DNA superhelicity affects the formation of transcription preinitiation complex on eukaryotic genes differently

Mitsuko Mizutani, Kiyoe Ura[1] and Susumu Hirose*
DNA Research Center, National Institute of Genetics and [1]Department of Genetics, The Graduate University for Advanced Studies, Mishima 411, Japan

Received March 12, 1991; Revised and Accepted May 8, 1991

ABSTRACT

***In vitro* transcription was reconstituted with HeLa cell transcription factors and RNA polymerase II, which were essentially free from DNA topoisomerase activities. DNA templates with defined negative superhelical densities were tested for transcription activity. Transcription of the *Bombyx mori* fibroin gene increases and plateaus from templates of increasing superhelicity, and transcription from the adenovirus 2 major late promoter rises and then falls, while transcription of the *Drosophila hsp70* gene remains unchanged. Dissection of transcription into pre and post-initiation steps by the use of Sarkosyl reveals that formation of a preinitiation complex on the fibroin gene or the adenovirus 2 major late promoter is slow on relaxed DNA and accelerated by DNA superhelicity. On the contrary, the preinitiation complex assembles rapidly on the *hsp70* gene irrespective of DNA topology. As is the case with the fibroin gene promoter, DNA superhelicity appears to facilitate the interaction of transcription factor IID to the adenovirus 2 major late promoter.**

INTRODUCTION

In prokaryotes, the superhelical state of DNA plays an important role in genetic processes including replication, transcription and recombination (1,2). Little is known about such a role in eukaryotes. Though chromatin DNA in eukaryotic cells is topologically underwound, bulk DNA is not under superhelical tension because the supercoils are constrained by histone-DNA interactions (3). This does not necessarily exclude the possibility that unconstrained supercoils are present in a small fraction of the chromatin and contribute to execution of DNA functions. Consistent with this notion, an extract prepared from posterior silk gland cells of *Bombyx mori* contains an activity which introduces unconstrained negative supercoils into closed circular DNA (4–6). Moreover, DNA supercoiling affects *in vitro* transcription of certain eukaryotic genes in this extract (5). Thus, transcription from the *B. mori* fibroin gene or the adenovirus 2 major late promoter (Ad2 MLP) is fully activated by partial supercoiling of DNA, whereas supercoiling has no effect on the transcription of *Drosophila hsp70* gene. Subsequent study shows that DNA supercoiling enhances the fibroin gene transcription by facilitating assembly of a transcription preinitiaion complex (6).

While these works demonstrated a profound effect of DNA supercoiling on the transcription of some eukaryotic genes, they had limitations. First, because supercoiling and transcription (or assembly of preinitiation complex) proceed simultaneously in the extract, we could not examine which is important the supercoiled state or the supercoiling process. Second, the extract contains a supercoiled DNA-relaxing activity as well as the supercoiling activity and the degree of supercoiling in the extract is determined by an equilibrium between them (4,5). Therefore, the supercoiling was always partial and we could not analyze how higher degree of supercoiling affects the transcription. Third, it was not known which transcription factor mediates the activation of transcription by supercoiling.

To overcome these limitations, we separated general transcription factors required for accurate initiation of transcription by RNA polymerase II from a HeLa cell nuclear extract. Reconstitution of a transcription system with these factors reveals that DNA supercoiling enhances the fibroin gene transcription by accelerating the interaction of transcription factor(TF)IID to the promoter (7). In the present study, we have improved the transcription system so that it is essentially free from DNA topoisomerase activities and compared transcription of DNA templates with various degrees of superhelicity. The results presented here show that the superhelical state of DNA but not the supercoiling process is important for the activation of transcription and that DNA superhelicity affects the formation of transcription preinitiation complex on eukaryotic genes differently.

