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# NONHISTONE PROTEIN HMG1 REMOVES THE TRANSCRIPTIONAL BLOCK CAUSED BY LEFT-HANDED Z-FORM SEGMENT IN A SUPERCOILED DNA

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The effect of HMG1 on the transcriptional block caused by left-handed Z-form in a highly negatively supercoiled DNA was examined using a supercoiled plasmid containing a $(CG)_{10}$ sequence downstream of promoters. The transcription by E. coli RNA polymerase was blocked at the boundary of alternating CG sequence in the Z-form. In the presence of HMG1, RNA polymerase could transcribe through the CG sequence resulting in the chain elongation of transcripts. The addition of HMG1 allowed the stalled RNA polymerase at the CG block to resume transcription. These suggest that HMG1 may remove the Z-block by flipping it back into the B-form. 

HMG1 and HMG2 are major nonhistone components in chromatin. The pig thymus HMG1 has the molecular weight of 24,785 deduced from the nucleotide sequence of the cDNA (1). In vitro, HMG1 and HMG2 have a preferential affinity for single-stranded DNA (2-5), destabilize or unwind DNA double helix (4,6-8), induce superhelicity in covalently closed circular DNA (6,9,10), and suppress in vitro nucleosome assembly (10). There are a few indications of their involvement in vivo in transcription or DNA replication (11).

In vitro, RNA polymerase could transcribe through an alternating CG sequence when it was in the B helical form, while the transcription was blocked when the template was negatively supercoiled to flip the CG sequence to the left-handed Z-form. In vivo, however, the sequence could be transcribed through, suggesting that there is some mechanism or factor to remove the Z-DNA block of transcriptional elongation in the cell (12). Here, using negatively supercoiled plasmid containing a $(CG)_{10}$ sequence downstream of the promoters, we show that HMG1 may function to remove the transcriptional block caused by left-handed Z-helical structure to allow the stalled RNA polymerase to continue transcription.

## MATERIALS AND METHODS

HMG1 preparation: HMG1 was prepared from pig thymus chromatin as described previously (8).

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Plasmid construction: The alternating CG sequences in various lengths were prepared by the self annealing of 5'-phosphorylated d[CGCGCGCGCG] (Pharmacia), followed by partial self-ligation. The sequences were inserted into the ScaI site of pBR322 DNA and transformed into E. coli HB101. A pBR322 DNA recombinant pBR322-$(CG)_{10}$, containing an alternating CG sequence of 20 bp in length, was cloned. The plasmid DNA was prepared as described previously (13,14).

Preparation of negatively supercoiled topoisomers: Plasmid pBR322-$(CG)_{10}$ circular topoisomers with increasing numbers of negative superhelical turns were prepared by adding various amounts of ethidium bromide to aliquots of plasmid in the presence of pig thymus DNA topoisomerase I. For the calculation of negative superhelical density, the linking difference of topoisomers ($\alpha$-$\alpha_0$) was obtained by band-counting methods (15); in which is the linking number for topoisomer, and $\alpha_0$ for form Ir DNA. The negative superhelical density ($-\sigma$) is then defined as specific linking difference: $-\sigma = -(\alpha-\alpha_0)/\alpha_0$.

Two-dimensional gel electrophoresis of DNA topoisomers: A mixture of negatively supercoiled DNA topoisomers was electrophoresed on a 1 % agarose gel. Then, after soaking in chloroquine, the gel was re-electrophoresed in the orthogonal direction and stained with ethidium bromide (16).

