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DNA Length Is a Critical Parameter for Eukaryotic Transcription In Vivo

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The organization of eukaryotic chromosomes into topological domains has led to the assumption that DNA topology and perhaps supercoiling are involved in eukaryotic nuclear processes. *Xenopus* oocytes provide a model system for studying the role of DNA topology in transcription. Linear plasmid templates for RNA polymerases (Pols) I and II are not transcribed in *Xenopus* oocytes, while circular templates are transcriptionally active. Here we show that circularity is not required for transcription of Pol I or Pol II promoters if the linear template is sufficiently long (>17 to 19 kb). The *Xenopus* rRNA (Pol I) promoter is active in central positions on a long linear template but is not transcribed when located near an end. Because supercoils generated by transcription could be retained by viscous drag against the long template, these results are consistent with a supercoiling requirement for this promoter. Surprisingly, the herpes simplex virus thymidine kinase (Pol II) promoter is active even 100 bp from the end of the long template, indicating that template length fulfills a critical parameter for transcription that is not consistent with a supercoiling requirement. These results show that DNA length has unrecognized importance for transcription in vivo.

Eukaryotic chromosomes are organized into topological domains, so that a linear eukaryotic chromosome behaves like a series of isolated closed loops (1, 5, 24). Because closed loops, like circular DNAs, can be supercoiled, these topological domains could potentially provide supercoiling for processes such as transcription. However, despite extensive investigation, the role of supercoiling and topological domains in eukaryotic transcription is not clear. The classical approach for demonstrating that a process requires supercoiling is to show that the activity varies as a function of superhelical density. This approach has demonstrated that DNA supercoiling is required in prokaryotes for initiation of DNA replication, site-specific recombination, and transcription of some genes (reviewed in reference 14). This kind of analysis is possible in prokaryotes because of the specificities of the prokaryotic topoisomerases. In prokaryotes, DNA gyrase introduces negative supercoils or relaxes positive supercoils and topoisomerase I relaxes only negative supercoils; thus, the entire genome is more negatively supercoiled in a topoisomerase I mutant. In contrast, eukaryotic topoisomerases relax positive and negative supercoils equally well. An additional complicating factor is that when supercoiled plasmids are introduced into cells, they are immediately relaxed by endogenous topoisomerases and subsequently re-supercoiled by packaging into nucleosomes. One approach to overcoming these difficulties is to study the dependence of transcription on supercoiling in vitro. Some eukaryotic genes are transcribed more efficiently in cell extracts if they are supercoiled (13, 20, 30), although the presence of topoisomerases in the extracts compromises the conclusion that supercoiling facilitates transcription. Furthermore, experiments show that the basal transcription factors IIE and IIH are not required in a minimal in vitro transcription reaction if the transcription template is negatively supercoiled (11, 23). However, in one in vitro study the transcriptional activity of the *Saccharomyces cerevisiae* rRNA promoter varied as a function of superhelical density in transcription extracts prepared from *top1 top2* mutants (29). This evidence strongly suggests that supercoiling facilitates transcription of this promoter.

There have also been suggestive results in vivo for the participation of supercoiling in eukaryotic transcription. Linearized plasmid DNAs are poorly transcribed in mammalian tissue culture cells compared with circular forms of the same templates (34), and linear plasmid templates for RNA polymerase (Pol) I and II genes are not transcribed at all in *Xenopus laevis* oocytes (12, 26). Even when circular templates in oocytes are allowed to establish transcription and are subsequently linearized by injection of a restriction enzyme, transcription is dramatically reduced or entirely abolished (12, 26). Linear and circular templates are equally stable in oocytes, so the failure of linear templates to be transcribed is not due to degradation of injected templates. Thus, circular templates fulfill a critical requirement for eukaryotic transcription in vivo. Nevertheless, it is not clear whether the requirement for circular templates reflects a supercoiling requirement or whether circularity per se is somehow required for transcription. For example, free DNA ends in linear templates could be inhibitory, perhaps because they provide an entry site for inhibitory proteins or initiate cooperative binding of an inhibitor. Indeed, capping free DNA ends has previously been shown to increase transcription of linear templates in transient transfection assays (6). To show a requirement for DNA supercoiling in vivo therefore requires that we address the issue of whether the ends of linear DNA are inhibitory and that we distinguish a requirement for template circularity from a requirement for DNA supercoiling.

One way to separate circularity from supercoiling is to generate supercoiled domains on a linear template. Transcription itself generates positive supercoils ahead of the Pol and negative supercoils behind it if two critical requirements are fulfilled (18). First, the RNA Pol must be restricted from rotating freely around the DNA helix. Second, the supercoils that are generated must not diffuse off the ends; this requirement can be fulfilled by tethering the DNA to restrict its free rotation. Transcription-driven supercoiling clearly occurs both in vitro and in vivo, but the effect of transcription-driven supercoiling on other cellular processes in vivo is uncertain (4, 10, 29, 31,