MATERIALS AND METHODS

DNA template

The following plasmid DNAs were used as templates for transcription. pFb205 carries the *B. mori* fibroin DNA segment *Hin*dIII-*Pvu*II (from −860 to +728 relative to the cap site) in the *Hin*dIII-*Pvu*II fragment of pBR322 (4). pFb238 carries the fibroin DNA segment *Eco*RI*-*Bgl*II (−238 to +585) in the

* To whom correspondence should be addressed

*Eco*RI-*Bam*HI fragment of pBR322. pML(C_2AT) contains the Ad2 MLP sequence (−400 to +10) in front of the guanosine-free fragment inserted into pUC13 (8). pAd500 contains the Ad2 MLP sequence (−500 to +33) in the *Eco*RI-*Bam*HI fragment of pBR322 (5). p56H8RIA contains the *Eco*RI fragment of the *Drosophila hsp70* gene in the *Eco*RI site of pBR322(9).

Transcription components

HeLa cell TFIIB (DEAE-Sepharose fraction), TFIID (phosphocellulose fraction), and TFIIE (phosphocellulose fraction) were prepared from the nuclear extract as described (7) with the following modification. Transcription factors were eluted from a heparin-Sepharose column with 0.36M KCl in place of 0.4M KCl. Strong activity of DNA topoisomerase I (10) present in the extract was still retained on the column at 0.36 M KCl. For a heat treatment of TFIIE, 50-μl portions of the preparation of TFIIE were placed into 0.5ml Eppendorf tubes on ice, then incubated at 48°C for 20 min and then returned to ice. RNA polymerase II was purified from HeLa cell nuclear pellets as described by Reinberg and Roeder (11).

Other materials

Calf thymus DNA topoisomerase I was purchased from Bethesda Research Laboratories. 3′-0-methyl-GTP was from Pharmacia LKB Biotechnology Inc. Other unlabeled nucleotides, Sarkosyl (N-lauroyl sarcosine sodium salt), polyvinyl alcohol (average molecular weight 10,000), and Sl nuclease were from Sigma. [α−^{32}P]UTP was from Amersham Corp.

DNA topoisomers

Topoisomers of plasmid DNA with defined negative superhelical densities were prepared as described by Singleton and Wells (12) except that calf thymus topoisomerase I was used instead of wheat germ enzyme. The mean negative superhelical density, $-\sigma$, was determined as described (12). Agarose gel electrophoresis of the topoisomers in the presence or absence of chloroquine was carried out as described by Shure et al.(13).

Transcription reaction

The reaction mixture (12.5μl) contained 12mM Hepes-NaOH, pH7.9, 60mM KCl, 0.6mM EDTA, 7.5mM $MgCl_2$, 0.6mM dithiothreitol, 12% glycerol, 2% polyvinyl alcohol, 0.6mM each of ATP, CTP, and GTP, 10μM [α-^{32} P]UTP (8−16×10^4 cpm/pmol), 100ng of DNA, 1.5μl of TFIIB, 1.5μl of TFIID, 1.5μl of heated TFIIE, and 2 units (as defined in Ref. 14) of RNA polymerase II. In the case of transcription from pML(C_2AT) DNA, GTP was replaced by 0.1mM 3′-0-methyl GTP. The mixture was incubated at 30°C for 60 min.

Complex formation

Two-stage reaction (7) was employed to measure the amounts of preinitiation complex formed. Preincubation mixture (10μl) contained 15mM Hepes-NaOH, pH7.9, 75mM KCl, 0.75mM EDTA, 9.4mM $MgCl_2$, 0.75mM dithiothreitol, 15% glycerol, 2.5% polyvinyl alcohol, 100ng of DNA, 1.5μl of TFIIB, 1.5μl of TFIID, 1.5μl of heated TFIIE, and 2 units (as defined in Ref.14) of RNA polymerase II. After incubation at 30°C for various times, Sarkosyl and nucleotides in water were added in 2.5μl. The reaction mixture for the second incubation (12.5μl) contained 0.2mM each of ATP, CTP and GTP, 10μM [α-^{32} P] UTP (8−16×10^4cpm/pmol), and 0.01% Sarkosyl in addition to the ingredients carried from the preincubation. In the case of transcription from pML(C_2AT) DNA, GTP was replaced by 0.1mM 3′-0-methyl GTP. The mixture was incubated at 30°C for 10 min to allow single round of transcription.