Transcription reaction and electrophoretic analysis of the transcripts: The transcription reaction mixture (50 µl) contained 30 mM Tris-HCl (pH 7.8), 50 mM KCl, 10 mM $MgCl_2$, 0.2 mM dithiothreitol, 0.1 mM EDTA, 50 µg/ml gelatin, 200 µM ATP, 200 µM GTP, 200 µM UTP and 500 µM CTP with 2 µCi of [$\alpha$-$^{32}$P]CTP, 5 µg heparin and 0.1 unit E. coli RNA polymerase holoenzyme (Pharmacia). After preincubation of 0.5 µg template DNA with RNA polymerase at 37 C° for 10 min, 2 µg HMG1 dissolved in 50 mM KCl, 30 mM Tris-HCl (pH 7.8) was added and the mixture was kept for 10 min. Reaction was initiated by the addition of nucleotide triphosphates containing [$\alpha$-$^{32}$P]CTP. After 2 min heparin was added, followed by the addition of 500 µM CTP after another 5 min. The complete reaction mixture was incubated for 10 min. The reaction was stopped by the addition of an equal volume of phenol, followed by extraction with chloroform and by precipitation with 3 volumes of ethanol. Ethanol-precipitated samples dissolved in 90 % formamide, 44.5 mM Tris-borate (pH 8.0), 1.25 mM EDTA, 0.025 % BPB, 0.025 % xylene cyanol, were electrophoresed on a 6 % polyacrylamide gel containing 8.3 M urea and then autoradiographed. The approximate nucleotide size of transcript was obtained from the separate electrophoresis on a 6 % polyacrylamide gel containing 98 % formamide (17).

For the time-course analysis of transcript in the presence of HMG1, pBR322-$(CG)_{10}$ DNA was preincubated with RNA polymerase for 5 min in a standard reaction mixture. The reaction was initiated by the addition of nucleotide triphosphates. After 2 min 1.2 µM rifampicin was added, followed by the addition of CTP after another 2 min. Then, after 5 min HMG1 was added and incubated for 10 min. The reaction mixture separated at various times before and after the addition of HMG1, was added with EDTA and SDS for stopping the reaction. The transcript was analyzed as described above.

## RESULTS AND DISCUSSION

To examine the effect of a left-handed Z-helical structure on transcription, we utilized the negative supercoiling of template plasmid pBR322-$(CG)_{10}$ to induce the B to Z transition of an alternating CG sequence of 20 bp positioned at the ScaI site (3846) on pBR322 DNA (18), shown in Fig. 1(A). The supercoiling-induced change in DNA helical structure can be evaluated directly from a two-dimensional gel pattern. As shown in Fig. 1(B), a discrete B-Z transition became apparent at a linking difference of -15 in the topoisomer distribution of pBR322-$(CG)_{10}$. Thus, the negative superhelical density ($-\sigma$) of 0.035 is the approximate turning point of B-Z transition of