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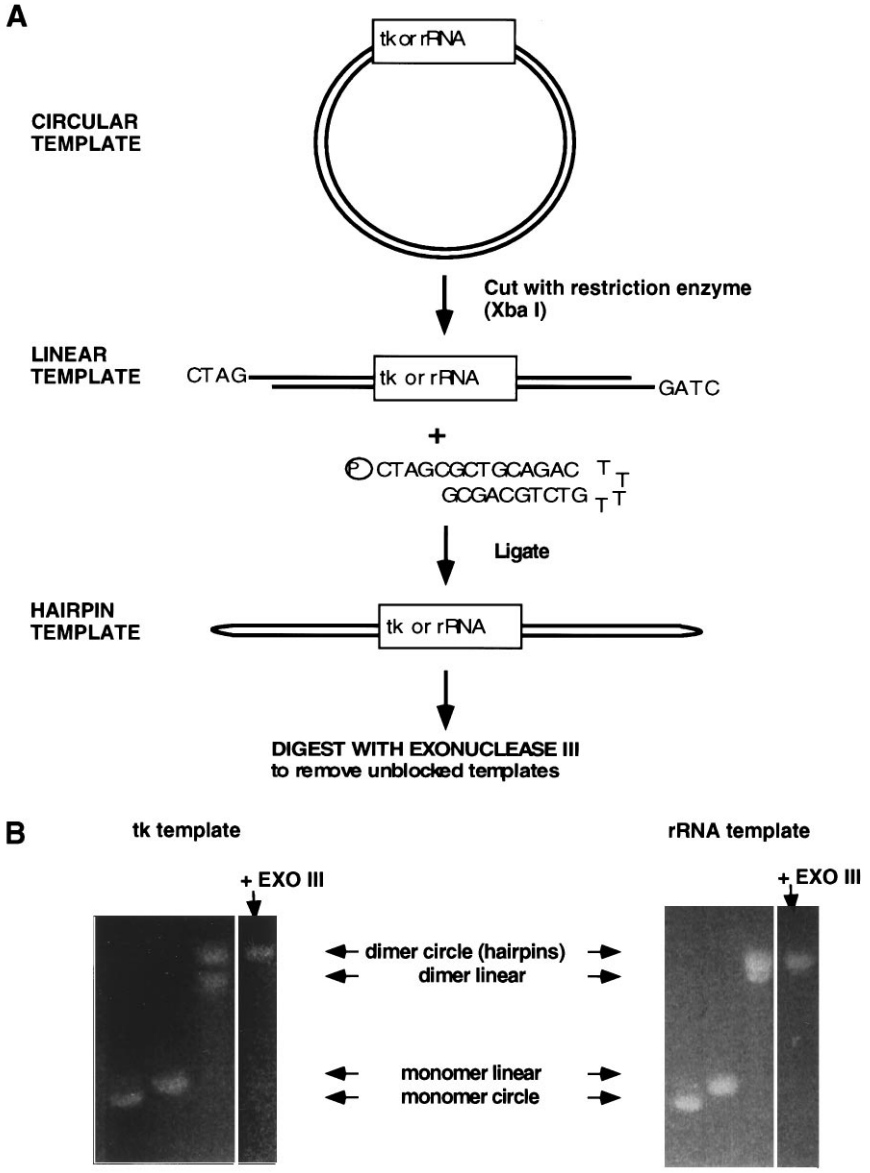

FIG. 1. Construction of hairpin-ended templates. (A) Diagram of method used to construct hairpin-ended templates. Hairpin-ended templates were made by cutting plasmids with a restriction enzyme and then ligating them to hairpin-forming oligonucleotides. This is described in detail in Materials and Methods. (B) Ethidium bromide-stained alkaline agarose gels of the hairpin-ended templates. The third lane of each gel shows the hairpin constructs immediately after the ligation step. The fourth lane of each gel shows the result of exonuclease III digestion of the samples in the third lanes.

37). Further, the importance of transcript length, DNA- and RNA-binding proteins, chromosomal context, the competing relaxation of supercoils by topoisomerases, and other aspects of the nuclear environment in restricting rotation of RNA Pol and in preventing diffusion of supercoils in vivo is not known.

We have previously separated template circularity from supercoiling by generating transcription-driven supercoils on a linear template in the *Xenopus* oocyte (7). While endogenous *Xenopus* Pol I or II cannot normally transcribe linear plasmid templates, T7 RNA Pol is able to do so when injected into oocytes. We showed that the *Xenopus* rRNA promoter can be activated on a linear template by transcription from a divergent upstream T7 RNA promoter. This and additional experiments from that study suggest that transcription-driven supercoiling can provide the minimum topological requirement for transcription for this promoter (7). These results further suggest that even a short linear DNA with no known tethers can, at least transiently, retain supercoils at sufficient density to activate a promoter.

In this study we investigate the importance of template topology and template length on transcription. We show that we cannot activate transcription on a linear plasmid by competing for possible inhibitory end-binding factors with DNA fragments nor by blocking the ends of linear plasmids with DNA hairpins. Most importantly, we show that both Pol I and Pol II promoters are transcribed in long linear templates provided by phage λ cloning vectors. We have varied the positions of the reporter genes within the λ templates by circular permutation in order to determine whether DNA flanking the promoters was required. The rRNA promoter is transcribed only when it is located far from the ends of the long linear template, suggesting that the role of DNA length for this promoter is to

