An Unpaired 3′ Terminus Stimulates recA Protein-promoted DNA Strand Exchange*

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Daniel A. Soltis and I. R. Lehman
From the Department of Biochemistry, Stanford University School of Medicine, Stanford, California 94305

In the presence of 100 mM NaCl, the efficient exchange of strands between a circular single strand and an homologous DNA duplex promoted by the recA and single-stranded DNA binding proteins of Escherichia coli requires an unpaired 3′ terminus. Of the duplex DNA tested, only those with 4 unpaired bases at the 3′ termini were effective. Without added NaCl, strand exchange proceeds efficiently with all duplex DNA termini examined including a nicked circular duplex. Thus, at approximately physiological salt concentrations, factors in addition to the recA and single-stranded DNA binding proteins are needed to promote efficient strand exchange. One such factor may be a DNA helicase.

The recA protein of Escherichia coli can promote the exchange of strands between homologous circular SS' and full-length linear duplex DNA molecules. The products are a circular duplex with a single strand interruption and a linear single-strand (1).

The reaction proceeds in two distinct phases. In the first, recA protein and circular SS DNA form a stable complex in the presence of ATP and SSB which is necessary for efficient strand exchange (2, 3). The recA protein-SS DNA complex then interacts with the linear duplex resulting in strand assimilation (2). In the second phase, the heteroduplex region formed during strand assimilation is expanded through recA protein-promoted branch migration which is polar and proceeds in a 3′ to 5′ direction relative to the minus strand of the linear duplex (4–7). In the experiments described here, we have examined the effect of duplex DNA termini on strand exchange, and have found that at 100 mM NaCl there is a strong preference for duplex DNAs with unpaired bases at the 3′ termini.

**EXPERIMENTAL PROCEDURES**

**Materials**

RecA protein (8) and SSB (3) were purified as described previously. E. coli helicase II and rep protein were the generous gifts of N. Dixon and N. Arai (Stanford University), respectively. Labeled and unla- beled αX circular single-stranded, closed circular, and linear duplex DNAs were prepared and their concentrations determined as described (1, 2). All DNA concentrations are given in nucleotides.

Restriction endonucleases PstI, Stul, and XhoI were purchased from New England Biolabs. DNase I was obtained from Worthington. H-labeled nucleotides were purchased from Amersham and ENHANCE was obtained from New England Nuclear. Phosphoenol- pyruvate, pyruvate kinase, and S1 nuclease were purchased from Sigma.

Singly nicked circular duplex αX DNA was prepared by treating the closed circular duplex with DNase I in the presence of ethidium bromide (9). The reaction mixture contained 10 mM Tris-HCl (pH 8.0), 1 mM MgCl₂, 1 mM EDTA, 0.1 mg/ml of bovine serum albumin, 66 μg/ml of ethidium bromide, 190 mM closed circular duplex αX DNA, and 1 μg/ml of DNase I. The reaction was incubated at 25 °C for 30 min and then stopped by phenol extraction.

**Methods**

All reactions were performed in 25 mM Tris-HCl (pH 7.2), 10 mM MgCl₂, 1 mM dithiothreitol, 5% glycerol, and where indicated, 100 mM NaCl. An ATP regeneration system was added as indicated and contained a final concentration of from 1.7 to 4.2 mM phosphoenolpyruvate and from 14 to 35 μg/ml of pyruvate kinase.

Reactions were performed with linear duplex DNA witnessed by the addition of a mixture of ATP and SSB after preincubination of all other components at 37 °C. Reactions containing nicked circular duplex DNAs were initiated by combining two separately preincubated mixtures. In one, nicked circular duplex DNA was incubated with SSB, ATP, and an ATP regenerating system at 37 °C for 30 min, and helicase II and rep protein were added as indicated. In the second preincubation, recA protein was incubated at 37 °C with circular SS DNA, and SSB and ATP were added to form the recA protein-SS DNA complex (2, 3). Thus, the reaction was started by mixing a preformed recA protein-SS DNA complex with the nicked circular duplex.

**Assay for Heteroduplex Formation**—This assay measures the incorporation of H-labeled SS DNA into S1 nuclease resistant heteroduplex. The procedure used is the same as described previously (2), except that the S1 nuclease digestions were performed at 45 °C. Since the nicked circular duplex was made by random nicking of closed circular duplex DNA, a procedure that should produce equal numbers of molecules with single nicks in either the plus or minus strand, only half of the nicked circular duplex DNAs will be capable of reacting. A agarose Gel Electrophoresis and Fluorography—Electrophoresis was performed in 0.8% agarose as previously described (1). After electrophoresis, the gel was blotted to remove excess water and then soaked in ENHANCE for approximately 1 h with shaking. The gel was then soaked in water for 1 h, after which it was dried down onto Whatman 3MM paper. The labeled bands were visualized by exposure of a sheet of Kodak XAR-5 x-ray film at –80 °C.

