Transcription-induced conformational change in a topologically closed DNA domain

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ABSTRACT

We have tested *in vitro* the occurrence of a B-to-Z transition in a region of alternating purines and pyrimidines as a consequence of transcription-induced negative supercoiling. By using a monoclonal antibody as a specific Z-DNA stabilizing agent, we demonstrate that the formation of left-handed DNA can transiently occur when a topologically unconstrained template is transcribed. The B-to-Z transition, observed in a sub-population of templates, appears to be induced by negative supercoiling generated in the wake of an elongating T7 RNA polymerase. Consistent with this, the presence of topoisomerases during the transcription period prevents the change in DNA conformation. These data agree with the 'twin-supercoiled-domain' model for transcription of Liu and Wang (1). Interestingly, our results suggest that the diffusion rate of transcription-induced superhelical twists must be relatively slow compared to their generation, and that under *in vitro* conditions localized transient supercoiling can reach unexpectedly high levels.

INTRODUCTION

Liu and Wang recently proposed the 'twin-supercoiled-domain' model for transcription (1) which was subsequently supported by a number of elegant studies using both pro- and eukaryotic systems (2–4). The demonstration of significant changes in DNA supercoiling, induced by elongating bacterial and eukaryotic RNA polymerases *in vitro* and *in vivo*, led us to search for a transcription-induced B-to-Z transition in a stretch of alternating purine and pyrimidine residues. In doing so, we utilized the potential of negative (−) supercoiling to induce and to stabilize local Z-DNA segments (5) and, in turn, we have taken Z-DNA formation as an indicator for torsional stress within a template. Our preliminary results indicated that such a structural transition was detectable in a circular template, most likely as a result of (−) supercoiling transiently generated in the wake of an elongating T7 RNA polymerase (T7 RNP) (6). In the present report, we extend these studies and demonstrate directly that a change in DNA conformation can indeed occur in a fraction of transcriptionally active templates.

For specific and rapid detection of Z-DNA, we utilize a monoclonal antibody (mAb), designated Z-D11, which is specifically directed against stretches of $d(CG)_n$ in the left-handed conformation (7,8). This particular antibody appears to be an optimal tool for our studies because it is well characterized and exhibits an unusually high binding affinity for left-handed $d(CG)_{16}$ (8). Furthermore, the DNA-bound antibody shows the capacity to block an elongating T7 RNP and thus prevents the enzyme from passing through a stretch of left-handed DNA. This transcriptional blockage is remarkably stable, even remaining when (−) supercoiling is released by linearization of the template subsequent to antibody binding (6).

In order to test for a B-to-Z transition in transcribed templates, we utilized this antibody's ability to stabilize Z-DNA and developed the experimental strategy schematized in Figure 1. We begin with a topologically unconstrained, i.e. completely relaxed, covalently closed circular plasmid, designated p101. This template contains a promoter consensus sequence for T7 RNP and a stretch of $d(CG)_{16}$ located 70 nucleotides downstream from the transcriptional start site. In Figure 1a, the alternating purine-pyrimidine stretch is marked by the letter B to indicate its right-handed conformation in the unconstrained template, which is now incubated with both T7 RNP and mAb Z-D11 under conditions optimal for transcription. Since no specific termination sites for T7 RNP are present in p101, the circular template can be continuously transcribed so that transcripts of several thousand nucleotides in length are generated within a few minutes (2).

Once a transcript has reached a critical length, its frictional drag could anchor the attached polymerase and thus prevents the elongating enzyme from rotation around the template axis (1,2). At this stage, the two polynucleotide chains of the template become overwound in front of and underwound behind the polymerase. Consequently, the double helix axis will start to rotate around itself as the enzyme tracks further along the template. The sense of helix axis rotation will be left-handed in front of and right-handed behind the enzyme, thus generating domains of (+) and (−) supercoiling, respectively (Fig. 1b).

