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Transcription Activates RecA-Promoted Homologous Pairing of Nucleosomal DNA

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RecA protein catalyzes the homologous pairing of a single-stranded circular DNA and a linear duplex DNA molecule. When the duplex is packaged into chromatin, formation of homologously paired complexes is blocked. We have established a system for studying the RecA-promoted reaction by using a duplex fragment containing a single-phased nucleosome. Under these conditions there is no reaction leading to formation of joint molecule complexes. However, transcription on the chromatin template activates the formation of complexes. Reaction is dependent on RNA synthesis and DNA sequence homology and proceeds regardless of the direction of transcription.

In general, chromatin is viewed as a repressive structure, down-regulating gene expression primarily by reducing the accessibility of *trans*-acting factors to their cognate binding sites (3, 8, 10, 27). Repression extends to other processes such as genetic recombination, for which it has been observed in vitro that strand exchange promoted by *Escherichia coli* RecA protein is inhibited by chromatin (20). This raises the question of how recombination occurs in eukaryotes, in which genomes are packaged into chromatin. One notion that seems reasonable is that the homologous pairing and strand exchange machinery could gain access to DNA if chromatin structure were disrupted. DNA replication might be considered one means by which chromatin structure is disrupted. Here the helix is unwound and the relationship between the histones and DNA is altered (see reference 2 and references therein). Transcription is another process which, intuitively, would seem likely to destabilize or alter chromatin structure. Unwinding of the duplex resulting from binding by transcription factors or from active transcription might be supposed to disrupt chromatin structure sufficiently that recombination could be initiated.

The activity of RNA polymerases on chromatin templates and the passage of RNA polymerase through nucleosomal particles has been the subject of intense investigation over the last several years. RNA polymerases from bacteriophages T7 and SP6 have been observed to transcribe through a nucleosome core assembled on a plasmid DNA sequence (13) or on a 5S RNA gene fragment (14). In similar in vitro studies with the eukaryotic enzyme RNA polymerase II or RNA polymerase III, transcriptional elongation was found to be inhibited by nucleosomes (16). The fate of the nucleosomes after polymerase transit through a nucleosome is a matter of controversy (4). In one well-studied case, nucleosomes were found to be completely displaced from a DNA template (13), whereas in another no net displacement was observed (14). Discrepancies in observations might result from differences in DNA templates, but it nevertheless remains clear that the process of transcription serves to alter the DNA structure, facilitating transient opening of the chromatin structure.

We are interested in learning about the mechanism by which homologous recombination occurs within the framework of chromatin. To this end we developed an experimental system in which to analyze recombination on a chromatin template mediated by *E. coli* RecA protein. The DNA substrates were a short duplex DNA upon which one nucleosome was positioned and a single-stranded circular DNA containing a sequence homologous to the linear fragment. The duplex DNA was readily transcribed by bacteriophage RNA polymerases regardless of nucleosome assembly. In the work described in this report we investigated the repression of recombination by nucleosomes and examined the activation of homologous pairing by transcription with bacteriophage RNA polymerases.

MATERIALS AND METHODS

DNA templates and nucleosome reconstitution. A 240-bp fragment containing a single copy of the *Xenopus laevis* 5S RNA gene (somatic type) and surrounding spacer DNA from plasmid pXbsf201 (21) was inserted into the expression vector pSP72 (Promega, Madison, Wis.) after digestion with *Hin*dIII and *Eco*RI. In one orientation, the SP6 promoter element is located 16 bases upstream from the 5′ end of the 5S fragment (not the coding sequence) while a T7 promoter is located 34 bases downstream from the 3′ end. Digestion of this plasmid with *Nde*I and *Eco*RI yields a 337-bp fragment. When transcribed by SP6 RNA polymerase, RNA molecules designated as plus stranded are produced. Digestion with *Hin*dIII and *Hpa*I yields a fragment that can be inserted into pSP72 under the control of T7 RNA polymerase, which generates minus-strand transcripts. Single-stranded circles originated from M13mp18 phage DNA into which the 240-bp *Hin*dIII-*Eco*RI 5S fragment had been inserted. For heterologous controls, ϕX174 phage DNA was substituted for M13 DNA and all bacteriophage DNA by definition is of positive polarity. Duplex DNA was radiolabeled at the 5′ end of DNA with T4 polynucleotide kinase and [γ-^{32}P]ATP or at the 3′ end with the Klenow fragment of DNA polymerase I and α-^{32}P- or α-^{35}S-labeled deoxynucleoside triphosphates (dNTPs). To tag specifically either the minus or plus strand, the plasmid was