A B

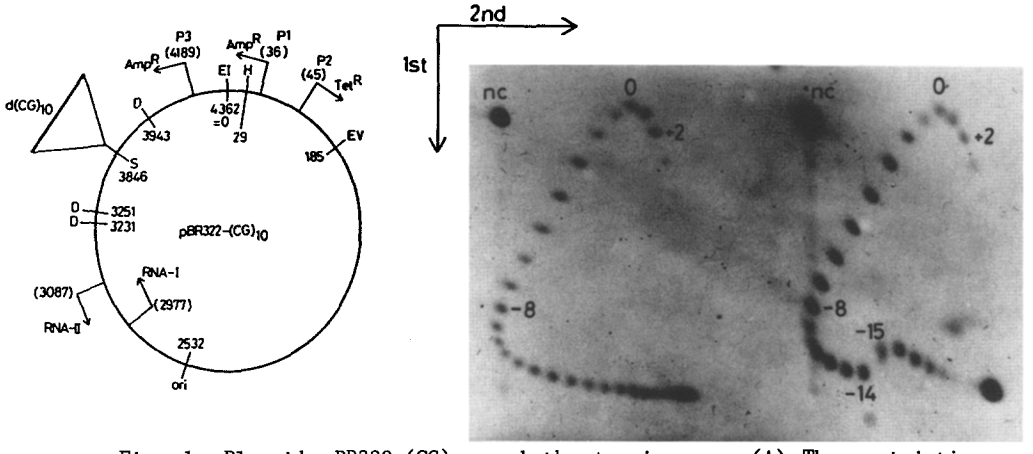


Fig. 1. Plasmid pBR322-$(CG)_{10}$ and the topoisomers. (A) The restriction sites and transcription maps. S, D, H, EI and EV denote the ScaI, DraI, HindIII, EcoRI and EcoRV restriction sites, respectively. Arrows indicate the directions of five transcripts. The nucleotide position number of each initiation site is indicated in parentheses. Numbering on the pBR322 DNA sequence, except for the $(CG)_{10}$ sequence inserted, is according to Sutcliffe (18). (B) Two-dimensional agarose gel electrophoretic patterns of a mixture of topoisomers of pBR322-$(CG)_{10}$ (right) and that of pBR322 (left). The directions of electrophoresis are indicated in the figure. nc denotes the nicked circular (form II) plasmid. The linking differences ( $\alpha - \alpha_0$ ) of selected topoisomers are indicated in the figure.

the topoisomers; the alternating CG sequence in the topoisomers of $-\sigma$ above 0.035 is in the Z-helical structure.

Fig. 2 shows the distribution of transcript by E. coli RNA polymerase holoenzyme using pBR322-$(CG)_{10}$ and pBR322 DNA templates with $-\sigma$ of 0.011, 0.057 and 0.12. Both the native form I plasmid DNAs have the $-\sigma$ of 0.057 (not shown). Under these conditions, the autoradiogram of transcribed RNAs yielded several major bands. When pBR322-$(CG)_{10}$ DNA was used as a template, the transcripts of 490 and 320 nucleotides were abundant for the template with $-\sigma = 0.057$ and 0.12 (lanes 5 & 6), but neither detected for the template with $-\sigma = 0.011$ (lane 4) nor for pBR322 DNA template (lanes 2 & 3), showing that these transcripts resulted from the insertion of alternating CG sequence. At least five promoters in pBR322 can be activated to give the majority of specific transcript in vitro by E. coli RNA polymerase holoenzyme (Fig. 1(A)). The $Amp^R$ gene has two promotors of P1 and P3 (19). The formation of Z-form structure causes the blockage of transcriptional elongation (12). Therefore, the transcription initiated at position 36 (for P1) and 4189 (for P3) and terminated near the proximal end of alternating CG sequence in the Z-form, may yield the nascent transcripts of 553 and 343 nucleotides or of a little shorter by piling up of earlier polymerases or nascent transcripts (12).

To confirm this, an experiment using pBR322 DNA linearized with restriction enzymes as a template was arranged. The DNA template linearized with ScaI, which was used for the insertion of a $(CG)_{10}$ sequence, gave a marked stimulation of RNAs similar in size to transcripts by the expected CG blockage