Analysis of RNA

RNA sythesized was recovered as described previously (15). Transcript from pML(C_2AT) DNA was electrophoresed through polyacrylamide gel containing 7M urea under denaturing conditions as described (15). Transcripts from other plasmid DNAs were analyzed by the modified Sl nuclease assay as described (4). Phage DNA of f1Fb38(4), M13Ad4(5),and M13hsp70(5) were used as hybridization partners for the fibroin gene, the Ad2 MLP, and the *hsp70* gene transcripts, respectively. The specific transcripts were visualized by autoradiography and excised, and their radioactivities were measured.

RESULTS

Establishment of transcription system free from DNA topoisomerase activities

General transcription factors (TFIIB, TFIID and TFIIE) and RNA polymerase II were partially purified from HeLa cells to reconstitute a transcription system. During the purification, care was taken to avoid contamination with DNA topoisomerase I and hence these transcription components were virtually free from topoisomerase I activity (data not shown). However, the preparation of TFIIE contained DNA topoisomerase II activity

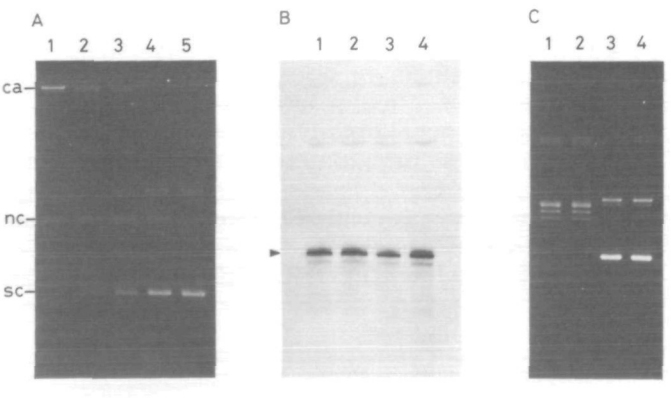

Figure 1. Selective inactivation of DNA topoisomerase II. **A**, heat inactivation of DNA topoisomerase II in the preparation of TFIIE. Supercoiled plasmid DNA of pML(C_2AT)(lane 5) was incubated with 1.5μl of TFIIE which had been heated at 48°C for 0 min (lane 1), 5 min (lane 2), 10 min (lane 3), or 20 min (lane 4). The incubation was carried out at 30°C for 60 min in the transcription mixture lacking TFIIB, TFIID, RNA polymerase II, and [α-^{32} P]UTP but containing unlabeled UTP. The reaction was terminated by the addition of EDTA, sodium dodecyl sulfate and proteinase K to 10mM, 0.5% and 1mg/ml, respectively. The mixture was incubated at 37°C for 30 min and electrophoresed in a 1% agarose gel. The gel was stained with ethidium bromide. sc, nc, and ca represent the positions of supercoiled circular DNA, nicked circular DNA, and catenanes, respectively. **B**, thermal stability of the TFIIE activity. *Hin*dIII-cut linear DNA of pML(C_2AT) was incubated at 30°C for 60 min in the transcription mixture containing 1.5μl of TFIIE which had been heated at 48°C for 0 min (lane 1), 5 min (lane 2), 10 min (lane 3), or 20 min (lane 4). The specific transcripts were analyzed as described under 'MATERIALS AND METHODS'. The arrowhead indicates the band of the correct transcript. **C**, topology of closed circular DNA after the transcription reaction. Relaxed closed circular DNA (lane 2) or supercoiled plasmid DNA of pML(C_2AT) (lane 4) was incubated at 30°C for 60 min in the transcription mixture lacking [α-^{32}P]UTP but containg unlabeled UTP. The DNA was recovered as described in **A** and electrophoresed in a 1% agarose gel. The gel was stained with ethidium bromide. Lanes 1 and 3 are input relaxed DNA and supercoiled DNA, respectively.