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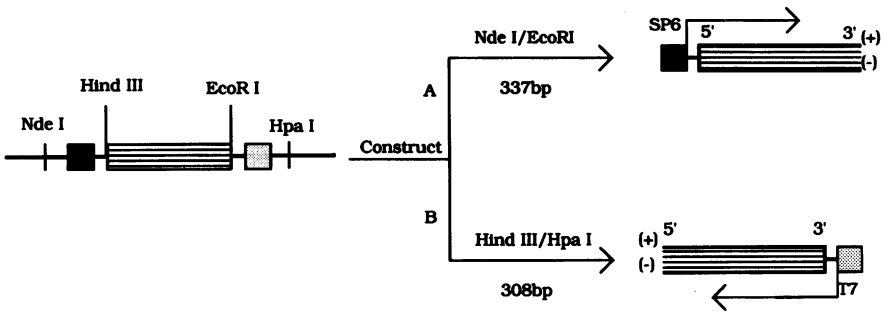

FIG. 1. Schematic of constructs A and B. An *Nde*I-*Hpa*I fragment containing the 5S RNA gene from *X. laevis* (striped bar), an SP6 RNA polymerase promoter element (black box), and a T7 RNA polymerase promoter element (stippled box) are diagrammed. Cleavage with restriction enzymes *Nde*I and *Eco*RI produces a 337-bp fragment (construct A) that contains the SP6 promoter and the gene. The direction of transcription is indicated by the arrow. Digestion with *Hin*dIII and *Hpa*I generates a 308-bp fragment containing the T7 promoter and the gene. The arrow indicates the direction of transcription. Plus (+) and minus (−) refer the polarities of the duplex strands in the strand transfer reaction.

cleaved first with either *Hin*dIII or *Eco*RI, labeled at the 3′ end with Klenow fragment DNA polymerase (minus strand) or at the 5′ end with T4 polynucleotide kinase (plus strand), and subsequently recut with the appropriate restriction enzyme, *Nde*I or *Hpa*I (see Fig. 1). Uniquely end-labeled DNA fragments were separated by electrophoresis and purified by electroelution and extraction with phenol and chloroform.

Nucleosome reconstitution was carried out by the methods of Lutter (15) and deMurcia et al. (6) with chicken erythrocytes depleted of histone H1 or H5 as the source of nucleosomal cores. Nucleosomes were assembled onto [^{32}P]DNA by using a 50-fold excess of histone octamers to DNA in a starting solution containing 2 M NaCl which was reduced sequentially to 0.2 M by dialysis. The excess of donor chromatin was used to avoid reversibility of the exchange reaction. Chromatin templates were purified by sucrose gradient centrifugation (5 to 30%) at 35,000 rpm in a Beckman SW40.1 rotor for 19 h at 4°C. The ^{32}P-labeled chromatin templates formed a peak halfway down the gradient, and 50 to 75% of the input DNA was recovered routinely. The efficiency of reconstitution was measured by band shift analysis in low-salt buffer (7) and by DNase I footprinting (5, 17, 29). For footprinting, samples were adjusted to 5 mM $MgCl_2$ when necessary, digested with DNase I (1 U of bovine pancreatic RNase-free DNase I [Boehringer Mannheim]), and reactions were stopped by addition of EDTA followed by phenol extraction and ethanol precipitation (28). The products were redissolved in formamide containing 1 mM EDTA, 0.05% xylene cyanol, and 0.03% bromophenol blue and electrophoresed through 8% polyacrylamide–7 M urea gels for 4 h (29). A sequencing-ladder size marker generated by using Sequenase 2.0 (U.S. Biochemical Corp.) and ddGTP was run in a parallel lane.

Joint-molecule formation and in vitro transcription. Reaction mixtures (20 μl) containing 0.038 μM ^{32}P-labeled duplex fragments (as nucleotide), 1.9 μM viral single-stranded circular DNA (as nucleotide), 0.65 μM RecA protein (U.S. Biochemical Corp.), 0.28 μM *E. coli* single-stranded DNA-binding protein (SSB) (U.S. Biochemical Corp.) in 20 mM Tris-HCl (pH 7.5)–10 mM $MgCl_2$–1.25 mM ATP–1 mM dithiothreitol were incubated at 37°C for various times. The reactions were terminated by the addition of sodium dodecyl sulfate (SDS) to 0.1% and incubated at 37°C for 60 min with proteinase K at a final concentration of 100 μg/ml. Electrophoresis was carried out in 0.8% agarose, after which gels were dried onto Whatman 3MM paper and autoradiographed.