If, during that stage of transcription, the stretch of $d(CG)_{16}$ is passed by an elongating polymerase, the purine-pyrimidine track becomes included into the (−) supercoiled domain built up behind the enzyme. Assuming that enough (−) torsional strain is generated, a B-to-Z transition will be induced. The alternate DNA conformation will then be bound and stabilized by the anti-Z-DNA antibody present in the reaction mixture (Fig. 1b to d) (8,9). A second consequence of the highly cooperative structural transition is a reduction in the number of (−) superhelical twists. A switch of the entire $d(CG)_{16}$ stretch from the right-handed into the left-

handed conformation will remove approximately five (−) supercoils (Fig. 1c) (10). Upon the addition of wheat germ topoisomerase type I those templates will be relaxed (Fig. 1d to e).

As previously demonstrated (6), further transcription of templates in which a structural transition has occured will be blocked at the Z-stretch when the polymerase encounters the bound antibody (Fig. 1e). After removal of the antibody by deproteinization, the stretch of $d(CG)_{16}$ flips back from the left-handed Z- into the right-handed B-conformation, which, in turn, re-introduces (−) superhelical twists into the template (Fig. 1f). This can be explained by both the preceding net decrease in template linking number (Lk) during topoisomerase I treatment and the increase in the number of helical twists concomitant with the Z-to-B transition.

Thus, we use analyses of topoisomer distributions to identify structural transitions in transcribed templates. Furthermore, we show by antibody-mediated blockage of T7 transcription that the antibody is in fact bound to the $d(CG)_{16}$ stretch in a fraction of topologically unconstrained templates. Our results demonstrate that transcription of topologically closed domains can generate a degree of torsional strain sufficient to induce conformational DNA changes like a B-to-Z transition.

MATERIALS AND METHODS

Plasmids and enzymes

Plasmids pTZ18R and p101 have been described (6). Briefly, pTZ18R (Pharmacia) contains a bacteriophage T7 promoter consensus sequence immediately upstream of a polylinker. In p101, an EcoR I-Hind III polylinker restriction fragment containing a 32 base-pair alternating d(CG) stretch at the BamH I site (10) replaced the polylinker fragment of pTZ18R. To position the potential Z-stretch further downstream from the promoter, a 56 base-pair pUC 18 polylinker, containing symmetrical EcoR I sites at its ends, was cloned into the respective site of pTZ18R.

T7 RNA polymerase was obtained from New England Biolabs. Wheat germ topoisomerase type I and RNAsin were obtained from Promega. Restriction enzymes were purchased from Boehringer Mannheim. The generation and purification of monoclonal anti-Z-DNA antibody Z-D11 has been described (7,8).

Topoisomerase reaction

Complete relaxation of p101 was achieved by incubating 1μg of supercoiled DNA with 2units of wheat germ topoisomerase in 50mM Tris-HCl (pH 7.9), 50mM NaCl, 1mM EDTA, 1mM Dithiothreitol (DTT), 20% (v/v) glycerol for 30 minutes at 37°C, followed by a second addition of enzyme and further incubation for 30 minutes. The reaction was stopped by the addition of EDTA and $NaDodSO_4$ to a final concentration of 25mM and 0.5% (w/v), respectively. DNA was purified by phenol/chloroform extraction, isolated by ethanol precipitation, and finally resuspended in 10mM Tris-HCl (pH 7.5), 1mM EDTA.

In vitro transcription assay

In order to follow the experimental strategy as depicted in Figure 1, transcription was carried out in 20μl of a buffer containing 40mM Tris-HCl (pH 8.0), 8mM $MgCl_2$, 50mM NaCl, 25mM DTT, 2mM spermidine, 10units RNAsin, 50units T7 RNA polymerase/μg of relaxed p101, 2μg mAb Z-D11, 0.5mM of each ATP, UTP, GTP, and CTP. Incubation was at 37°C in the presence or absence of monoclonal antibody Z-D11 as indicated. After 15 minutes, 8units wheat germ topoisomerase type I was added per μg of template, and incubation at 37°C was continued for 5 minutes. The reaction was stopped by the addition of both $NaDodSO_4$ (0.5% w/v) and EDTA (25mM). The reaction mixture was then treated with RNAse (20μg/ml)