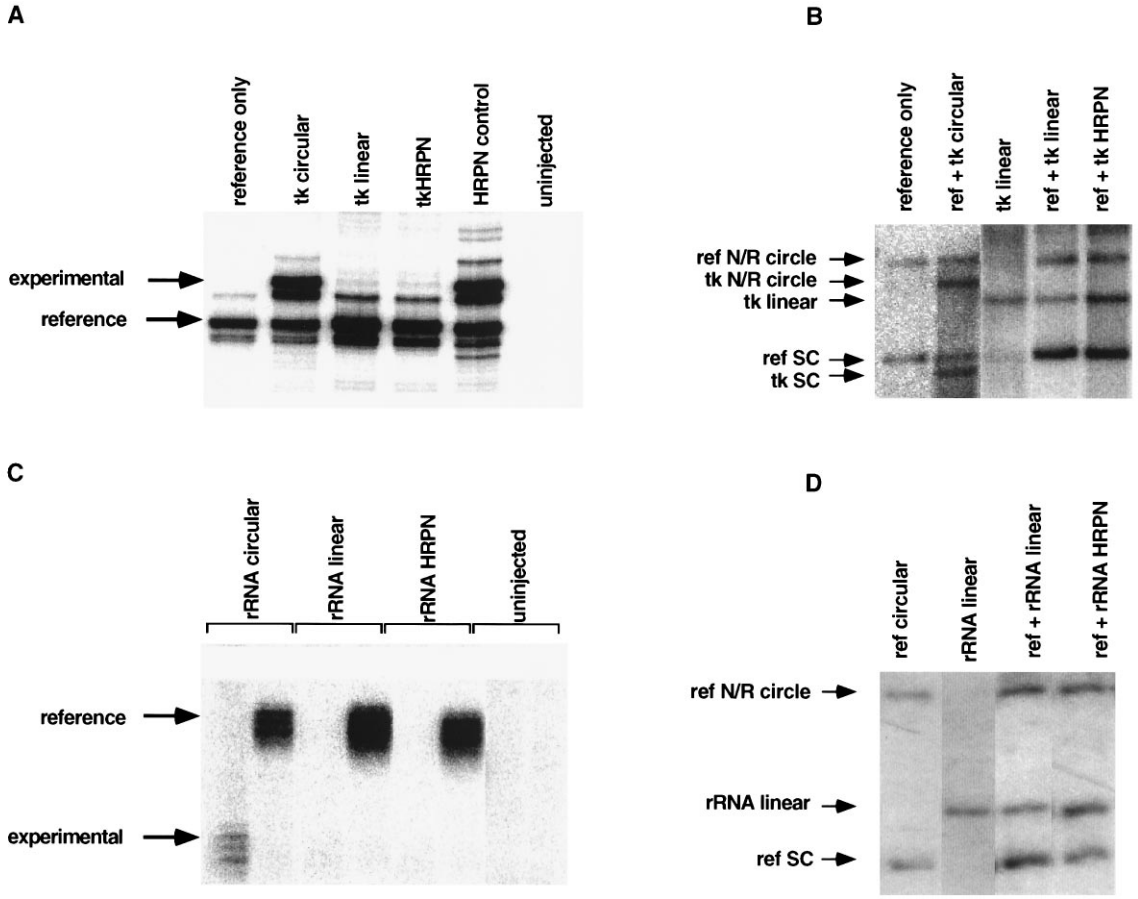

FIG. 2. Linear plasmid templates with hairpin ends are not transcribed. (A) Primer extension analysis of TK transcription by Pol II. The positions of the TK (experimental) and the circular reference plasmid (reference) primer extension products are indicated by arrows. The band between the reference and experimental signals is seen in the absence of experimental templates (see reference only lane) and is an artifact of the primer extension. (B) Southern blot of injected TK templates after recovery from oocytes. (C) S1 nuclease analysis of rRNA transcription. The positions of the ψ40 (experimental) and the circular reference plasmid (reference) S1-protected signals are indicated by arrows. (D) Southern blot of injected rRNA templates after recovery from oocytes. HRPN, hairpin; N/R, nicked/relaxed; SC, supercoiled.

prevent or retard diffusion of transcription-driven supercoils. However, the thymidine kinase (TK) promoter is active whether it is centrally located or near an end, indicating that the essential function of DNA length for this promoter is not, or is not solely, to maintain supercoiling. Instead, DNA length serves some other essential role in transcription. We discuss several possibilities for the function of DNA length in transcription, including the idea that DNA length overcomes a kinetic barrier to assembly of the transcription complex by facilitated diffusion of transcription factors to the promoter.

MATERIALS AND METHODS

Template construction. To make the λ templates, the 760-bp rRNA minigene, ψ40 (16), was cloned into the *Eco*RI site of λgt11. The 2-kb herpes simplex virus TK gene (19) was cloned into the *Sal*I and *Xba*I sites of λgt22A. To make the multimerized plasmid template, two different plasmids containing the TK gene (pBR322 and pBluescript) were linearized with the restriction enzyme *Xba*I and then ligated to form concatamers. Ligation products were separated by agarose gel electrophoresis, and high-molecular-weight products were isolated from the gel. These gel-purified products were then used to transform DH5α cells. The multimer plasmid was linearized with a restriction enzyme (*Xho*I) that cut in only one of the two plasmids.

Hairpin-ended templates were made by cutting plasmids containing either the TK gene or the rRNA minigene with the restriction enzyme *Xba*I and then ligating the linearized plasmids to hairpin-forming oligonucleotides (5′-CTAGC GCTGCAGACTTTTTGTCTGCAGCG-3′). The ligated samples were then digested with exonuclease III to remove nicked and unligated material. Hairpin-ended templates were analyzed on agarose gels under alkaline conditions (0.05 M NaOH, 1 mM EDTA), in which hairpin-ended templates run with the mobility of single-stranded dimer circles. Nicked hairpin templates run as linear dimers and are therefore separable on alkaline gels. All detectable nicked or unligated material was digested by exonuclease III.

Analysis of oocyte injection experiments. All templates were microinjected into the nuclei of *X. laevis* oocytes in a volume of 20 nl; 0.5 ng of linear and circular plasmid templates and reference templates was injected unless otherwise specified. Long linear templates were injected at approximately 5 ng in order to achieve an equimolar ratio of experimental reporter gene to reference. rRNA templates were coinjected with 50 μg of α-amanitin per ml. Otherwise, injections and harvesting of oocyte nucleic acids were performed as described elsewhere (16).

Two assays were employed to detect transcription from injected templates. Transcription from the TK gene and its corresponding reference, ψ*tk*, was detected by primer extension, as described previously (19). Transcription from the rRNA minigene and reference was detected by S1 nuclease protection, as described elsewhere (16). All transcription levels are normalized to the level of transcription from the circular plasmid reference, which is given a value of one. The normalized transcription levels also take into account any deviations from an equal molar ratio in the injected templates (determined by Southern blotting [see below]) and, in the case of the S1 protection assays, differences in the specific activities of the S1 probes. Values for normalized transcription represent the averages for at least three experiments, and the standard errors are given. Transcription assays and Southern blots were quantitated with a Phosphor Imager (Molecular Dynamics).

The molar ratios of the competing reporter genes and the stability of injected templates were verified by Southern blotting of injected samples. All templates used in this study were stable over the entire course of an injection experiment (as long as 24 h at room temperature). In the cases in which the templates were not injected at exact equal molar ratios, the differences in template levels were taken into account in the final values for normalized transcription. The com-