**RESULTS**

**Effect of Duplex DNA Termini on Strand Exchange**—The effect of linear duplex DNA termini on strand exchange in the presence of 100 mM NaCl and SSB is shown in Fig. 1. Closed circular αX DNA cleaved with the PstI enzyme which generates 4 unpaired bases at the 3′ termini was significantly more reactive than DNAs cleaved with either XhoI which produces 5′ termini with 4 unpaired bases, or Stul which generates blunt ends. Similar results were obtained for strand exchange reactions performed in the presence of 100 mM NaCl, but without SSB. We had reported earlier that the rate and extent of heteroduplex formation in the presence of SSB and in the absence of added 100 mM NaCl were nearly the same for all three duplex DNA termini (10). Since branch migration proceeds in a 3′ to 5′ direction on the minus strand

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1 The abbreviations used are: SS, single-stranded; SSB, E. coli single-stranded DNA binding protein; αX, bacteriophage αX174.
of the linear duplex (4–7), these findings suggest that an unpaired 3' terminus is required for efficient strand exchange. Presumably, the added NaCl stabilizes the duplex DNA termini, so that only the PstI-cleaved DNA possesses the requisite unpaired 3' terminus.

Exchange of Strands between Circular SS and Nicked Circular Duplex DNAs—RecA protein can promote the exchange of strands between 3'H-labeled circular SS and nicked circular duplex DNAs (Fig. 2). In the presence of SSB, this reaction proceeded nearly to completion although at a somewhat lower rate than strand exchange reactions performed with a linear duplex (not shown). The products were a nicked circular duplex, into which the 3'H-labeled circular SS DNA had been incorporated, and a displaced circular single strand (Fig. 3). These results again suggest that an unpaired 3' terminus is necessary for efficient strand exchange in the presence of 100 mM NaCl, and that addition of NaCl stabilizes the duplex structure of the nicked circular duplex DNA, thereby preventing interaction with the circular SS DNA.

Effect of DNA Helicases on Strand Exchange—When E. coli DNA helicase II and rep protein were added to a strand exchange reaction performed in the presence of 100 mM NaCl, there was a 3-fold stimulation in the rate and extent of heteroduplex formation (Fig. 2). An approximate 3-fold increase was also observed in the amount of labeled nicked circular duplex formed (Fig. 3). The stimulation of heteroduplex formation by helicase II and rep protein was additive and may result from the unwinding of the 3' terminus at the nick in the circular duplex DNA by these proteins. The addition of more helicase II and/or rep protein, however, did not substantially increase the rate or extent of reaction (data not shown). Additional recA protein and purified recBC nuclease, which can also unwind duplex DNA (11-14), had no effect (data not shown). We do not know what factors limit the extent of heteroduplex formation, but the effect of helicase II and rep protein does appear to be dependent on the added NaCl since they did not influence strand exchange in the absence of added NaCl (data not shown). Thus, although helicase II and rep protein are able to stimulate heteroduplex formation between circular SS and duplex DNAs in the presence of 100 mM NaCl, it would appear that other factors are necessary to promote this reaction fully.
Our principal conclusion is that an unpaired 3' terminus on the minus strand of the duplex DNA is required for recA protein to promote the efficient exchange of strands between circular SS and duplex DNAs. This conclusion is based on the assumption that addition of 100 mM NaCl stabilizes basepairing at the 3' termini, thereby inhibiting initiation of strand exchange. In the absence of NaCl, the 3' termini may exist in an equilibrium between paired and unpaired forms, such that the unpaired termini can react during strand exchange. Since an increase in ionic strength is known to raise the melting point of duplex DNA, this assumption is not unreasonable.

The finding that an unpaired 3' terminus is required for strand exchange would suggest that strand assimilation involves the initial pairing of two complementary SS DNAs over a rather short region. Inasmuch as strand exchange proceeds efficiently in the presence of 100 mM NaCl on duplex DNAs with 4 unpaired bases at the 3' termini, but not on DNAs with 4 unpaired bases at the 5' termini, or on DNA with blunt ends, 4 unpaired bases at the 3' end of the duplex DNA would appear to be sufficient to initiate productive strand exchange. However, a more detailed study is clearly required to evaluate the effect of length of unpaired 3' termini and the role of helicases in the initiation of strand exchange.

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