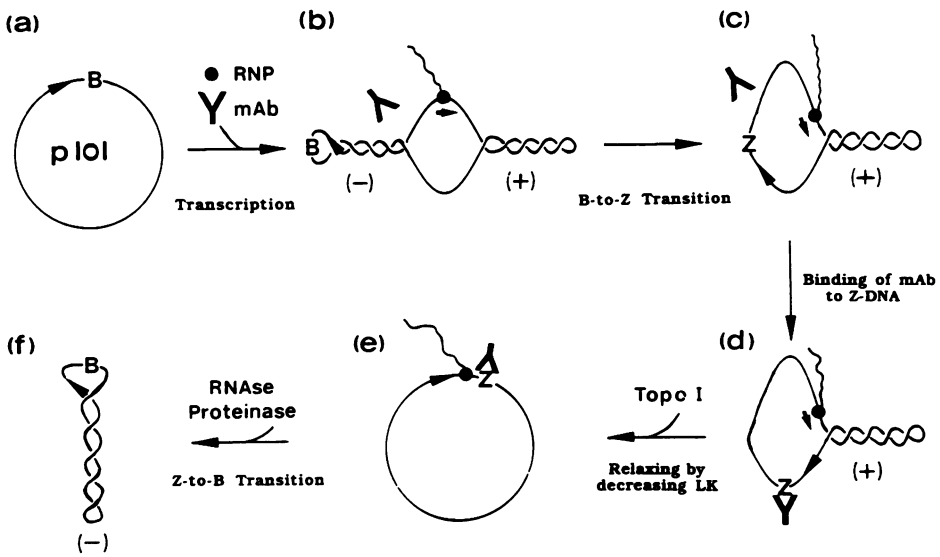

Figure 1. Schematic representation of the experimental strategy to test for a transcription-driven B-to-Z transition. A detailed description of the experimental outline is given in the Introduction. The double-stranded circular template, p101, is represented by a single-lined circle, where the arrowhead marks the position of the bacteriophage T7 promoter. The stretch of $d(CG)_{16}$ in the right-handed conformation and its position relative to the promoter is indicated by the letter B. In the left-handed conformation, however, the stretch is marked by the letter Z. Once the T7 RNA polymerase (RNP; ·) is transcribing the template in the presence of monoclonal antibody Z-D11(mAb ; Y), the double helix axis might start to rotate around itself. This will generate domains of positive (+) supercoiling in front of and negative (−) supercoiling behind the enzyme. The small arrow beneath the elongating polymerase indicates the direction of transcription. In this illustration, the transcription-induced supercoils are shown in a plectonemic, interwound configuration. It is equally likely that they could exist in an alternative solenoidal configuration.

for 30 minutes followed by proteinase K digestion (20μg/ml) for additional 20 minutes at 37°C. DNA was purified by phenol/chloroform extraction and ethanol precipitation.

The 'T7 RNP-footprint' of monoclonal antibody on templates bound to protein A-Sepharose CL4B (Fig.5) was performed as follows: After the primary transcription reaction to induce a structural transition recognized and stabilized by antibody binding and the subsequent topoisomerase treatment, the reaction mixture was incubated with 20μl pre-swollen protein A-Sepharose-CL4B (Pharmacia) for 15 minutes at room temperature with occasional agitation to keep the resin in solution. The resin was then washed with 20 volumes of 40mM Tris-HCl (pH8.0), 8mM $MgCl_2$, 50mM NaCl, 25mM DTT, and 2mM spermidine. After 5 to 7 washing cycles in a batch procedure, the resin was resuspended in 50μl of the same buffer and nucleoside triphosphates were added to a final concentration of 0.3mM, except for GTP which was kept at 0.05mM along with 10μCi of α[^{32}P] GTP (Amersham). After the addition of 50units T7 RNP and 10units RNAsin, the mixture was incubated for the secondary transcription reaction at 37°C for 15 minutes, and transcription was stopped by the addition of 0.5% (w/v) $NaDodSO_4$. Nucleic acids were purified by proteinase K digestion, phenol/chloroform extraction, and finally ethanol precipitation in order to remove unincorporated NTPs (6).