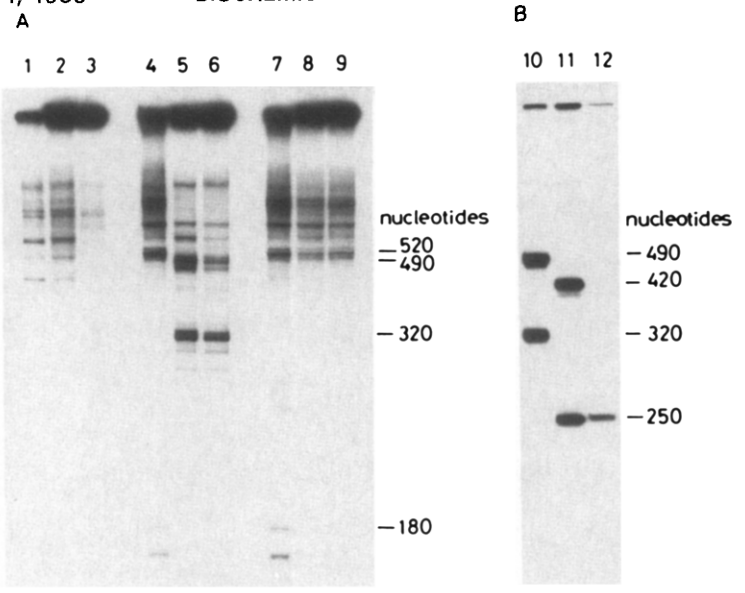


Fig. 2. Autoradiogram of transcription products of pBR322-$(CG)_{10}$ after separation on a denaturing sequence gel. (A) Effect of HMG1 on the transcription with the templates of different negative superhelical density ($-\sigma$). The transcription was templated on pBR322-$(CG)_{10}$ in the absence (lanes 4-6) or presence (lanes 7-9) of HMG1, and on pBR322 in the absence of HMG1 (lanes 1-3). The $-\bar{\sigma}$ of template is 0.011 (lanes 1,4 & 7), 0.057 (lanes 2,5 & 8) and 0.12 (lanes 3, 6 & 9). The nucleotide sizes of major transcripts referred to the length of denatured DNA restriction fragments are indicated on the right of the figure. (B) The transcripts templated on the linearized pBR322 DNA. Linearized template DNAs were prepared by digesting pBR322 DNA with ScaI (lane 10), DraI (lane 11), and HindIII + DraI (lane 12). The transcription was carried out in the absence of HMG1.

(Fig. 2(B), lane 10). The transcripts templated on the DNA linearized by DraI and HindIII + DraI (lanes 11 & 12) supported these estimations. Thus, E. coli RNA polymerase can transcribe through the alternating CG sequence when it is in the B-form. However, when plasmid template is sufficiently negatively supercoiled such that the CG sequence flips to left-handed Z-form, E. coli RNA polymerase and its nascent transcript may stop at the roadblock created by the Z-DNA.

The effect of HMG1 on the transcription templated on the pBR322-$(CG)_{10}$ DNA is shown in Fig. 2(A). As a whole, the autoradiograms for template with $-\sigma \approx 0.057$ and 0.12 (lanes 8 & 9) were similar to those for template with $-\sigma = 0.011$ in the presence (lane 7) or absence (lane 4) of HMG1. The transcripts of 490 and 320 nucleotides were not observed for the template with $-\sigma \approx 0.057$ and 0.12 in the presence of HMG1, whereas the products of 520 nucleotides and of higher molecular size were observed. These results suggest that E. coli RNA polymerase can transcribe through the alternating CG sequence in negatively supercoiled DNA in the presence of HMG1; HMG1 may flip back the alternating CG sequence in the Z-form to the B-form structure. To confirm this, an experiment was performed whereby the polymerase was first stopped at the Z-DNA block followed by the inhibition of re-initiation reaction by the

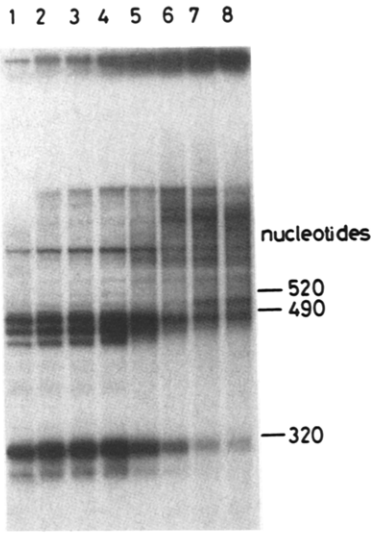


Fig. 3. Autoradiogram of transcription products of pBR322-$(CG)_{10}$ before and after the addition of HMG1. An aliquot of the reaction mixture was removed for the analysis at 10 sec, 1, 2, and 5 min of the transcription reaction in the absence of HMG1 (lanes 1-4) respectively, or at 10 sec, 1, 5, and 10 min of the reaction after the addition of HMG1 (lanes 5-8) respectively.

addition of rifampicin. HMG1 was then added to the reaction mixture to test whether it removes the Z-block. If the nascent transcripts are not released at the Z-block, continued transcription through the new right-handed alternating CG sequence should resume until a transcriptional termination site is encountered. The transcripts produced from supercoiled pBR322-$(CG)_{10}$ DNA ($-\sigma = 0.057$) after increasing incubation times are shown in Fig. 3. The pattern hardly changed for the incubation times between 10 sec and 5 min (lanes 1-4); the predominant transcripts were RNAs of 490 and 320 nucleotides. When HMG1 was added, these RNAs were rapidly chased into the RNAs of 520 nucleotides and the longer transcripts (lanes 5-8). Thus, in the presence of HMG1, the CG sequence even in the increased negative density may be in the B-form structure; the removal of Z-form block by HMG1 allows the stalled RNA polymerase to continue transcription.