as revealed by relaxation and catenation of supercoiled circular DNA in the presence of ATP (Fig. 1A, lane 1). Because DNA topoisomerase II is a relatively heat-labile enzyme (16), we tried to inactivate it by heating. As shown in Fig. 1A, we found that the activity was completely inactivated by incubating the preparation of TFIIE at 48°C for 20 min. In contrast with this, TFIIE activity as determined in a complementation assay with other transcription components tolerated the heat treatment (Fig. 1B). When transcription reactions were carried out by using TFIIB, TFIID, RNA polymerase II and heated TFIIE, supercoiled circular DNA remained supercoiled and relaxed closed circular DNA remained relaxed (Fig. 1C). These results show that topology of closed circular DNA is maintained during transcription in this system. When transcription reactions were carried out at various DNA concentrations, transcription activity increased with increasing concentration of DNA up to 8 μg/ml. However, the ratio of transcription activity on supercoiled DNA to that on relaxed circular DNA was constant if compared at the same DNA concentration (data not shown).

Transcription of DNA with defined superhelicity

DNA templates containing defined negative superhelical densities were prepared by incubating plasmid DNA with DNA topoisomerase I in the presence of varying amounts of ethidium bromide. The distribution of topoisomers in each preparation was examined by agarose gel electrophoresis in the presence or absence of chloroquine. For example, Fig. 2 shows electrophoretic separation of the pML(C_2AT) topoisomers. The topoisomers with lower superhelical turns were resolved in the normal gel (Fig. 2A), while those with higher superhelical turns were resolved in the chloroquine gel (Fig. 2B).

Each topoisomer population was tested for transcription activity in the reconstituted system described above and the results are shown in Fig. 3. Transcription of the *B. mori* fibroin gene on pFb205 DNA increases as the template σ value changes from 0 to -0.036 and then keeps the maximum level upon further increase in negative superhelicity of the template (Fig. 3A, the σ value of plasmid DNA isolated from *Escherichia coli* is approximately -0.06). Transcription of the Ad2 MLP on pML(C_2AT) DNA also increases as the σ value changes from 0 to -0.036 but then decreases by further increase in negative superhelicity (Fig. 3B). Whereas transcription of the *Drosophila hsp70* gene on p56H8RIA DNA appears to be unaffected by negative superhelicity of the template (Fig. 3C). pFb205 and pML(C_2AT) contain different vector sequences and these sequences could affect transcription of the fibroin gene and the Ad2 MLP differently in the above experiments. However, this possibility is excluded because another constructs of the fibroin gene (pFb238) and the Ad2 MLP (pAd500), which contain exactly the same vector sequence (see MATERIALS AND METHODS), gave the same results as pFb205 and pML(C_2AT), respectively (data not shown). The above experiments were performed by using population of topoisomers. However, essentially the same results were obtained when representative single topoisomers of pML(C_2AT) were isolated and tested for transcription activity (data not shown). These results illustrate the different effects of DNA superhelicity on the transcription of three genes and confirm our previous conclusion (5) that eukaryotic promoters require various degrees of DNA supercoiling for optimal function. Furthermore, these results obtained under the conditions in which topology of the template DNA is maintained suggest that the superhelical state of DNA but not the supercoiling process is important for the activation of transcription.