Transcription reaction conditions were identical, except that 0.5 mM GTP, UTP, and CTP were included. [^{32}P]RNA was generated by using [α-^{32}P]GTP in the reaction. SP6 or T7 polymerase (10 U; Promega) was incubated with the duplex or nucleosomal template for 30 min prior to the start of the homologous pairing reaction. During this period, RecA protein and SSB were preincubated with the single-stranded circle for 5 min; then the mixtures were combined. Runoff RNA was analyzed by polyacrylamide gel electrophoresis (8% polyacrylamide containing 7 M urea) and quantitated by scintillating counting of the excised band by the method of Wolffe et al. (30). In our hands, 10 U of SP6 or T7 polymerase generated approximately 30 transcripts per template per h, which is comparable to the rate measured by Losa and Brown (14).

RESULTS

A single-phased nucleosome inhibits RecA-promoted homologous pairing. RecA protein catalyzes the homologous pairing and strand transfer of a single strand from a short duplex molecule onto the complementary region of a single-stranded circle, creating a heteroduplex molecule and displacing the homologous strand of the original duplex. We were interested in studying this reaction with a chromatin template and used a duplex fragment containing one copy of the somatic-type 5S RNA gene from *X. laevis*, since it is well established that a single nucleosome can be precisely positioned on this fragment (22). We constructed the duplex such that an SP6 promoter element is located at the 5′ end of the top strand while the center of the nucleosome is located at approximately the first nucleotide of the gene. The total size of the fragment is 337 bp (Fig. 1). In Fig. 1, the top strand of each fragment is designated as plus and is the homolog of the plus strand in the single-stranded circular phage DNA, while the bottom strand is minus and is the complement of the same sequence in the single-stranded circular phage DNA. The M13 single-stranded circular DNA contains the 5S RNA gene sequence (240 bases) and serves as the other pairing partner in the reaction. The orientation of the top strand in both fragments is indicated so that the direction of RNA polymerase movement (arrows) can be envisioned. RNA synthesis promoted by SP6 polymerase generates plus-strand RNA, whereas T7 RNA polymerase produces minus-strand RNA. This assay is a well-established system in which to monitor strand transfer activity of DNA recombinases. The concentration of DNA used in the reaction is based on optimization studies conducted originally by McCarthy et al. (16) and later by our laboratory with naked DNA as substrates.