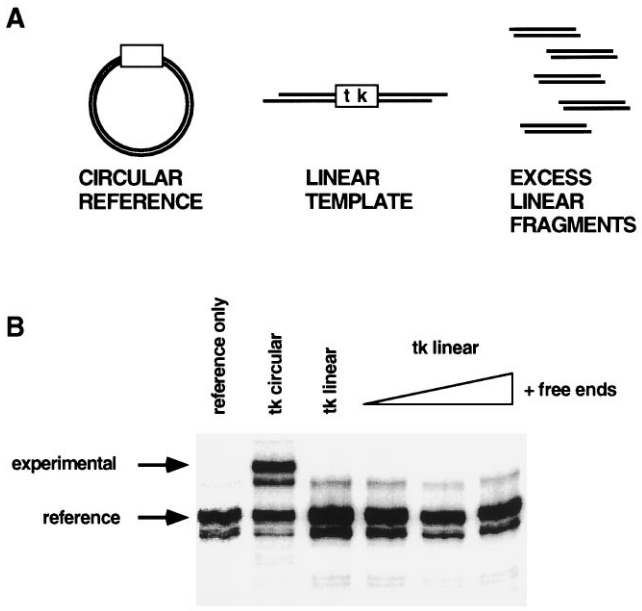

FIG. 3. Free DNA ends do not inhibit transcription. (A) Schematic of experiment. Linear transcription template was coinjected with a circular reference plasmid and various amounts of excess linear fragments (with *Eco*RI or *Sal*I termini). (B) Primer extension analysis of TK transcription with (tk linear + free ends) and without (tk linear) excess free DNA ends. The triangle indicates increasing molar excess of free ends of 2-fold, 20-fold, and 200-fold (left to right).

pleteness of digestion of λ templates was determined by digesting the injected λ DNAs with *Hin*dIII or *Pvu*I to produce multiple small fragments. These digested samples were then Southern blotted and probed with *Hin*dIII or *Pvu*I fragments that contain the sites for the enzymes used for circular permutation or deletion of λ templates. Full-size *Hin*dIII or *Pvu*I fragments would be seen only if digestion by the first enzyme(s) was incomplete. Injection samples in which these full-size fragments were detectable were discarded.

RESULTS

Free DNA ends do not inhibit transcription. We first tested the possibility that linear templates are not transcribed because of the presence of free DNA ends in these templates. Free DNA ends could inhibit transcription because of binding or entry of proteins at the ends. To test this possibility, we blocked the free ends of linear templates by preparing linear plasmid templates with hairpin ends. The construction of these templates is shown in Fig. 1. Plasmids were cut with a restriction enzyme (*Xba*I) and then ligated to self-complementary oligonucleotides. These oligonucleotides form hairpin structures with a 5′ single-strand extension that is complementary to the restriction site. After ligation, the DNA is treated with exonuclease III to degrade any unligated or nicked templates (Fig. 1A; see Materials and Methods). The constructs were analyzed on denaturing alkaline agarose gels, as shown in Fig. 1B. With these gels it is possible to separate both monomer- and dimer-length circles and linear DNAs. Dimer-length circles correspond to hairpin-ended templates. Dimer-length linear DNAs correspond to nicked hairpin templates or single-hairpin templates. These templates are sensitive to exonuclease III digestion (Fig. 1B, +EXO III). The identities of the bands on the alkaline gels were confirmed by end labelling the hairpin oligonucleotides with ^{32}P and subsequently exposing the alkaline gels to film (data not shown).

In this and all other experiments reported here we have tested two different transcription templates: the herpes simplex

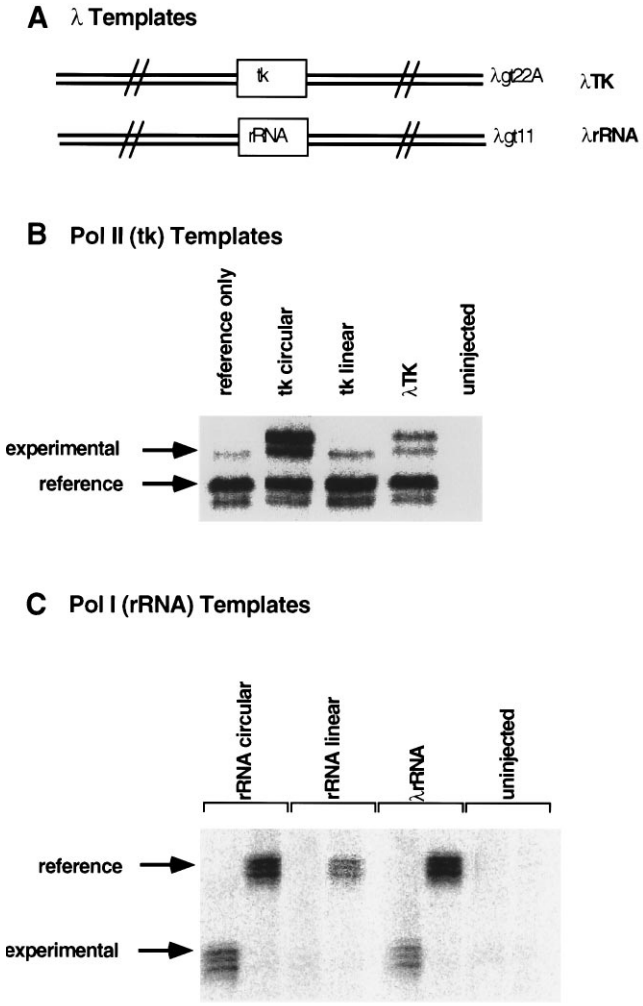

FIG. 4. TK and rRNA promoters in linear λ templates are transcribed. (A) Templates used in the injection assay. The reporter genes used in previous experiments were cloned into λ vectors to create 45-kb linear templates. λ templates were injected at a molar ratio equal to that of the circular reference plasmids. (B) Primer extension analysis of TK transcription. (C) S1 nuclease analysis of rRNA transcription.

virus TK gene, transcribed by RNA Pol II, and the *X. laevis* 40S rRNA gene (rRNA) transcribed by RNA Pol I. In all cases, the molar ratios and stability of injected templates were verified by Southern blotting (Fig. 2B and D and data not shown; see Materials and Methods).