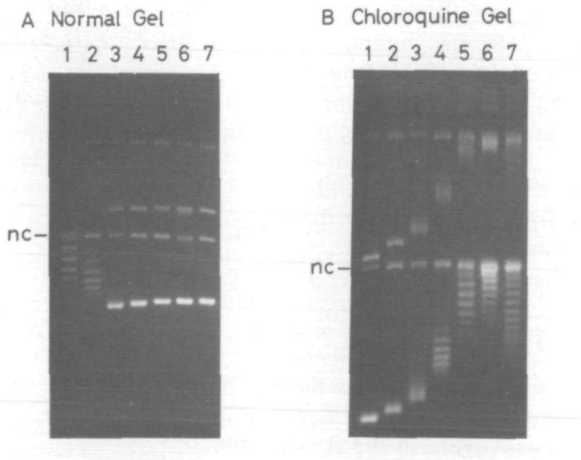

Figure 2. Agarose gel electrophoresis of DNA topoisomers. Portions (125ng in **A** and 250ng in **B**) of each topoisomer population of pML(C_2AT) DNA were electrophoresed in a 1% agarose gel with (**B**) or without (**A**) 15μg/ml of chloroquine as described under 'MATERIALS AND METHODS'. Lane 1, $\sigma = 0$; Lane 2, $\sigma = -0.021$; Lane 3, $\sigma = -0.036$; Lane 4, $\sigma = -0.051$; Lane 5, $\sigma = -0.071$; Lane 6, $\sigma = -0.091$; Lane 7, $\sigma = -0.100$. In this chloroquine gel, topoisomers in lanes 1 to 5 are migrating as positively supercoiled DNA whereas those in lane 7 are migrating as negatively supercoiled DNA as determined by comparison with other gel containing less chloroquine. nc represents the position of nicked circular DNA.

DNA superhelicity affects the formation of transcription preinitiation complex

Previously, we showed that DNA supercoiling enhances the fibroin gene transcription by facilitating the assembly of transcription preinitiation complex (6,7). This conclusion was based on the results of rather complicated experiments in which DNA supercoiling and the formation of preinitiation complex proceeded simultaneously. Therefore, we reinvestigated the effect of DNA topology on the assembly of preinitiation complex by using transcription components lacking DNA topoisomerase activities. First, DNA was preincubated with transcription factors

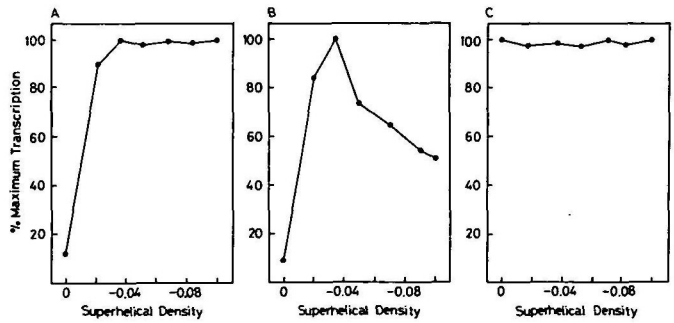

Figure 3. *In vitro* transcription from templates of varying superhelical density. **A**, transcription of the fibroin gene on pFb205 DNA. **B**, transcription from the Ad2 MLP on pML(C_2AT) DNA. **C**, transcription of the *hsp70* gene on p56H8RIA DNA. Each topoisomer preparation was tested for transcription activity as described under 'MATERIALS AND METHODS'. The activity was expressed as percentage of the maximal value for transcript of each gene and plotted versus superhelical density of the DNA template. The maximal values were 3,800cpm in **A**, 5,900cpm in **B**, and 5,400cpm in **C**.

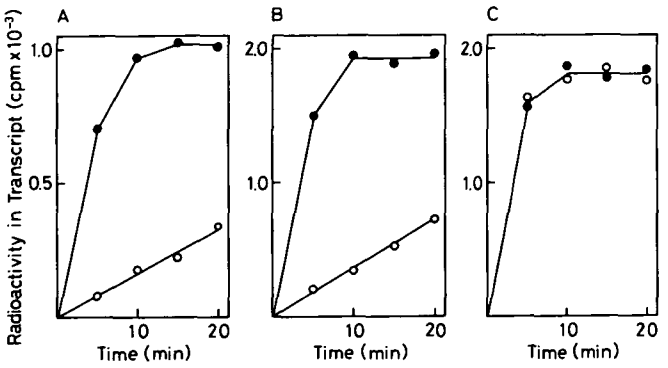

Figure 4. Time required for the assembly of preinitiation complex. **A**, relaxed closed circular DNA (○) or supercoiled plasmid DNA (σ = −0.06) of pFb205 (●) was preincubated for the indicated times and preinitiation complexes formed were assayed as described under 'MATERIALS AND METHODS'. **B**, same as in **A** except that relaxed closed circular DNA (○) or partially superhelical DNA (σ = −0.036) of pML(C_2 AT) (●) was used as the template. *C*, same as in *A* except that relaxed closed circular DNA (○) or supercoiled plasmid DNA (σ = −0.06) of p56H8RIA (●) was used as the template.