When the minus strand of the duplex molecule is labeled

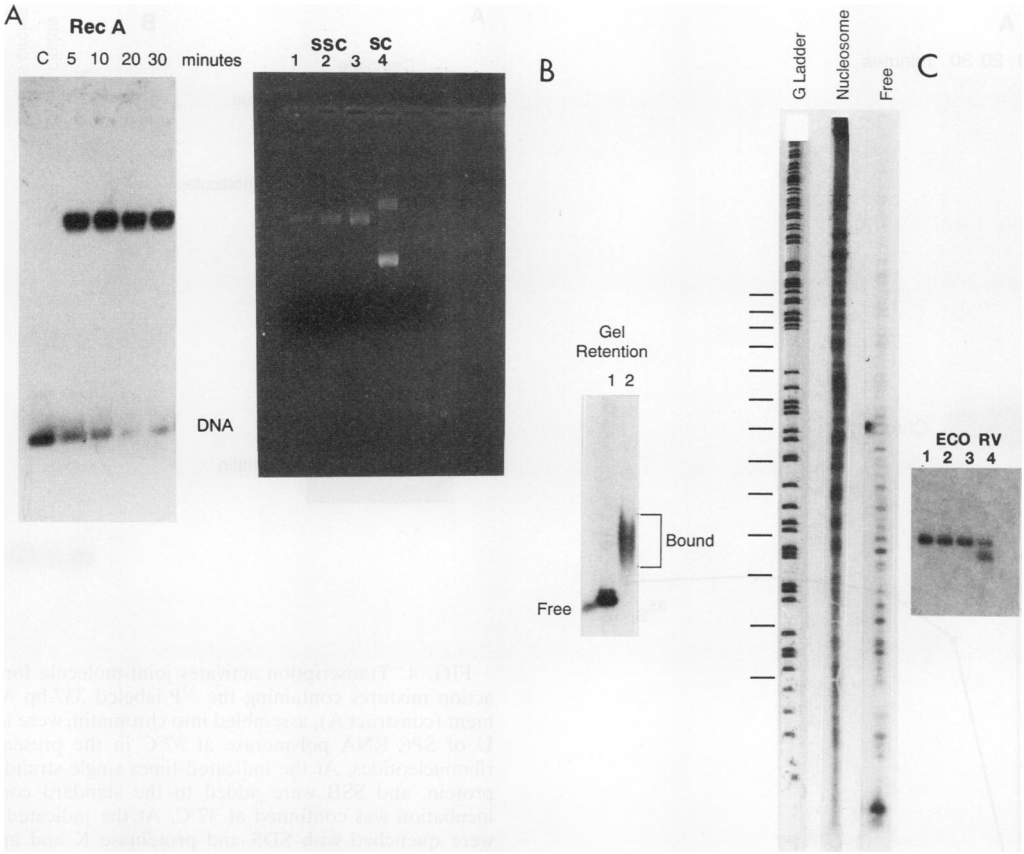

FIG. 2. Joint-molecule formation promoted by RecA protein and assembly of nucleosomes. (A) Standard reactions containing ^{32}P-labeled 337-bp *Nde*I-*Eco*RI duplex DNA (construct A) were carried out as described in Materials and Methods. In these reactions, only the minus strand of the duplex was radioactively labeled at the 5′ end. DNA, position of ^{32}P-labeled duplex fragment; C, no RecA added. In parallel lanes, 75 pmol (lane 1), 150 pmol (lane 2), and 300 pmol (lane 3) of M13 phage DNA (as nucleotide) were electrophoresed, stained with ethidium bromide, and photographed under UV light. SSC, single-stranded circles; SC, supercoiled form I M13 DNA (lane 4). (B) In the left panel, 150 pmol of the ^{32}P-labeled *Nde*I-*Eco*RI DNA fragment (construct A) was incubated with a 50-fold excess (2.5 μg) of chicken erythrocyte chromatin fiber fragments stripped of H1 by the method of Lutter (15). The nucleosomal cores assembled on the fragment were purified by sucrose gradient centrifugation, and the nucleosomal DNA was electrophoresed through 0.8% agarose. Lanes: 1, nucleosomal DNA digested with proteinase K (100 μg/ml) in the presence of SDS (0.1%) for 2 h at 37°C; 2, nucleosomal DNA, not deproteinized. In the right panel, reaction mixtures (10 μl) containing either 4.5 pmol of ^{32}P-labeled construct A reconstituted into chromatin or 1.5 ng of ^{32}P-labeled fragment (construct A) alone and DNase I were incubated in the presence of 10 mM $MgCl_2$ for 2 min or 30 s, respectively at 37°C. Reactions were terminated by the addition of 10 mM EDTA, and the DNA fragments were precipitated with ethanol. The precipitated DNA was resuspended in 99% formamide–1 mM EDTA–0.03% xylene cyanol–0.03% bromophenol blue and heated to 80°C for 5 min. The digested fragments were electrophoresed through 8% polyacrylamide containing 7 M urea and processed for autoradiography. Nucleosome, reconstituted nucleosomal template digested with DNase I for 2 min; Free, free DNA digested with DNase I for 30 s; G ladder, ^{32}P-labeled G sequencing ladder. The bars indicate 10-base periodicity. (C) The nucleosomal template described in panel A was incubated with 10 U of *Eco*RV for 2 h at 37°C. The reaction was terminated with SDS and proteinase K, and the radiolabeled fragment was electrophoresed through 1% agarose at 100 V for 2 h. Lanes: 1, uncut free DNA; 2, deproteinized but uncut nucleosomal DNA; 3, nucleosomal DNA treated with *Eco*RV and then deproteinized; 4, free DNA cleaved by *Eco*RV.

with ^{32}P, the RecA protein-promoted transfer of this strand to the circle can be monitored after agarose gel electrophoresis (Fig. 2A). The mobility of the joint molecule in agarose coincides with that of the single-stranded circle, as evidenced by the ethidium-stained gel. The same labeled DNA fragment was reconstituted into chromatin by direct exchange of H1-depleted histone octamers from chicken erythrocyte chromatin (6, 15). All of the labeled DNA was assembled into a nucleosomal structure as evidenced by a gel retardation assay and by DNase I digestion, which revealed a characteristic 10-bp periodicity indicative of the presence of a nucleosome (Fig. 2B). Nucleosomal DNA was also confirmed by digestion with micrococcal nuclease (data not shown) and by restriction enzyme cleavage (Fig. 2C). This particular template has a unique *Eco*RV site at the position bound by the nucleosome. Hence, *Eco*RV cleavage is evident only when the DNA template is not nucleosomal (Fig. 2C, lane 4).