Hairpin-ended templates for each promoter were coinjected with the appropriate circular reference template, and transcription was assayed (Fig. 2A and C). Neither the 6-kb TK template nor the 4-kb rRNA template is detectably transcribed when linearized (Fig. 2A, tk linear; Fig. 2C, rRNA linear). The limit of detection for the rRNA S1 assay is approximately 1% of the signal seen for circular templates. The limit of detection for the TK primer extension assay is at least 0.1% of the signal seen for the circular reference. Blocking the ends of these templates with hairpins does not allow them to be transcribed (Fig. 2A, tk HRPN; Fig. 2C, rRNA HRPN). As a control, the same plasmids were linearized, self-ligated to recircularize, and digested with exonuclease III to mimic the end-blocking procedure. These control templates were transcribed normally, showing that this procedure itself does not render templates

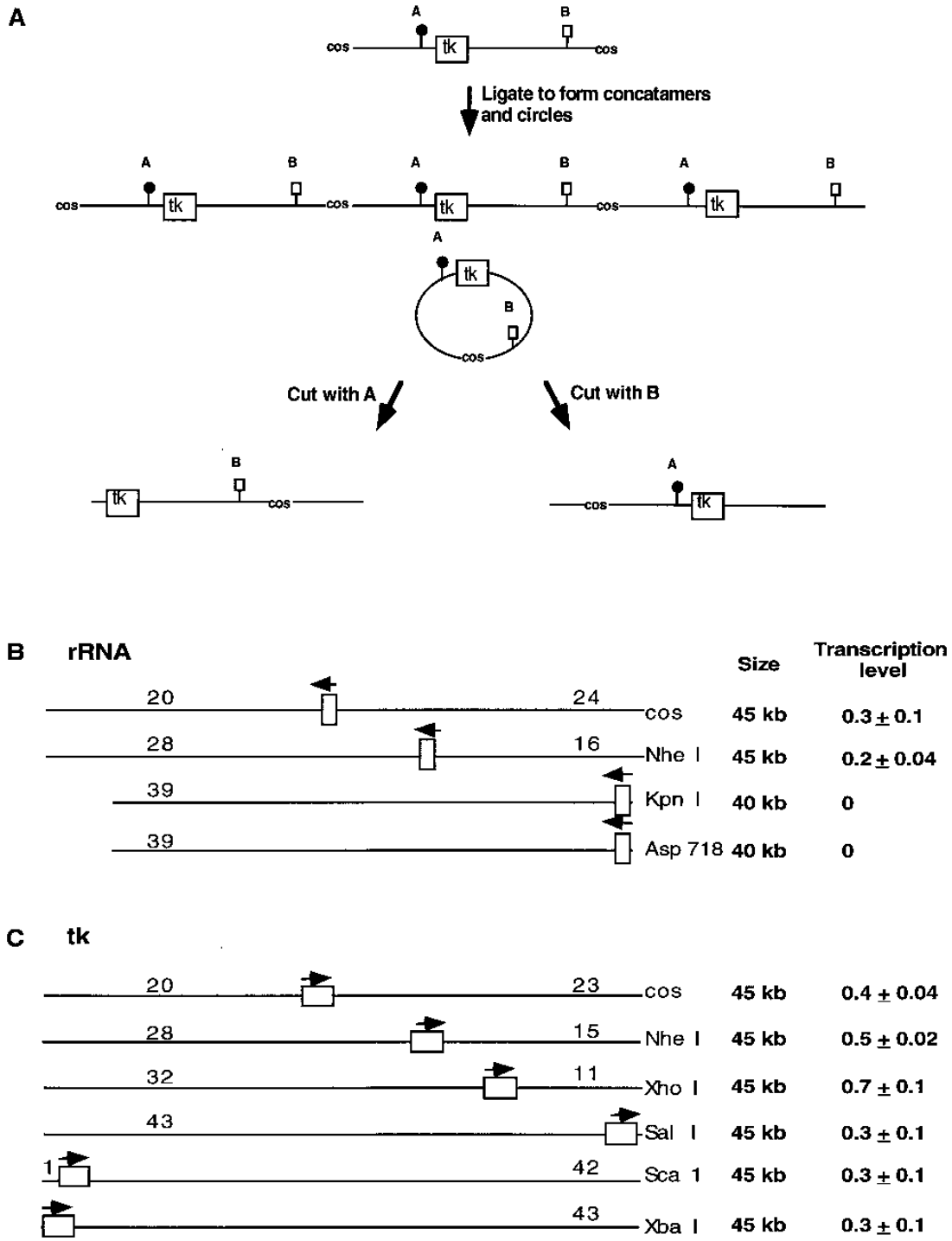

FIG. 5. Transcription of circular permutations of λ templates. (A) Diagram of the procedure and consequences of circular permutation of the λ templates. Circular permutations were made by ligating the cos sites of λ templates to form concatemers and then digesting the concatemers with the restriction enzymes indicated. Linearization of injected templates was determined by Southern blotting. (B) Summary of transcription of λTK circular permutations. The direction of TK transcription (arrow) and length of flanking DNA in kilobases (numbers above templates) are indicated. Transcription of λ templates was normalized to transcription of the circular reference template. Values represent the averages from at least three experiments, and standard errors are given. (C) Summary of transcription of λrRNA circular permutations.

inactive (Fig. 2A, HRPN control). Southern blots of the injected templates are also shown (TK templates, Fig. 2B; rRNA templates, Fig. 2D). The hairpin-ended templates were still resistant to exonuclease III digestion after reisolation from the oocytes (data not shown).

We next attempted to compete for inhibitory end-binding factors by coinjecting a linear template with increasing concentrations of free ends. No transcription was detected from linear TK templates in the presence of a 2-, 20-, or 200-fold molar excess of free ends (Fig. 3). The linear rRNA template also was