Table I. Requirements for rapid assembly of preinitiation complex

No	Protein added at 0 time	Template	UMP incorporated (fmol)
1	Complete	pML(C_2AT),psc	104
2	FIIB	pML(C_2AT),psc	7
3	FIID	pML(C_2AT),psc	92
4	FIIE	pML(C_2AT),psc	6
5	NA polymerase II	pML(C_2AT),psc	8
6	FIID	pML(C_2AT),rc	21
7	Complete	p56H8RIA,sc	93
8	FIIB	p56H8RIA,sc	6
9	FIID	p56H8RIA,sc	91
10	FIIE	p56H8RIA,sc	8
11	RA polymerase II	p56H8RIA,sc	6
12	TFIID	p56H8RIA,rc	89

DNA was incubated in the standard preincubation mixture (complete), or the mixture containing an indicated transcription component for 10 min. Then the missing components were added and 30-s later Sarkosyl was added to 0.01%. Then transcription was started by the addition of nucleotides. Amounts of UMP incorporated were calculated from radioactivity of the specific transcript and the specific radioactivity of [α-^{32}P]UTP. psc, partially supercoiled DNA (σ = −0.036); rc, relaxed circular DNA; sc, supercoiled DNA (σ = −0.06).

and RNA polymerase II. At various times, 0.01% Sarkosyl was added to prevent further formation of the preinitiation complex. The extent of complex formation at each time was then measured by allowing a single round of transcription. As shown in Fig. 4A, superhelical DNA (σ = −0.06) supports more rapid formation of the preinitiation complex on the fibroin gene promoter than relaxed DNA does. Similar analysis performed on the Ad2 MLP reveals that the formation of preinitiation complex proceeds more rapidly on partially superhelical DNA (σ = −0.036) than on relaxed DNA (Fig. 4B). Note that the superhelical template of this σ value gives the maximum level of transcription from this promoter (see the section above). The assembly of preinitiation complex on superhelical DNA with σ value of −0.06 occurs faster than that on relaxed DNA but slower than that on partially superhelical DNA with σ value of−0.036 (data not shown). In the case of *hsp70* gene, the preinitiation complex assembles rapidly on both superhelical (σ = −0.06) and relaxed DNA (Fig. 4C).

When transcription was started synchronously from the preinitiation complex assembled on the fibroin gene promoter, the Sl nuclease protection band of expected size could not be seen at first but became detectable within 1 min after addition of nucleotides on both superhelical and relaxed DNA (data not shown). Similar results were obtained for transcription of the Ad2 MLP and the *hsp70* gene. Thus, the start of RNA chain polymerization and the subsequent elongation appear to be rapid processes irrespective of DNA topology once the preinitiation complex is formed. From these results we conclude that DNA superhelicity enhances transcription of certain genes by accelerating the formation of transcription preinitiation complex but it does not affect transcription of other gene on which the preinitiation complex assembles rapidly even in the relaxed state.

DNA superhelicity facilitates interaction of TFIID to the Ad2 MLP

In the case of the fibroin gene, TFIID and supercoiled DNA are essential for the rapid assembly of preinitiation complex (7). To determine which transcription factor is responsible for the rapid assembly of preinitiation complex on the superhelical template of Ad2 MLP, each transcription factor was incubated with pML(C_2AT) DNA for 10 min. After addition of the remaining factors, the incubation was continued and 30-s later, 0.01% Sarkosyl was added. The amounts of preinitiation complex formed were measured by a single round of transcription. When partially superhelical DNA (σ = −0.036) was preincubated with TFIID, the amounts of transcript were almost the same level as control where all transcription components were present during the preincubation (Table I, No 3 versus No 1). Similar results were obtained when purified yeast TFIID was used in place of partially purified human TFIID (data not shown). No significant level of transcript was detectable if the preincubation contained only TFIIB, TFIIE or RNA polymerase II (No 2, 4 and 5). Only a small amount of preinitiation complex was formed on relaxed DNA even if it had been preincubated with TFIID (No 6). Theses results suggest that the functional binding of TFIID to the promoter is the rate limiting step in the assembly of preinitiation complex on the Ad2 MLP and that DNA superhelicity facilitates the step. This is in sharp contrast with the *hsp70* gene. In this case, significant amounts of preinitiation complex were formed on both superhelical and relaxed DNA if the preincubation contained TFIID (No 9 and 12). These results suggest that DNA superhelicity has no effect on the interaction of TFIID to the *hsp70* gene promoter.