Gel electrophoresis

The analysis of transcription products was essentially as described (11) using denaturing polyacrylamide gels containing 8M urea. Topoisomer distributions were displayed in 0.8% agarose gels which were run in TAE-buffer (40mM Tris-acetate, pH8.1; 2mM EDTA). To achieve an optimal separation of topoisomers, the TAE-buffer contained chloroquine (Sigma) at a final concentration of 1.5μM. DNA was visualized by ethidium bromide staining. It should be noted that the topoisomers migrate as positively supercoiled DNA. Thus the fastest migrating species are characterized by higher linking numbers than the slower migrating forms.

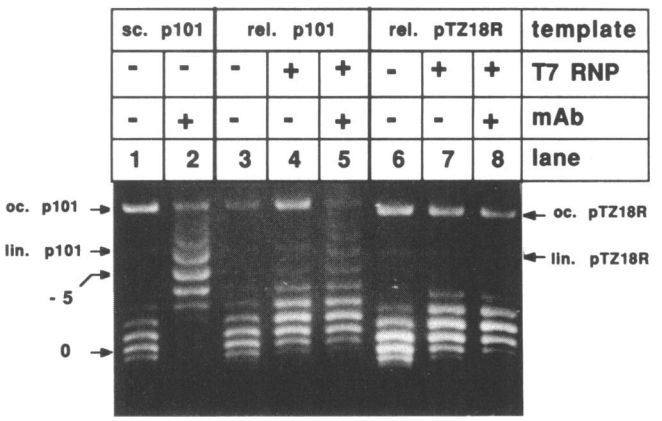

Figure 2. A reduction in template linking number indicates a B-to-Z transition in the $d(CG)_{16}$ stretch. Topologically unconstrained plasmids p101 (rel. p101) and pTZ18R (rel. pTZ18R) are transcribed by T7 RNP in the presence (lanes 5 and 8) or absence (lanes 4 and 7) of monoclonal antibody (mAb) Z-D11. In addition, both templates were incubated under identical conditions except that T7 RNP was omitted from the reaction mixture (lanes 3 and 6). To identify a reduction in the linking number due to a B-to-Z transition, negatively supercoiled p101 (sc. p101) was incubated under identical conditions in the presence (lane 2) or absence (lane 1) of monoclonal Z-D11. After transcription, the samples were further incubated with topoisomerase type I, and treated with RNAse and proteinase K. DNA was purified and prepared for gel electrophoresis which was carried out in the presence of chloroquine to achieve maximum separation. The topoisomers migrate as positively supercoiled DNA and are visualized by ethidium bromide staining. The arrows accompanied by numbers on the left side of the photograph indicate the linking differences of the respective topoisomers. oc., Open circular; lin., linear.

RESULTS

A reduction in template linking number indicates a transcription-induced B-to-Z transition

To identify a change in template Lk induced by a B-to-Z transition, as depicted in Figure 1, p101 at a 'native' superhelical density ($\sigma = -0.07$) is incubated in a control experiment either in the presence or absence of mAb Z-D11. The antibody is bound for 15 minutes under conditions identical to those used for *in vitro* transcription, after which the DNA is relaxed to completion by the addition of topoisomerases. Due to its high affinity binding the antibody can stabilize left-handed DNA during topoisomerase treatment (8,9). After deproteinization, however, the Z-DNA in p101 flips back into the right-handed B conformation thereby inducing the formation of about five compensatory (−) superhelical twists (Fig. 2, lane 2) (10). In comparison, DNA that is relaxed in the absence of mAb Z-D11 cannot retain the $d(CG)_{16}$ stretch in the left-handed conformation during topoisomerase treatment and thus migrates as a population of completely relaxed topoisomers with a linking difference centered around 0 (Fig. 2, lanes 1).