RecA protein did not form joint molecules in reactions containing the purified chromatin substrate (Fig. 3A). To ensure that this inhibition was not due to free histones or contaminating proteins binding the single-stranded molecule, we performed the reaction with an internal positive control. ^{35}S-labeled naked DNA duplex template was added together with the ^{32}P-labeled chromatin fragment to the reaction. After the deproteinized mixture was separated by electrophoresis, the band at the position of the single-stranded circle was located by staining with ethidium bromide, cut out of the gel, and analyzed by scintillation counting with window settings adjusted so as to identify the energy spectrum of ^{35}S versus ^{32}P. As shown in Fig. 3B, ^{35}S-labeled DNA was found exclusively in

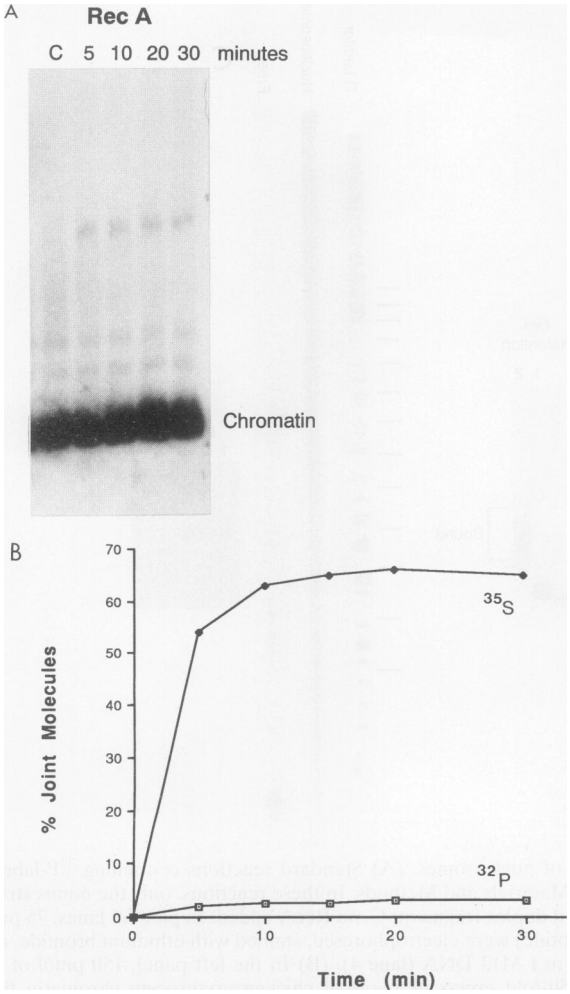

FIG. 3. DNA packaged into chromatin does not form joint molecules. (A) Standard reaction conditions were used, except that the duplex fragment (construct A) had been assembled into chromatin and purified by sucrose gradient centrifugation. After the reaction, DNA was deproteinized with SDS and proteinase K. Joint-molecule formation was detected by autoradiography. C, no RecA added; Chromatin, position of deproteinized chromatin pairing substrate. (B) Standard reaction mixtures containing [^{32}P]chromatin template or ^{35}S-labeled 337-bp (*Nde*I-*Eco*RI) duplex DNA (construct A) were incubated with RecA protein. The location of joint molecules was determined by staining the gel with ethidium bromide and locating the position of single-stranded circular DNA. The bands representing joint molecules and duplex fragment from each lane were cut out and quantitated by scintillation counting with window settings adjusted to discriminate between β-particle emission from ^{35}S and ^{32}P. The ordinate represents the percentage of the total counts per minute recovered migrating at the position of joint molecules.