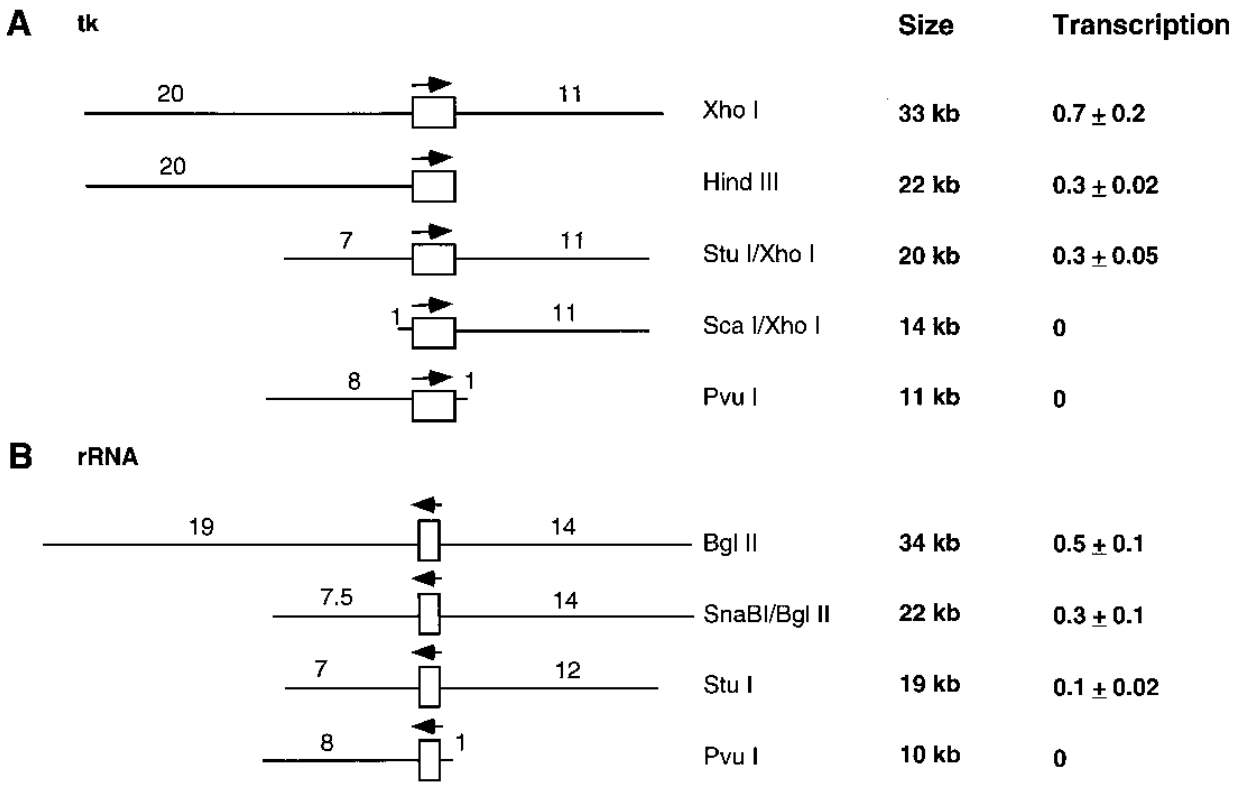

FIG. 6. Transcription of shortened λ templates. Shortened templates were made by digesting λ templates with the restriction enzymes indicated, and the resultant mixture of DNA fragments was coinjected with the reference plasmid into oocytes. (A) Summary of transcription of shortened λTK templates. (B) Summary of transcription of shortened λrRNA templates. The direction of TK and rRNA transcription (arrows), the length of flanking DNA in kilobases (numbers above templates), and total template length are indicated.

not activated by competing free ends (data not shown). Since no transcription was detected even at the highest levels of competitor free ends and since hairpin-ended linear plasmids are not transcriptionally active, it is unlikely that the failure of short linear templates to be transcribed is due to the binding of inhibitory factors to their free ends.

Long linear templates are transcriptionally active. We next tested whether template length was a factor in transcription of linear templates. We reasoned that long DNA arms flanking a transcription template might act as a barrier to diffusion of supercoils due to friction against the mass of DNA and proteins. If this were the case, then negative supercoils generated behind a Pol during transcription from a promoter in a long template might be sustained long enough to facilitate further initiation events.

Long linear templates were constructed by cloning either the rRNA or the TK reporter gene into phage λ vectors (Fig. 4A). These templates are approximately 45 kb in length, and the reporter genes are centrally located within the vector DNA when linearized at the cos sites. The λ templates were coinjected with an equimolar amount of circular reference plasmid, and transcription was assayed (Fig. 4B and C). In dramatic contrast to linear plasmids, both the rRNA and TK genes are transcribed on long linear templates (Fig. 4B, λTK; Fig. 4C, λrRNA).

The position of the rRNA promoter within a linear template is critical for transcription. If transcription on the long linear templates resulted from the formation of localized domains of supercoiling due to a diffusion barrier provided by long DNA arms, then transcription of a promoter in a long linear template would be expected to depend on its position within the template. Specifically, a promoter located near the end of a template will not be transcribed as efficiently, since supercoils diffuse rapidly off a short linear stretch of DNA. We tested the importance of promoter position within the λ templates by making circular permutations of the λ templates (Fig. 5A). The cos ends of λTK or λrRNA were ligated in vitro to form a mixture of concatemers and circular templates. Aliquots of these mixtures were each digested with specific restriction endonucleases to create linear templates in which the position of the reporter gene varies with respect to the ends of the templates. This circular permutation not only allowed us to test the position dependence of transcription in these templates but also allowed us to eliminate the possibility that the transcripts detected from the λ templates in Fig. 3 arose from templates in which the λ cos sites had annealed in vivo. The cos ends of the λ templates have much longer overhangs (12 bp) compared to those generated by restriction enzyme cleavage of plasmid DNAs. In the circularly permuted templates, the cos sites are ligated and located internally, while the template ends are generated by restriction enzymes like the linear plasmid templates. Permuted λrRNA templates were coinjected with circular reference plasmids, and transcription was assayed and quantitated as described above (Fig. 5B). Templates in which the rRNA promoter is still flanked with long DNA arms but that have restriction enzyme overhangs instead of long complementary ends from the cos sites are transcribed with efficiency equal to that of the original λ templates (Fig. 5B, cos versus *Nhe*I). Therefore, the difference between transcription on λ templates and linear plasmid templates is not due to