The experimental strategy depicted in Figure 1 is tested by the following experiment: Negatively supercoiled p101 is first relaxed to completion by wheat germ topoisomerase to remove all Z-DNA segments. After purification, the templates are incubated with a molar excess of T7 RNP in the absence or presence of mAb Z-D11 for 15 minutes. The transcription period is followed by further incubation with wheat germ topoisomerase for 5 minutes, after which the reaction is stopped by the addition of

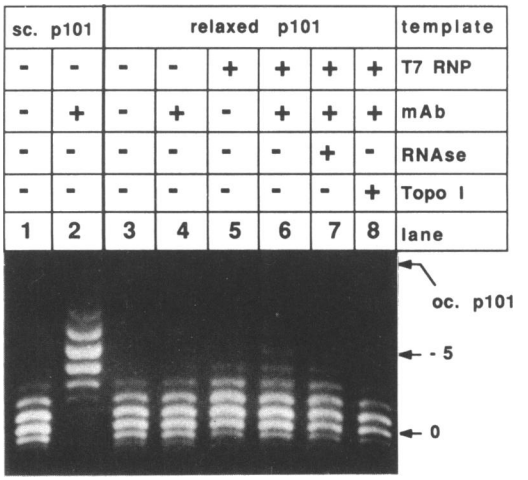

Figure 3. Characterization of the B-to-Z transition. To investigate the transcription-induced structural transition in more detail, relaxed p101 was incubated in the presence or absence of various agents as indicated (lanes 3 to 8). The control for a reduction in template linking number induced by a structural transition (lanes 1 and 2) is identical to that shown in Figure 2 and obtained with (−) supercoiled p101. oc., Open circular; T7 RNP, T7 RNA polymerase; mAb, monoclonal Antibody; Topo I, wheat germ topoisomerase type I.

$NaDodSO_4$. The result of such an experiment (Fig. 2, lane 5) shows that a decrease in template linking number, comparable to the control in lane 2, is indeed observed in a subset of template molecules when p101 is transcribed and subsequently treated with topoisomerase in the presence of mAb Z-D11. This shift in topoisomer distribution is not observed when the antibody was absent during the reactions (lane 4). Furthermore, this shift is also not detectable when parent plasmid pTZ18R, lacking the stretch of $d(CG)_{16}$, is utilized either in the absence or presence of Z-D11 (Fig. 2, lanes 7 and 8). We take these results to indicate that the antibody specifically recognizes and stabilizes a B-to-Z transition of the $d(CG)_{16}$ stretch in a subset of transcriptionally active p101 molecules.

We noticed that the linking number in both DNAs, p101 and pTZ18R, is reduced, on average, by about one when the templates are transcribed and subsequently treated by wheat germ topoisomerase (compare lane 3 with 4, and lane 6 with 7). One reason for this linking number change induced by transcription alone could be a limited duplex unwinding upon formation of an open complex. This was first observed with *E.coli* RNA polymerase, where it was found that about 15 base-pairs in the template are melted in an open complex (12). More complex mechanisms, however, are possible. For example, specific wrapping of the template around the enzyme might be compensated by positive torsional strain outside of the nucleoprotein complex thus generating a substrate for topoisomerases.

The appearance of a fraction of topoisomers exhibiting a reduction in template Lk is indicative of a structural transition as a consequence of transcription. This is directly demonstrated in Figure 3, where the incubation of the antibody with the template alone is not sufficient to cause a decrease in Lk upon topoisomerase treatment (compare lanes 3 and 4). In contrast, when the antibody is present during transcription, topoisomers with reduced linking numbers become again detectable (compare lanes 5 and 6). This result demonstrates that unconstrained p101 does not *per se* contain a stretch of left-handed DNA which can be recognized by mAb Z-D11, nor does the antibody itself induce such a structural transition during 15 minutes of incubation with p101. Furthermore, the presence of RNAse (lane 7) or wheat germ topoisomerase type I (lane 8) during transcription completely prevents the observed change in template Lk.