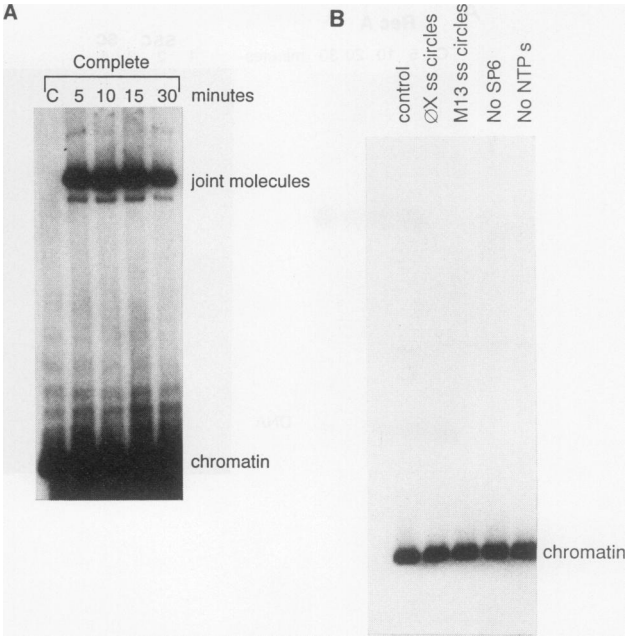

FIG. 4. Transcription activates joint-molecule formation. (A) Reaction mixtures containing the ^{32}P-labeled 337-bp *Nde*I-*Eco*RI fragment (construct A), assembled into chromatin, were incubated with 10 U of SP6 RNA polymerase at 37°C in the presence of unlabeled ribonucleotides. At the indicated times single-stranded circles, RecA protein, and SSB were added to the standard concentrations and incubation was continued at 37°C. At the indicated times, reactions were quenched with SDS and proteinase K and analyzed for joint molecules. Complete, homologous reaction substrates; C, no RecA added; chromatin, position of deproteinized duplex substrate. (B) Reaction conditions were identical to those described for panel A except for the indicated modification. Lanes: Control, no RecA protein; ϕX ss circles, ϕX174 phage DNA replaced M13mp18 5S phage DNA; M13 ss circles, wild-type M13 phage DNA replaced M13mp18 5S phage DNA; No SP6, no SP6 RNA polymerase added; No NTPs, reaction lacked ribonucleotides.

the joint molecule, indicating that the ^{35}S-labeled template was a participant in the strand transfer reaction. From these data, we conclude that the inhibition of pairing by nucleosomal DNA is not a result of nonspecific DNA binding or template destruction and that a single nucleosome positioned on the DNA fragment is sufficient to block the homologous pairing of that fragment.

Transcription activates homologous pairing on chromatin. Naked DNA was transcribed more efficiently than DNA assembled into chromatin (approximately sevenfold) with SP6 RNA polymerase at low levels. However, as the level of SP6 or T7 RNA polymerase was increased, the transcriptional activity from the chromatin templates rose to 70% of that observed with the naked DNA template. When purified nucleosomal templates in a homologous pairing reaction were transcribed with enough SP6 polymerase to generate approximately 30 transcripts per template per h, the inhibition of pairing was overcome (Fig. 4). RecA protein was able to promote joint-molecule formation between the nucleosomal template and the single-stranded circle when the chromatin fragment was transcriptionally active. As performed, both strands of the duplex were radiolabeled, so that only half of the radioactivity would be expected to appear in the product if transfer went to completion. As is evident in Fig. 4, the pairing reaction is complete within 5 min. We have noted no difference in the response over a range of ATP concentration up to 3 mM or in the presence of an ATP-regenerating system. Controls confirmed that pairing was a consequence of active transcription. First, the reaction was dependent on DNA homology. When ϕX174 phage DNA or M13 phage DNA was used as the single-stranded circle, no joint molecules were observed. Second, when SP6 polymerase or the NTPs were eliminated, there was no reaction (Fig. 4). Third, transcription in the opposite direction also activated pairing. For this control we used T7