DISCUSSION

The present study shows that the negative superhelicity of DNA can have different effects on the transcription of eukaryotic genes. Thus, transcription of the *B. mori* fibroin gene increases and plateaus from templates of increasing superhelicity, that from the Ad2 MLP increases and then decreases, whereas that of the *Drosophila hsp70* gene keeps a constant level. The dependency of transcription on the superhelical state of DNA appears to be determined by the mode of assembly of the transcription preinitiation complex. The complex assembles slowly on relaxed templates of the fibroin gene and the Ad2 MLP. DNA superhelicity enhances transcription of these genes by accelerating formation of the complex. In contrast with this, the complex on

the *hsp70* gene assembles rapidly irrespective of template topology and hence DNA superhelicity does not affect transcription of the gene.

In an analogy from transcription of prokaryotic genes (see Ref. 17 for review), transcription initiation of eukaryotic genes is thought to involve local unwinding of the DNA double helix within the promoter region. Then, it is reasonable to expect that the negative superhelicity of template DNA facilitates the process in some cases. However, the decline in transcription from the Ad2 MLP at σ values more negative than -0.036 is unexpected. Followings are the possible explanation for the phenomenon. First, the superhelical tension might be dissipated by generation of stress-absorbing conformations like cruciforms. It has been reported that a cruciform is not stable in relaxed DNA but becomes stable in supercoiled DNA with σ values more negative than -0.03 (18). Second, cruciforms or local unwinding of DNA might develop outside the promoter region and sequester the transcription components such as RNA polymerase II. Third, transcription initiation might be aborted by extensive unwinding of DNA around the promoter region.

Why does the preinitiation complex assemble rapidly on the *hsp70* gene promoter even in relaxed DNA? High affinity of TFIID to this promoter might allow rapid assembly of the complex. Consistent with this notion, Wu has shown the strong protection of the TATA box region of the *hsp70* gene from the exonuclease attack in nuclei of uninduced cells (19). Alternatively, this promoter might be able to induce local unwinding of DNA easily without any help of superhelical tension, resulting in rapid assembly of preinitiation complex. However, the promoter region of the *hsp70* gene is not necessarily rich in A T base pair (20). Nucleotide sequence of the TATA box of the *hsp70* gene is TATAAAT. Interestingly, the human beta intereferon gene carrying the TATA box sequence of TATAAAT also showed rapid assembly of the preinitiation complex on both relaxed and supercoiled DNA. In all other promoters tested thus far (e. g. silk fibroin gene, sericin gene, Ad2 MLP, chicken conalbumin gene, and rabbit beta globin gene), supercoiled DNA supported more rapid assembly of the preinitiation complex than relaxed DNA did (this work and our unpublished results). These genes have the TATA box sequence of PyATAAAA. Therefore, the TATAAAT sequence seems to be important for rapid assembly of the complex on relaxed DNA.

Whatever the mechanism may be, the observed difference in time course of preinitiation complex formation may have relevance to control of gene expression. Assembly of the complex is a rate- limiting step in transcription of the fibroin gene, the Ad2 MLP, and the adenovirus 5 E4 gene (6,7,21,22, this work, and our unpublished results), suggesting that the step can be one of the major targets for the regulation of gene expression. This notion is supported by our finding that DNA supercoiling enhances transcription of these genes by accelerating the assembly of preinitiation complex (6,7, this work, and our unpublished results). Other researchers also demonstrate that trans-acting factors stimulate transcription of the Ad2 MLP, the adenovirus 5 E4 gene, and the artificial promoter carrying the progesterone response elements by facilitating the assembly of preinitiation complex (23, 24, 25). On the contrary, rapid assembly of preinitiation complex on the *hsp70* gene suggests that expression of this particular gene is regulated at a step other than the formation of preinitiation complex. Indeed, Rougvie and Lis have proposed a control mechanism for transcription of this gene that acts during chain elongation of RNA (26). Presumably, the special device is adopted to achieve quick response to the stress stimulus.