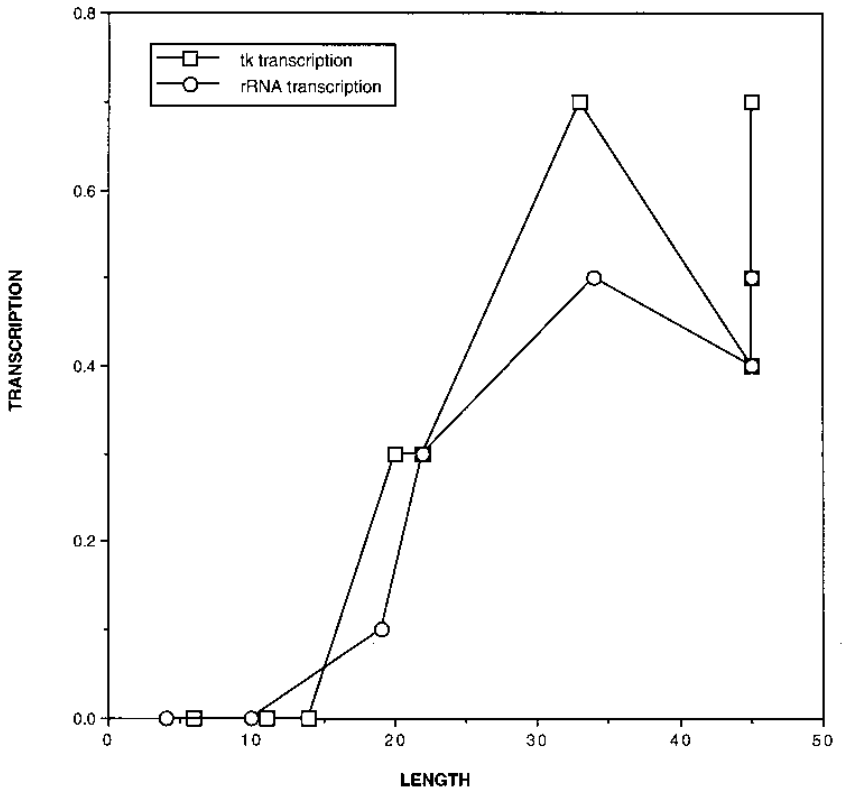

FIG. 7. Transcription increases with increasing length. Plot of transcription level versus template length for λTK and λrRNA templates. Units of transcription are arbitrary. The plot includes data from circular permutations and shortened templates.

reannealing of the ends. We have also prevented annealing of cos sites directly by filling in the cos overhangs with Klenow Pol, and we see that these templates are transcribed to the same extent as those shown in Fig. 4 (data not shown).

The position of the rRNA promoter was critical for transcription in the long template, however. This promoter is not transcribed when located within 100 bp of the end of the template (Fig. 5B, *Kpn*I and *Asp*718), regardless of whether the end has a 3′ or 5′ overhang. This observation is consistent with our original idea that long linear templates might allow domains of supercoiling to accumulate and that this makes transcription possible on a linear template.

Transcription of the TK promoter is independent of position. To our surprise, TK and rRNA promoters do not have the same dependence on position within the λ template. In contrast to the rRNA promoter, moving the TK promoter close to the end of the λ template had only modest effects on transcription. Transcription is only decreased about twofold in TK templates in which the TK gene is between 100 bp and 1 kb from an end (Fig. 5C, *Sal*I, *Sca*I, and *Xba*I). Transcription was observed whether the 5′ or 3′ end of the gene was very close to an end, suggesting that transcription from this promoter is not dependent on supercoiling. DNA length is apparently providing some other critical function for this promoter.

A minimum length is required for a linear template to be transcriptionally active. Since 45-kb linear λ templates are transcribed but 6-kb linear plasmids are not, we tested templates of intermediate sizes for transcriptional activity. The λ*tk* and λrRNA templates were digested with restriction enzymes to create shortened templates, and the mixture of DNA fragments was injected into oocytes. The shortened templates and a summary of the transcription data are shown in Fig. 6. Both the TK and rRNA promoters are active in templates as small as 20 kb (Fig. 6A, *Xho*I, *Hin*dIII, and *Stu*I-*Xho*I; Fig. 6B, *Bgl*II, *Sna*BI-*Bgl*II, and *Stu*I). However, neither promoter is active in templates in the 10- to 14-kb range (Fig. 6A, *Sca*I-*Xho*I and *Pvu*I; Fig. 6B, *Pvu*I).

Once a critical minimum length is reached, the level of transcription increases with increasing length for the shortened λ templates (Fig. 7). We have not tested templates longer than 45 kb, but we note that transcription of these long linear templates is still less than for a circular template. The minimum length is similar for λ*tk* and λrRNA, and the shape of the curve is also similar. Since transcription does increase with length in these templates, it seems that length alone can determine efficiency of transcription of a template.

Linear plasmid multimers of sufficient size are transcribed. To confirm that length rather than a sequence context was the critical parameter for transcription of the λ templates, we constructed a long TK template by multimerizing plasmids that are not transcribed as linear monomers (Fig. 8A). Two different TK gene-containing plasmids (pBR322 and pBluescript) were linearized with *Xba*I and ligated to form concatemers. Neither of these TK gene-containing plasmids is transcribed as a linear monomer (TK gene in pBR322, Fig. 2; TK gene in pBluescript, data not shown). The ligated material was transformed into *Escherichia coli*, and multimers having only one pBluescript vector were selected. Thus, the multimerized plasmid shown can be linearized by digestion with any restriction enzyme having a site unique to pBluescript.

Transcription of the 17-kb multimer TK template is shown in Fig. 8B. The linear plasmid multimer is transcribed at ap-