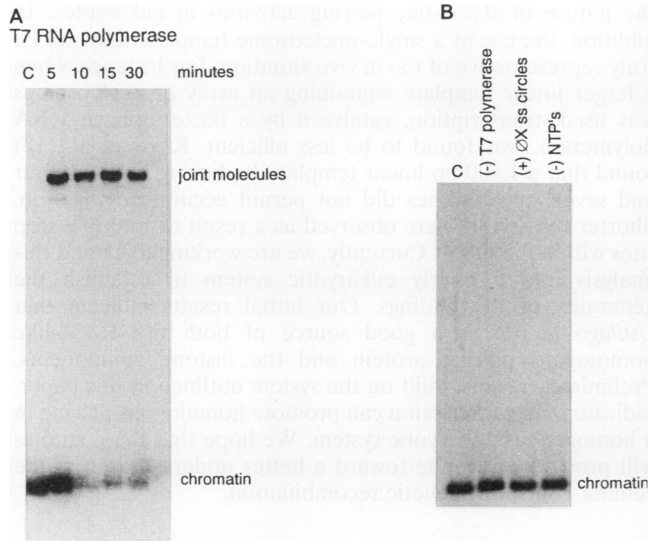

FIG. 5. Transcription of the positive strand of the nucleosomal duplex DNA activates joint-molecule formation. (A) Standard reaction mixtures were used except that construct B (*Hin*dIII-*Hpa*I 308-bp fragment) under the transcriptional control of T7 RNA polymerase was used in place of construct A; only the minus strand of the nucleosomal DNA was radioactively labeled. The reactions proceeded for the indicated times after the addition of RecA protein, *E. coli* SSB, and M13mp18 5S phage DNA. C, no RecA added. (B) Reactions were as in panel A with the following modifications; C, no RecA protein added; (−) T7 polymerase, no T7 RNA polymerase added; (+) ϕX ss circles, ϕX phage DNA was used in place of M13mp18 5S phage DNA.

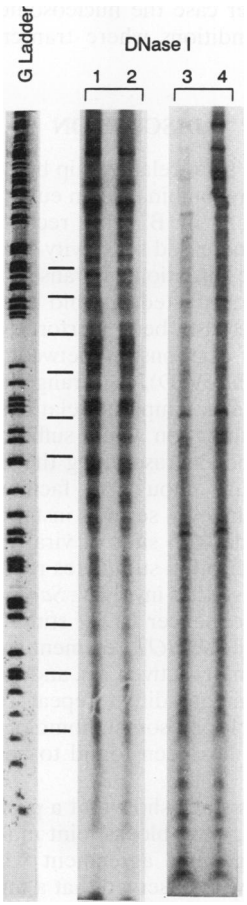

FIG. 6. Fate of the nucleosome. Reaction mixtures (20 μl) containing 3.0 pmol of ^{32}P-labeled *Nde*I-*Eco*RI DNA fragment reconstituted into chromatin were incubated with SP6 RNA polymerase and NTPs for 30 min. The reaction was terminated by the addition of 10 mM EDTA, and the fragments were precipitated with ethanol and processed for DNase I footprint analysis as described in Materials and Methods. Identical reactions were set up in which DNase I analysis was carried out on reconstituted template to which RNA polymerase, RecA protein, and NTPs were added together, and incubation was continued for 30 min. These mixtures were digested with DNase I for 1 min at 37°C and processed as described in Materials and Methods. In a third set of reactions, reconstituted templates that had been preincubated with SP6 RNA polymerase and ribonucleotides for 30 min were mixed with RecA protein to 0.65 μM and 150 pmol of single-stranded circles, and the reactions were incubated for 30 min at 37°C (lane 3). At this point, DNase I was added for 1 min and the reaction was terminated by the addition of 10 mM EDTA followed by ethanol precipitation. A similar DNase I reaction was carried out on templates in which the SP6 RNA polymerase, NTPs, RecA protein, and single-stranded circle were added simultaneously. This mixture was incubated for 30 min, at which time DNase I was added for 1 min. Footprints were visualized after the processing, as described in Materials and Methods. G Ladder, sequencing markers of G residues; the bars represent 10-base intervals.

RNA polymerase to transcribe the minus strand (Fig. 5). The duplex fragment was uniquely labeled only on the 5′ end of the minus strand and assembled into a nucleosome. T7 RNA polymerase was added, and the reaction was allowed to proceed for 30 min. The pairing reaction was then started by the addition of RecA protein, single-stranded circular DNA, and SSB. The results show that transcription by T7 RNA polymerase also activated RecA protein-promoted pairing in a homology-dependent, NTP-dependent manner. Thus transcription in either direction activates pairing.

Preliminary transcription of nucleosomal template was not absolutely necessary to activate joint-molecule formation. When all components were present at time zero, joint-molecule formation was observed. The level of product, however, was lower than that obtained when the nucleosomal template was preincubated with the RNA polymerase. This reduction may be due in part to the sequestration of RNA polymerase by single-stranded DNA. Hence, we cannot be sure that the lower levels of product formation reflect a change only in the accessibility of the nucleosomal DNA. It is possible that fewer templates become transcriptionally active and thus unavailable for DNA pairing when both the strand transfer and transcription reactions were begun simultaneously.

