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A Role for RNA Synthesis in Homologous Pairing Events

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The relationship between RNA synthesis and homologous pairing in vitro, catalyzed by RecA protein, was examined by using an established strand transfer assay system. When a short DNA duplex is mixed with single-stranded circles, RecA protein promotes the transfer of the minus strand of the duplex onto the complementary region of the plus-strand circle, with the displacement of the plus strand of the duplex. However, if minus-strand RNA is synthesized from the duplex pairing partner, joint molecules containing the RNA transcript, the plus strand of the DNA duplex, and the plus-strand circle are also observed to form. This reaction, which is dependent on RNA polymerase, sequence homology, and RecA protein, produces a joint molecule that can be dissolved by treatment with RNase H but not RNase A. Under these reaction conditions, product molecules form even when the length of shared homology between duplex and circle is reduced to 15 bp.

Among the most important phases of the recombination process is the homologous alignment of DNA molecules. The formation of this structure is a requirement for progression through the exchange of genetic material based on sequence homology. Once alignment has occurred, heteroduplexed molecules are created and subsequent resolution completes the recombination cycle. Much of the information about homologous pairing has been gathered from studies on the action of the *Escherichia coli* RecA protein (see reviews, see references 3 and 21). This remarkable 37,842-Da protein promotes the homologous pairing of a wide variety of DNA substrates, including duplex linear DNA and single-stranded circles. This duo has been used extensively in the in vitro characterization of homologous recombination, and the results of these experiments contributed to the identification of certain stages within the genetic exchange process.

One of the reaction parameters surrounding the duplex by circle pairing reaction is the length of homology shared by the two molecules. In recombination assays in which RecA protein acted as the recombinase, the lower limit of complementarity was originally defined to lie between 30 and 151 bp (6). The lower range was established because 30 bp of homology was insufficient to facilitate pairing even when reaction conditions that approached V_{max} were used. Hence, only stable reaction intermediates are formed when the length of homology is this low. Recently, Hsieh et al. (8) reported that RecA protein could catalyze the formation of low levels of joint molecules when there was 38 bp of homology between pairing partners. This same group extended this analysis by using a nonhydrolyzable ATP analog, 5′-[γ-thio]ATP, in pairing reactions (9). These workers found that stable joint molecules were formed when there is as little as 26 bases of homology between the DNA substrates. Furthermore, synaptic complexes which require the presence of RecA protein for stability were formed when 8 or 15 bases of shared homology was present.

We have been examining the process of homologous pairing on chromatin templates by using the duplex DNA–single-stranded circle pairing reaction. Our data indicate that a positioned nucleosome inhibits strand transfer by reducing the accessibility of RecA for the homologous regions within the duplex (14, 15). Subsequently, we demonstrated that transcription through the chromatin template destabilizes the nucleosome, facilitating RecA-promoted joint molecule formation. During the course of our preliminary studies on DNA templates devoid of nucleosomes, we detected the RNA transcript itself in the products of the strand transfer reaction. In some of these reactions, the positive strand of the duplex molecule was transferred in conjunction with the RNA transcript, forming a complex that consisted of plus-strand linear duplex DNA, minus-strand RNA, and plus-strand DNA circle. The creation of this complex was found to be dependent on RecA protein, single-stranded circles, minus-strand RNA, and DNA homology.

In this report, we focus on the length of DNA homology required to facilitate the (co)transfer of the positive-strand of the naked DNA duplex onto the single-stranded circle, RNA-mediated complex formation under reaction conditions that include ATP. In agreement with Gonda and Radding (5, 6) and Hsieh et al. (8), we find that a lower limit of DNA homology exists for the minus-strand transfer onto the plus-strand circle by the action of RecA protein. This barrier approaches 72 bases of homology under our reaction conditions. Plus-strand transfer which is dependent on RNA synthesis, however, occurs when the region of homology is as low as 15 bp.

MATERIALS AND METHODS

DNA templates and proteins. The 224-bp switch region ($S_{\gamma 1}$) of the γ constant region gene was a kind gift from Y. Chen (Temple University). This fragment was cloned into the expression vector pSP72 (Promega Biotec) via the *Pst-Bam*HI compatible sites. Such an arrangement places the fragment 49 bases away from a T7 RNA polymerase promoter. A construct containing this fragment and the T7 promoter was isolated by a *Pst*I-*Hpa*I double digestion, producing a 308-bp duplex DNA fragment. Radiolabeling of either the negative or positive strand was accomplished by the action of T4 polynucleotide kinase (U.S. Biochemicals). The 5′ end of the *Pst*I (recessed) or *Hpa*I (blunt) end was radiolabeled with [γ-^{32}P]ATP by T4 polynucleotide kinase. To accomplish the labeling of only one strand, the plasmid was cleaved first with either *Pst*I or *Hpa*I, radiolabeled, and then recut with the other restriction enzyme.

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