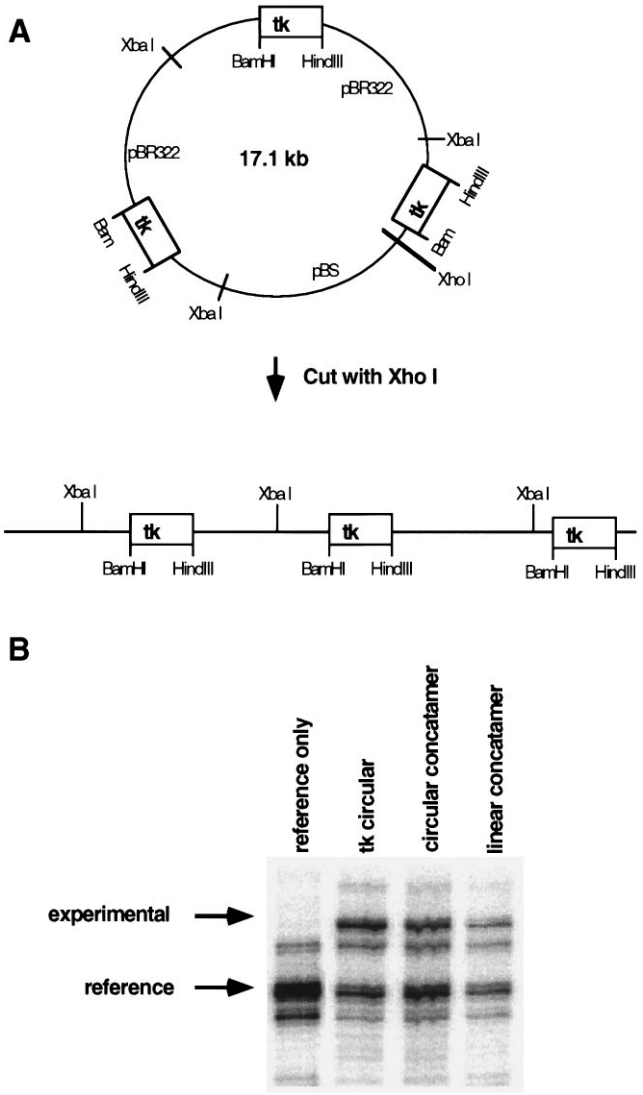

FIG. 8. Linear plasmid multimers are transcribed. (A) Construction of the TK multimer template. Two different plasmids containing the TK gene (pBR322 and pBluescript SK) were linearized with the restriction enzyme *Xba*I and then ligated to form concatemers. Ligation products were separated by agarose gel electrophoresis, and high-molecular-weight products were isolated from the gel. These gel-purified products were then used to transform *E. coli*. The multimer plasmid was linearized with a restriction enzyme (*Xho*I) that cuts in pBluescript only. (B) Primer extension analysis of TK transcription of multimerized templates.

proximately one-third of the level of the circular reference plasmid (normalized with respect to the copy number of TK genes in the construct). This level of transcription is similar to that of the long TK template provided by the λ vector. This indicates that length alone provides an essential function for transcription of linear templates.

DISCUSSION

We have shown that long linear templates are transcribed by both Pol I and Pol II with an efficiency comparable to that of circular plasmids. Transcription is not due to specific sequences in the λ vector, because linear plasmid multimers of sufficient length are transcribed. Thus, a linear plasmid template that is transcriptionally inactive can be activated simply by increasing its length. The minimum length required for transcription of either RNA Pol I or Pol II templates is between 14 and 17 kb, only a three- to fivefold increase over the length of typical plasmids. A similar effect is observed in tissue culture transfection experiments, in which circular templates are preferred to linear templates when the templates are at low concentrations, but at high DNA concentrations, linear templates are transcribed. In these cases, the high DNA concentration leads to concatemerization of the linear plasmids (25, 33, 34). Because there is no end-joining activity in *Xenopus* oocytes, short linear templates do not form concatemers even when they are injected at high concentrations in our assays.

It is clear from the experiments reported here that DNA length facilitates transcription of RNA Pol I and II genes in different ways. Transcription of the *Xenopus* rRNA promoter is dependent on the position of the promoter with respect to the ends of the DNA, consistent with the idea that a topological domain can be created on a large linear template. The viscous drag against a large DNA molecule is apparently sufficient to prevent transcription-generated supercoils from diffusing rapidly off the end of the DNA, so that DNA length creates a topological domain. These results, especially when taken with previous data showing that transcription-driven supercoiling can activate the rRNA promoter (7), suggest that this promoter requires supercoiling. The two studies taken together are the best evidence to date that supercoiling is required for any function in vivo in a eukaryote.

In contrast to rRNA transcription, the transcription of the TK promoter is independent of its position within the λ template. Templates in which either the 5′ or 3′ end of the TK gene is near the end are transcribed with efficiency similar to the efficiency of those in which the TK promoter is centrally located within the λ DNA. These observations suggest that long DNA serves different functions for the two genes tested and separates transcription of the two genes mechanistically. This distinction is further supported by the experiments using T7 transcription to activate eukaryotic promoters. The TK promoter could not be activated by transcription from a divergent T7 promoter (6a).

If the results with TK are not consistent with a supercoiling requirement, then what is the advantage to having a long template for transcription? A classic problem in molecular biology is how a sequence-specific DNA-binding protein finds its target sequence within the vast excess of nonspecific DNA (17, 32). Given the relative affinities of sequence-specific DNA-binding proteins for their target sequences and for nonspecific DNA, these proteins would never find their targets if the nonspecific DNA acted only as a competitor. Our experiments show that the additional nonspecific DNA present in *cis* on the long templates facilitates transcription and therefore cannot be considered a competitor in the traditional sense. There is previous evidence that DNA length can facilitate a process. The rate at which site-specific DNA-binding proteins find their sites is increased (8, 15, 35, 36). For example, the association of *lac* repressor with an operator sequence is approximately 10-fold faster in a λ template than in an oligonucleotide duplex (2, 27, 28). This rate is about 1,000 times faster than one would expect for a diffusion-controlled macromolecular association. The increased rate of association on long DNA is proposed to occur by a process of facilitated diffusion in which DNA-binding proteins associate with nonspecific DNA and either slide or hop along the DNA contour or are directly transferred from one segment of DNA to another (3, 32). This reduces a three-dimensional search to a one- or two-dimensional search. Thus, the activation of the TK promoter on long templates fulfills one criterion discussed by von

Hippel and Berg as indicative of a process in which facilitated diffusion is important: the rate of the process is increased when template length is increased (32). Although our experiments do not address which step or steps in the transcription process are rate limiting in these different templates, it is possible that the increased rate at which sequence-specific transcriptional activators find their targets facilitates assembly of the transcription complex, perhaps by providing a surface for nucleation of the complex. There are other possible roles for a long template, such as attachment to the nuclear architecture or targeting to a nuclear compartment rich in transcription factors. Attachment, for example, could create DNA loops that mimic the topological domains of the chromosome. In either case, our results show that such attachment or targeting must be triggered solely by DNA length, not specific sequences.

There are other examples of the importance of DNA length in cellular processes. DNA length is important for chromosome segregation and nuclear assembly (9, 21, 22), though the role of DNA length is not understood for either process. It is clear that the dynamics of long DNAs play a critical role in many nuclear activities, and studies of these dynamics will lead to greater understanding not only of these processes but of the structure and function of the chromosome itself.

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