ACKNOWLEDGEMENTS

We thank Dr. R.G.Roeder for the gift of pML(C_2AT), Dr. K.Nagata for critical reading of the manuscript, and J. Mihara for preparation of the manuscript. The project has been partially supported by Grants-in-Aid for Scientific Research from the Ministry of Education, Science and Culture, and by the Joint Studies Program of The Graduate University for Advanced Studies.

REFERENCES

1. Gellert,M. (1981) *Annu. Rev. Biochem. 50*, 879–910
2. Wang,J.C. (1985) *Annu. Rev. Biochem. 54*, 665–697
3. Sinden,R.R., Carlson,J.O., and Pettijohn,D.E. (1980) *Cell. 21*, 773–783
4. Hirose,S., Tsuda,M., and Suzuki,Y. (1985) *J. Biol. Chem. 260*, 10557–10562
5. Hirose,S., and Suzuki,Y. (1988) *Proc. Natl. Acad. Sci. U.S.A. 85*,718–722
6. Tabuchi,H., and Hirose,S. (1988) *J. Biol. Chem. 263*, 15282–15287
7. Mizutani,M., Ohta,T., Watanabe,H., Handa,H., and Hirose,S. (1991) *Proc. Natl. Acad. Sci. U.S.A. 88*, 718–722
8. Sawadogo,M., and Roeder,R.G. (1985) *Proc. Natl. Acad. Sci. U.S.A. 82*, 4394–4398
9. Moran,L., Mirault,M.-E., Tissieres,A., Lis,J., Schedl,P., Artavanis-Tsakonas,S., and Gehring,W.J. (1979) *Cell. 17*, 1–18
10. Liu,L.F. (1983) *Methods in Enzymol. 100*, 133–137
11. Reinberg,D., and Roeder,R.G. (1987) *J. Biol. Chem. 262*, 3310–3321
12. Singleton,C.K., and Wells,R.D. (1982) *Anal. Biochem. 122*, 253–257
13. Shure,M., Pulleyblank,D.E., and Vinograd,J. (1977) *Nucl. Acids. Res. 4*, 1183–1205
14. Watanabe,H., Imai,T., Sharp,P.A., and Handa,H. (1988) *Mol. Cell. Biol. 8*, 1290–1300
15. Tsujimoto,Y., Hirose,S., Tsuda,M., and Suzuki,Y. (1981) *Proc. Natl. Acad. Sci. U.S.A. 78*, 4838–4842
16. Osheroff,N., Shelton,E.R., and Brutlag,D.L. (1983) *J. Biol. Chem. 258*, 9536–9543
17. Chamberlin,M. (1976) in *RNA Polymerase* (Losick,R., and Chamberlin,M., eds) pp. 17–67, Cold Spring Harbor Press, Cold Spring Harbor,NY
18. Courey,A.J., and Wang,J.C. (1983) *Cell 33*, 817–829
19. Wu,C. (1984) *Nature 309*, 229–234
20. Torok,I., and Karch,F. (1980) *Nucl. Acids. Res. 8*,3105–3123
21. Fire, A., Samuels,M., and Sharp,P.A. (1984) *J. Biol. Chem. 259*, 2509–2516
22. Howley,D.K., and Roeder,R.G. (1985) *J. Biol. Chem. 260*, 8163–8172
23. Sawadogo,M., and Roeder,R.G. (1985) *Cell 43*, 165–175
24. Horikoshi,M., Hai,T., Lin,Y.-S., Green,M.R., and Roeder,R.G. (1988) *Cell 54*, 1033–1042
25. Klein-Hitpass,L., Tsai,S.Y., Weigel,N.L., Allan,G.F., Riley,D., Rodoriguez,R., Schrader,W.T., Tsai,M. J., and O'Malley,B.W. (1990) *Cell 60*, 247–257
26. Rougvie,A.E., and Lis,J.T. (1988) *Cell 54*, 795–804