We conclude that levels of unconstrained (−) supercoiling sufficient to drive a B-to-Z transition in a template are generated in the wake of a transcribing T7 RNP. The reversal of this effect by RNAse reveals this transition process to be critically dependent on the presence of a minimal transcript length, in agreement with a previous study where the generation of oppositely supercoiled domains by T7 RNPs was only detectable when the transcript length exceeded about 7000 nucleotides (2).

Mapping of antibody binding in a sub-population of templates

To demonstrate that the antibody is specifically bound to a sub-population of topoisomers, unconstrained p101 was transcribed in the presence of mAb Z-D11, followed by topoisomerase treatment exactly as described above. A fraction of the reaction mixture was removed and the DNA analyzed by gel electrophoresis (Fig. 4, lane 3). As above, it is obvious that a sub-population of templates in this sample exhibits a reduced

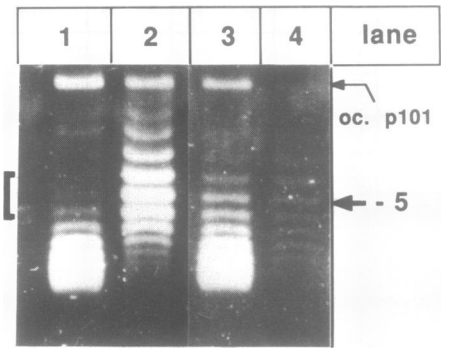

Figure 4. Isolation of antibody-DNA complexes by immuno-affinity chromatoragphy. Plasmid p101 was incubated with T7 RNA polymerase, followed by topoisomerase treatment as depicted in Figure 1. A fraction of the reaction mixture was removed and the topoisomer distribution analyzed (lane 3). The remainder of the sample was incubated with protein A-Sepharose and, after adsorption and excessive washing, the resin-bound nucleoprotein complexes were proteinase K digested and analyzed on the same gel (lane 4). The controls in lane 1 and 2 are identical to those described in Figure 2. The bracket on the left side of the Figure highlights the position of the relevant topoisomers with linking differences between −3 and −7. oc., Open circular.

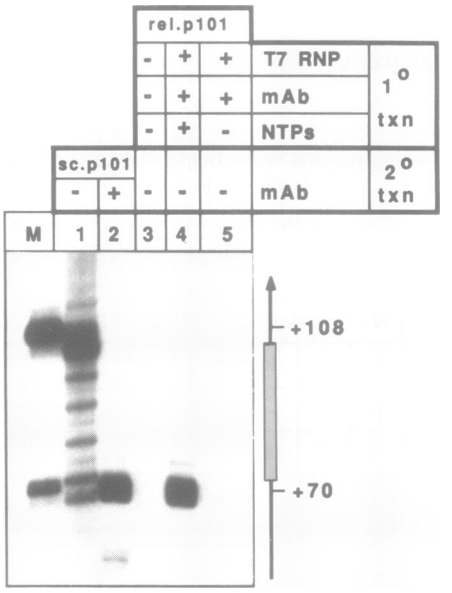

Figure 5. Mapping of the antibody binding site on transcriptionally active p101. In order to localize the binding site of monoclonal antibody (mAb) Z-D11 on templates with a reduced linking number, the experimental outline depicted in Figure 1 was followed with relaxed p101 (rel. p101). The template was incubated in a primary transcription reaction (1° txn) either alone (lane 3), or in the presence of T7 RNA polymerase, mAb Z-D11, and NTPs (lane 4). In a negative control (lane 5), the addition of NTPs was omitted in order to prevent transcription during the primary reaction. The samples were then incubated for additional five minutes with excess topoisomerase, after which the resin was added. After multiple washing steps, the resuspended resin from each sample was incubated for the secondary transcription reaction (2° txn) with freshly added T7 RNP and NTPs, including $\alpha[^{32}P]$ GTP. Transcription products were isolated, prepared for denaturing gel electrophoresis and visualized by autoradiography. As controls, (−) supercoiled p101 (sc.p101) was incubated with (lane 2) or without (lane 1) mAb Z-D11 and subsequently transcribed by T7 RNP during secondary transcription reaction in the presence of labeled NTPs. To identify the position of the $d(CG)_{16}$ stretch in p101 by transcript length, the template was partially digested with BamH I, which cuts at positions corresponding to both B-to-Z junctions. These run-off transcripts are +70 and +108 nucleotides in length (lane M). This is indicated on the right side of the autoradiogram, where the stippled vertical box marks the position of the stretch of left-handed DNA.

linking number (compare the distribution of the non-transcribed templates in lane 1 with that in lane 3).