Fate of the nucleosome. The structure of the nucleosome was monitored by DNase I footprinting as a function of transcription and RecA-promoted DNA pairing (Fig. 6). A nucleosomal footprint was obtained when the template had been preincubated with RNA polymerase for 30 min with no addition of RecA protein and single-stranded circle (lane 1; compare with Fig. 1B) or when the RNA polymerase was present with RecA protein but in the absence of single-stranded circle (lane 2). The nucleosomal digestion pattern was disrupted under conditions of complete reaction when the transcriptionally active template was mixed with RecA protein and single-stranded DNA and incubation was continued for 30 min (lanes 3 and 4). The pattern resembles free DNA digestion by DNase I (Fig. 2). The template was cleaved more efficiently when RNA polymerase was added 30 min prior to the addition of RecA and the circle (lane 3). Since more of the nucleosomal DNA is likely to be transcriptionally active, such a result is

predictable. In either case the nucleosome structure appears disrupted under conditions where transcription and pairing were active.

DISCUSSION

There is evidence for a relationship between transcriptional activity and genetic recombination in eukaryotes. In studies on V(D)J rearrangement in B cells, recombination has been correlated with an increased sensitivity to endonuclease activity as well as to the initiation of transcription (1, 9, 19, 24). Experiments with transfected plasmid constructs suitable for recombination have also been performed, and the results suggested that the relationship between transcription and recombination in the V(D)J rearrangement events is only temporal (11). Even a temporal relationship between transcription and recombination would suffice because the movement of the RNA polymerase along the DNA would disturb nucleosomal structure enough to facilitate the binding of DNA-pairing proteins. In several instances, sites of active transcription coincide with sites of viral integration (23, 25). Evidence that transcription stimulates recombination has also been obtained from studies involving *Saccharomyces cerevisiae*. The recombination enhancer *HOT1* stimulates recombination of an adjacent gene if the *HOT1* element and the rDNA repeat unit are transcriptionally active (28), and transcription has also been shown to stimulate direct repeat recombination (26). Furthermore, intrachromosomal homologous recombination in mammalian cells has been found to be enhanced by gene expression (18).

The results of this study show that a positioned nucleosome on a short DNA fragment blocks joint molecule formation by RecA protein, in general agreement with the findings of Ramdas et al. (20), who observed that a much longer chromatin template was not active in RecA-promoted pairing reactions. The important finding reported in this paper is that transcription activates the homologous pairing reaction promoted by RecA protein on the chromatin template, thereby providing an in vitro system for analysis of the relationship between transcription and recombination. The pairing reaction is dependent on DNA homology and occurs regardless of which duplex strand is transcribed. Under the conditions of reaction, the mechanism of activated homologous pairing involves a process that disrupts nucleosomal structure. As discussed by Clark and Felsenfeld (4), RNA polymerase may break histone-DNA contacts. Thus, once a critical level of contacts is broken, the DNA would become available for interaction with the RecA protein single-stranded DNA filament. As described above, we found that the nucleosome is not totally displaced as a function of transcription but is lost during or after the strand transfer event. We hope to detail the structural nature of the nucleosome immediately before and after recombination begins, but this analysis requires a more sophisticated approach than DNase I digestions. The goal of the current study was to disrupt nucleosomal DNA enough to permit homologous pairing reactions to occur.

The experimental system used here was designed as a minimal system for studying the effect of transcription on recombination within the context of chromatin. SP6 and T7 RNA polymerases were used because efficient transcription of chromatin was required. RecA protein was used because it is the prototype homologous pairing protein, and the chromatin template consisting of a short duplex with a single phased nucleosome was used because it represents the simplest possible nucleosomal template. However, the system may be excessively simplified, because there is still uncertainty about the nature of RecA-like pairing activities in eukaryotes. In addition, the use of a single-nucleosome template may not be truly representative of the in vivo situation. For instance, when a larger linear template containing an array of nucleosomes was used, transcription, catalyzed by a bacteriophage RNA polymerase, was found to be less efficient. Kirov et al. (12) found that a 1,400-bp linear template harboring between four and seven nucleosomes did not permit accurate elongation. Shorter transcripts were observed as a result of multiple stop sites within the DNA. Currently, we are working to extend this analysis into a purely eukaryotic system to establish the generality of the findings. Our initial results indicate that *Ustilago maydis* is a good source of both the RecA-like homologous pairing protein and the histone components. Preliminary results, built on the system outlined in this paper, indicate that transcription can promote homologous pairing in a homogenous eukaryotic system. We hope that these studies will provide an avenue toward a better understanding of the cellular control of genetic recombination.

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