As shown in Fig. 7, addition of ATPγS appears to cause a transient acceleration of the branch migration rate, increasing the amount of full-length DNA observed immediately after its addition. However, the final level of protection is greatly reduced with respect to a control in which ATPγS is absent.

To test whether the complex of ATPγS and uvsX protein binds tightly enough to double-stranded DNA to protect it from BamHI digestion and thereby invalidate our assay, a control experiment was carried out under exactly the same conditions with a heterologous single-stranded DNA circle (φX174 DNA) substituted for the single-stranded M13 DNA. Since only a trace of nuclease protection was observed (2 vs. 24% for the M13 DNA circle; data not shown), we conclude that the accelerated protection of the radioactive DNA seen in Fig. 7 is the result of branch migration.

The Strand Separation Activity of the uvsX Protein—The search for homology during synapsis requires access to the DNA base pairs in the double helix, and a recombinase such as the uvsX or recA protein might be expected to show some strand separation activity even in the absence of an invading single-stranded substrate. Indeed, the binding of recA protein to a nicked circular DNA double helix in the presence of ATP or ATPγS results in extensive DNA unwinding (34, 35). The recA protein can also dissociate small oligonucleotides that are base paired to a large single-stranded DNA molecule (36).

To investigate a possible strand separation activity of the uvsX protein, we annealed a short 5' end-labeled synthetic DNA oligonucleotide to single-stranded M13 DNA and examined its protein-catalyzed dissociation as judged by polyacrylamide electrophoresis and autoradiography of reaction aliquots. In the absence of added protein, almost all of the labeled 26-nucleotide-long molecule remained bound to the single-stranded DNA circle after a 5-min incubation at 37 °C. The T4 dda protein is a very active DNA helicase (37, 38), and when a small amount was added during this incubation the oligonucleotide was completely dissociated from the DNA single strand as expected. In the presence of the uvsX protein and ATP, about 40% of the oligonucleotide was dissociated. The observed strand separation required the presence of ATP, although the nonhydrolyzable ATP analogue ATPγS also stimulated the strand separation activity of the uvsX protein to a lesser extent (Fig. 8). When the same experiments were repeated with a 57-nucleotide long molecule complementary to the same region of M13 DNA, no uvsX protein-dependent melting was observed (data not shown).

Although more than half of the 26-nucleotide long molecule remains unmelted in the Fig. 8 assays, the strand separation reaction reaches this limit after only a few minutes of incubation, and the reaction cannot be driven further by the addition of more uvsX protein. It, therefore, seems that an equilibrium between uvsX protein-catalyzed oligonucleotide dissociation and oligonucleotide reassociation is rapidly established in these reactions and that the reassociation rate of the 57-nucleotide long molecule is so rapid as to mask its slow rate of melting by the uvsX protein.

The Role of the Gene 32 Helix-stabilizing Protein in Branch Migration—We found previously that the gene 32 protein greatly accelerates the rate of uvsX protein-catalyzed pairing between homologous double and single-stranded DNAs (11). This protein is therefore present in all of our standard reactions that assay for strand exchange. Does the gene 32 protein also play a role in the branch migration reaction? Unfortunately, the very low rate of synapsis observed in the absence of the gene 32 protein prevents us from performing the obvious experiment of carrying out the entire assay in the complete absence of 32 protein. To approach this question, we therefore incubated the single-stranded and double-stranded DNAs together in the presence of both the uvsX and gene 32 proteins for a period sufficient to permit nearly complete pairing. A large excess of polyriboinosine (poly rl) was then added and the sample incubated and assayed for branch migration as in Fig. 4. The gene 32 protein binds with very high affinity to this RNA polymer (39), but the uvsX protein does not, as evidenced by the inability of poly rl to support the single-stranded DNA-dependent ATPase activity of the uvsX protein or to inhibit this activity when added as a competitor (data not shown).

The results obtained in this experiment depend upon the reaction conditions, particularly the amount of single-stranded DNA employed. Under our standard conditions (2:3:1 molar ratio of single-stranded to double-stranded DNA), the addition of excess poly rl inhibited branch migration dramatically (Fig. 9). This result can be explained in two ways. Either the gene 32 protein is required for branch migration, or the large amount of unpaired single-stranded DNA freed from 32 protein binding competes for uvsX protein, thereby decreasing the effective concentration of this protein available to promote branch migration. In an attempt to
clarify this point, the experiment was repeated using a 1:1 molar ratio of single-stranded to double-stranded DNA. Under these conditions the initial pairing reaction is much slower (data not shown), thereby delaying the start of branch migration and increasing the time necessary to observe an extensive amount of strand exchange beyond the BamHI site. Now the addition of poly rl inhibits the reaction to a lesser extent, and significant branch migration is observed even in the presence of poly rl (Fig. 9). Therefore, we conclude that, if the gene 32 protein plays a direct role in branch migration, it must either be required in very small amounts or have only a small (~2-fold) stimulatory effect.