The remainder of the same sample was incubated with protein A-Sepharose resin to isolate the molecules complexed with the anti-Z-DNA antibody. After excessive washing, the resin-bound DNA was purified and its topoisomer distribution analyzed. Compared to the bulk of DNA shown in lane 3, most templates that bind preferentially to the immuno-affinity resin exhibit a reduced template linking number (lane 4), which resembles that obtained by relaxing (−) supercoiled p101 after antibody binding (lane 2). We have quantitated using densitometry the topoisomer distribution in different experiments of the type shown in Figures 2 and 4. We estimate that about 10% of the templates can undergo a structural transition due to transcription.

In order to identify the antibody binding site(s) in the resin-bound fraction of templates, we tested whether transcription can be blocked at the site where the antibody should be bound. For this purpose, an experiment identical to that described in Figure 4 was repeated, whereby T7 RNP plus mAb Z-D11 were incubated with relaxed p101 in a primary transcription reaction (1° txn). After topoisomerase treatment, the antibody-complexed molecules are isolated using protein A-Sepharose. The purified resin is then incubated in a secondary transcription reaction (2° txn) with freshly added T7 RNP, where the transcripts generated on the resin-bound templates are labeled by incorporation of $\alpha[^{32}P]$GTP.

Analysis of these transcripts reveals a strong transcriptional block at the promoter-proximal B-to-Z junction around position +70 (Fig. 5, lane 4; the positions of both B-Z junctions is shown in lane M and indicated on the right side of the autoradiogram). In contrast, no antibody-mediated block of transcription is detectable in the 2° txn when transcription and template supercoiling during 1° txn are prohibited due to the absence of NTPs (lane 5). The position where T7 RNPs appear to terminate transcription in lane 4 is identical to that observed when mAb Z-D11 is bound to the Z-stretch in (−) supercoiled p101 prior to the 2° txn (lane 2). The influence of Z-DNA alone on transcription is shown in lane 1. In agreement with our previous observations (6), the main obstacles for a sub-population of elongating T7 RNPs are the two B-to-Z junctions and, to a lesser extent, the Z-stretch itself. From these results we conclude that mAb Z-D11 is bound to the $d(CG)_{16}$ stretch in a fraction of transcriptionally active and topologically unconstrained templates. In addition, antibody binding is strictly dependent on preceding transcription processes, which agrees with the results presented in Figure 3.

DISCUSSION

In this report we present two lines of evidence that a change in conformation from B- into Z-DNA can occur when topologically unconstrained templates are transcribed by T7 RNP *in vitro*. First, the alternate DNA conformation becomes detectable by a reduction in template linking number due to its stabilization by anti-Z-DNA antibodies during topoisomerase treatment. This reduction in Lk is qualitatively similar to that seen with (−) supercoiled p101. Second, we show by transcriptional blockage of T7 RNP that the monoclonal antibody is indeed bound to a position corresponding to the $d(CG)_{16}$ stretch in templates with a reduced Lk, and we demonstrate that this binding is strictly dependent on a preceding transcription process.

By incubation of the antibody with the template in the absence of T7 RNP (Fig. 3), we excluded the possibility that a fraction of templates, although topologically unconstrained, already contained the purine-pyrimide stretch in an alternate conformation. Furthermore, we could demonstrate in this experiment that the presence of the antibody does not induce a conformational transition; nor does it shift the equilibrium towards the Z-conformation in relaxed p101 during the 15 minutes incubation. This was observed in a previous study using linear or open circular DNAs and different anti-Z-DNA antibodies, although this effect was seen after much longer incubation periods (9).