HMG1 and HMG2 can distinguish between single- and double-stranded DNA (2-5). They preferentially bind to the nuclease S1 sensitive structure such as cruciform and B-Z junction present in the negatively supercoiled form I DNA (5). They can destabilize or unwind DNA double helix (4, 6-8). On the basis of these findings, it is likely that HMG1 binds to the single-stranded DNA region of B-Z junction in the negatively supercoiled DNA in the first instance. The cooperative binding of HMG1, then, unwinds the alternating CG sequence and the neighboring B-formed region resulting in the consequent flip back of the alternating CG sequence from Z to B form. RNA polymerase may transcribe the B-formed DNA strand in spite of the presence of HMG1 to elongate the chain length terminating in the intrinsic termination code.

In contrast to the transcriptional block by the Z-helical structure of alternating CG sequence in vitro, transcription inside the E. coli cell can proceed through the CG sequence. The simplest interpretation of this result is that inside the E. coli cell the CG sequence is in the right-handed B-form and therefore provide no impediment for transcriptional elongation (12). HMG1-like proteins contained in the E. coli cell may have a similar function to those of HMG1 presented here. The eukaryotic DNA also contains the alternating purine-pyrimidine sequences readily formative of left-handed Z-helical structure in negative supercoiling due to the torsional stress by DNA anchoring on the nuclear matrix structure (20). In vivo, HMG1 may function to remove such a Z-block to elongate the chain length in the transcription reaction.

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REFERENCES

1. Tsuda, K., Kikuchi, M., Waga, S., and Yoshida, M. (1987) Submitted.
2. Bidney, D.L., and Reeck, G.R. (1978) Biochem. Biophys. Res. Commun. 85, 1211-1218.
3. Isackson, P.J., Fishback, J.L., Bidney, D.L., and Reeck, G.R. (1979) J. Biol. Chem. 254, 5569-5572.
4. Yoshida, M., and Shimura, K. (1984) J. Biochem. 95, 117-124.
5. Hamada, H., and Bustin, M. (1985) Biochemistry 24, 1428-1433.
6. Javaherian, K., Sadeghi, M., and Liu, L.F. (1979) Nucleic Acids Res. 6, 3569-3580.
7. Makiguchi, K., Chida, Y., Yoshida, M., and Shimura, K. (1984) J. Biochem. 95, 423-429.
8. Yoshida, M. (1987) J. Biochem. 101, 175-180.
9. Mathis, D.J., Kindelis, A., and Spadafora, C. (1980) Nucleic Acids Res. 8, 2577-2590.
10. Waga, S., Mizuno, S., and Yoshida, M (1987) Submitted.
11. Einck, L., and Bustin, M. (1985) Exp. Cell Res. 156, 295-310.
12. Peck, L.J., and Wang, J.C. (1985) Cell 40, 129-137.
13. Birnboim, H.C., and Doly, J. (1979) Nucleic Acids Res. 7, 1513-1523.
14. Maniatis, T., Fritsch, E.F., and Sambrook, J. (1982) Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory, New York.
15. Keller, W. (1975) Proc. Natl. Acad. Sci. USA 72, 4876-4880.
16. Peck, L.J., and Wang, J.C. (1983) Proc. Natl. Acad. Sci. USA 80, 6206-6210.
17. Maniatis, T., Jeffrey, A., and Van deSande, H. (1975) Biochemistry 14, 3787-3794.
18. Sutcliffe, J.G. (1978) Cold Spring Harbor Symp. Quant. Biol. 43, 77-90.
19. Brosius, J., Cate, R.L., and Perlmutter, A.P. (1982) J. Biol. Chem. 257, 9205-9210.
20. Mirkovitch, J., Mirault, M.-E., and Laemmli, U.K. (1984) Cell 39, 223-232.