**DISCUSSION**

A mechanistic appreciation of the processes of protein-mediated DNA pairing and directed branch migration will require an understanding of the basic chemistry of the nucleoprotein filaments central to these reactions. We have provided evidence here that the uvsX protein-DNA filaments are very dynamic structures, rapidly exchanging protein components with those free in solution. We believe that our results rule out those mechanisms that require the formation of a stable uvsX-protein DNA complex that remains intact throughout the reaction.

Consideration of the different DNA-binding affinities of the ATP- and ADP-complexed forms of the uvsX protein, and its potent DNA-dependent ATPase activity, suggests the scheme illustrated in Fig. 10 as a possible explanation of our results. In this view only uvsX protein monomers that are bound to ATP can polymerize into the nucleoprotein filaments. The bound ATP is hydrolyzed in the polymer after a brief time delay, creating ADP-bound subunits. The coupling of ATP hydrolysis to the polymerization process permits the critical concentration of the uvsX protein (the minimum concentration required for net assembly) to be different at the two ends of the filament (40). Moreover, because of cooperative interactions between the uvsX protein subunits in the filament, even the weak-binding ADP form of the uvsX protein is expected to dissociate more readily from the filament only at a filament end. Therefore, net assembly of the filament can occur at the head, and net disassembly at the tail, resulting in “treadmilling.” This idea is modeled after the behavior of purified actin and tubulin in vitro (40, 41), has also been considered for recA protein-DNA interactions (32). A treadmilling filament of this type could be envisioned to drive polar branch migration via 5' to 3' growth of the protein polymer along the displaced strand into the branch point, causing opening of the double helix and allowing the invading DNA strand to extend its base pairing with its complement there. Although the strand separation activity of
the uvsX protein is modest (Fig. 8), the overall process of branch migration involves no free energy change, so that only local melting may be needed to drive the branch. Alternatively, the uvsX protein may manipulate all three strands at the branch in a more complicated manner, actively promoting the annealing of the invading strand, as well as peeling off the displaced one.

The mechanism just suggested can explain the observed rapid inhibition of branch migration by protein dilution or the addition of excess heterologous single-stranded DNA, since a high concentration of free uvsX protein monomer is required to maintain the nucleoprotein polymer. But how can we explain our finding that the addition of ATPyS to a reaction that is initially incubated with ATP results in a transient acceleration of the branch migration rate in a fraction of the DNA molecules (Fig. 7)?

We note that the head of a growing filament will generally possess a relatively stable "cap" of ATP-bound subunits. However, occasionally the ATP cap will be lost at the head end due to the ATP hydrolysis rate exceeding the rate of filament growth there. In this case the filament can collapse and disappear, analogous to the dynamic instability scheme proposed to account for the observed behavior of microtubules (42). But, according to the Fig. 10 scheme, the binding of ATPyS will lock the uvsX protein in its high DNA affinity conformation, block all filament decomposition due to collapse at the growing end, and allow the nucleoprotein filaments to grow irreversibly until the supply of free uvsX protein monomers is exhausted.

The T4 dda protein stimulates the rate of uvsX protein-mediated branch migration by severalfold (17). In terms of the Fig. 10 scheme, we propose that this 5' to 3' DNA helicase acts to melt the double helix in advance of the branch, allowing more facile growth of the uvsX protein filament in its wake and thereby increasing the rate of the directed branch movement.

It is perhaps useful to review here our general explanation for the existence of the ATPase activity of the uvsX protein. Studies on the recA protein reveal that the first stage of homologous pairing, formation of a paramecic (noninterwound) complex between homologous regions of single-stranded and double-stranded DNAs, can occur in the presence of ATPyS (43). We have shown here that directional branch migration can be catalyzed by the uvsX protein under the same conditions (Fig. 7). Thus, it would appear that the two most fundamental aspects of recombinase function do not require extensive ATP hydrolysis. However, ATP (or ATPyS) binding is required to generate a high affinity DNA-binding site on the recombinase, permitting a long protein-DNA filament to form on single-stranded DNA. In theory, a uvsX protein molecule can add much more quickly to one end of such a polymer than the other, causing a directional assembly of the protein-DNA filament even without ATP hydrolysis (40). A directional assembly of this type presumably explains our observation of accelerated directional branch migration after ATPyS addition. However, because the equilibrium constant for monomer addition must be the same at both ends of the polymer, a protein-DNA filament that assembles rapidly at one end will remain stably polymerized unless ATP is hydrolyzed (40). In our view the continuous catalysis of directional branch migration inside the cell requires both rapid assembly at one end of the filament and disassembly elsewhere in the filament, and it is this requirement that necessitates the ATPase activity. For a related, but different view, see Ref. 44.

A number of experiments in the recA system seem to indicate that the type of treadmilling suggested in Fig. 10 does not occur (32, 45). How different are the uvsX and recA proteins? Some of the apparent differences may be due to the different experimental conditions used. Alternatively, the T4 and E. coli proteins may operate by somewhat different mechanisms, since the limited degree of homology between their amino acid sequences leaves a great deal of room for diversification of function (14). These are important issues that can only be resolved by further studies.

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DNA Strand Exchange Catalyzed by T4 Proteins

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