We estimate that, under our conditions, a structural transition occurs in about 10% of the templates present in the reaction mixture. This proportion might in fact be much higher when related to active templates, since it seems likely that only a subset of DNA molecules are continuously transcribed. Previous studies have shown that a B-to-Z transition occurs in a stretch of $d(CG)_{16}$ around a superhelical density of −0.033, and furthermore that mAb Z-D11 can bind to the Z-stretch at this specific linking difference (6,10). It appears, therefore, that a fraction of templates contain such a degree of (−) supercoiling. This must have been transiently built up behind the translocating enzyme during the 15 minutes transcription period. However, the level of (−) supercoiling behind the RNP is likely to be insufficient to induce Z-DNA formation in all active templates. This could be due, for example, to premature termination of transcription, which would negatively affect the steady-state level of transcription-induced template supercoiling.

Detailed kinetic studies have revealed that the rate for a B-to-Z transition shows a broad range depending on the degree of (−) supercoiling (8,13). In a plasmid at a relatively high superhelical density (−0.07), the transition from B- to Z-DNA can occur in less than a few minutes. However, in plasmids at a moderate superhelical density (−0.05), the transition rate is slowed down by a factor of about 10. Based on the length of our transcription reaction, the actual level of (−) supercoiling generated by transcription is likely to exceed −0.033. This implies that a diffusion of (−) and (+) supercoils, which would lead to the cancellation of oppositely supercoiled domains in a circular template, is considerably slower than their generation. Thus, our experiments support the idea that the superhelical twisting of the template behind and in front of a translocating protein is localized in close proximity to the enzyme (2). The supercoils might, in fact, be crowded into only small domains of the template, which would consequently increase the local superhelical densities in these regions. Our system could be used to determine the maximal level of transcription-induced (−) supercoiling by varying the length of the potential Z-DNA stretch. It has been shown that the length dependence for B-to-Z transitions in stretches of $d(CG)_n$ is inversely related to the level of (−) supercoiling (14). However, it is also possible that a translocating polymerase interferes more directly with transitions in DNA conformation by, for example, its strand separation activity. This could very likely play a role in a transcription-induced formation of a different type of alternate DNA conformation, namely a cruciform, which requires the transient melting of base-pairs (for a review see ref. 15).

Our results demonstrate for the first time that a B-to-Z transition can indeed occur in actively transcribed templates *in vitro*, as originally postulated by Liu and Wang (1,2). There is increasing evidence that under certain conditions transcription-driven local changes in DNA conformation might also occur *in vivo* (see for

examples refs. 16,17). However, the situation *in vivo* is more complex because of the cellular environment and the abundance of topoisomerases able to release torsional strain. Indeed, we demonstrate that the presence of excess topoisomerase during the transcription period (Fig. 3) prevents the $d(CG)_{16}$ stretch from adopting the left-handed conformation. Thus, once (−) supercoiling starts to build up in the wake of a transcribing polymerase, it could either be relaxed by topoisomerases or serve as an energetic drive for structural transitions in appropriate regions. Such a scenario is supported by our recent finding that the enzymatic activities of both RNA polymerase II and topoisomerase type I are quantitatively linked (18). Important parameters involved in transcription-induced structural transitions *in vivo* might be the relative abundance of topoisomerases, which varies during the cell cycle (19), and the accessibility of the templates to enzymatic attack.

The relaxation time for a B-to-Z transition in $d(CG)_{16}$ has been measured to be in the range of 10 to 15 minutes (8). In order to play a biological role, the stability of Z-DNA in topologically unconstrained DNA has to be increased. This is achieved by the anti-Z-DNA antibody in our *in vitro* model system. The identification and characterization of Z-DNA binding proteins is important to elucidate potential physiological roles of Z-DNA. Since the transition in DNA conformation is accompanied by a change in DNA supercoiling, an additional role for an alternate DNA conformation, induced by transcription or other protein tracking processes (20, 21), could also include the topological communication between different DNA segments (for a review, see ref. 22).

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