Proc. Nat. Acad. Sci. USA
Vol. 70, No. 11, pp. 3077–3081, November 1973

Effect of Circularity and Superhelicity on Transcription from Bacteriophage λ DNA

(RNA synthesis/RNA polymerase)

PETER BQTCHAN, JAMES C. WANG*, AND HARRISON ECHOLS

Department of Molecular Biology and *Department of Chemistry, University of California, Berkeley, Calif. 94720

Communicated by Norman Davidson, July 18, 1973

ABSTRACT **We studied RNA synthesis *in vitro* from closed-circular λ DNA molecules with varying degrees of superhelicity. The four circular templates examined had *0*, *−50*, *−110*, and *−160* superhelical turns under the conditions of the transcription assay. We found that the total amount of RNA synthesis increases as the template acquires more negative superhelical turns. This increased transcription results from more frequent initiation of RNA chains. Transcription of circular DNA with no superhelical turns appears to mimic RNA synthesis *in vivo* more closely than transcription from either highly superhelical or linear DNA with regard to two criteria: preferential transcription of the region corresponding to early genes and sensitivity to repression by λ *cI* protein. We suggest that the physical basis for the increased initiation of RNA chains from superhelical DNA is the fact that unwinding events are energetically favored on a DNA molecule with negative superhelical turns. Possible general mechanisms are: (*a*) RNA polymerase must unwind the DNA duplex as a prelude to initiation; (*b*) the DNA itself must assume a new conformation at the promoter site which requires an unwinding of the DNA duplex.**

Most *in vitro* experiments with RNA polymerase of *Escherichia coli* have been done with linear viral DNA as template (1). However, many viral DNA molecules exist in a circular form *in vivo* during transcription by RNA polymerase. These circular DNA molecules are typically extracted from the cell in a superhelical configuration (2). For example, λ DNA has about 140 negative superhelical turns (3). For this reason, we undertook a study of the influence of circularity and superhelicity on the magnitude and specificity of transcription from λ DNA by RNA polymerase.

Previous studies of superhelical φX DNA (4) and SV40 DNA (5; P. Chambon, personal communication) have indicated that the integrity of the circular molecule or the superhelicity itself might influence the magnitude of transcription, because the introduction of a single-strand break (which allows unwinding) reduced the magnitude of RNA synthesis. In this report, we show that the amount of RNA synthesis from covalently-closed, circular λ DNA increases as the degree of superhelicity increases; we also present results that indicate that these quantitative alterations are associated with changes in the initiation pattern.

MATERIALS AND METHODS

[^{3}H]CTP was obtained from Schwarz/Mann, Orangeburg, New York and [^{32}P]CTP from New England Nuclear, Boston, Mass. RNase and DNase (code DPFF) were supplied by Worthington Biochemical Corp., Freehold, N.J., and 38% formaldehyde was from Mallinckrodt, Evanston, Ill.

The purification of RNA polymerase followed the method of Burgess (6) through $(NH_4)_2SO_4$ precipitation. Subsequent steps followed the agarose and DEAE-cellulose procedure of Wu *et al.* (7), with two modifications. Before agarose chromatography the extract was dialyzed for 4 hr against 2 liters of the Buffer A described by Burgess (6). After DEAE-cellulose chromatography, the high-salt glycerol gradient step of Burgess (6) was used. The enzyme was stored in Buffer A, in 0.5 M KCl and 50% glycerol. Na dodecyl sulfate–polyacrylamide gel electrophoresis revealed five protein species: the β, β′, σ, and α subunits (6) and one other protein (τ) typically found in RNA polymerase preparations (6).

The λ *cI* protein (λ repressor), a gift of Dr. S. Ghosh, was prepared by the method of Wu *et al.* (7). Hybridization procedures have been described (7), except that hybridization was done at 65° for 5 hr with 16 μg/ml of heat-denatured DNA. The wash buffer was 0.3 M NaCl–0.03 M Na-citrate (pH 7.3).

Circular templates used in this study were prepared from DNA extracted from purified phage. The cyclization and the covalent closure of the DNA in the presence of ethidium bromide were done by published procedures (8). The four templates obtained in this way had *0*, *−50*, *−110*, and *−160* superhelical turns under the conditions of the transcription assay.

The standard transcription assay (0.1 ml) contained: 0.05 M KCl, 0.1 mM dithiothreitol, 0.1 mM EDTA, 20 μg/ml of bovine-serum albumin (Armour), 0.01 M $MgCl_2$, 0.02 M Tris·HCl (pH 7.9 at 25°), 0.33 mM GTP, UTP, and ATP, 0.08 mM [^{3}H]CTP, and various amounts of RNA polymerase and λb2 DNA. The order of additions were: (*i*) DNA was first added to the buffer; (*ii*) RNA polymerase was then added to the mixture and incubated at 17° for 5 min; (*iii*) the reaction was then started by addition of the ribonucleoside triphosphates at 35°. The reaction was stopped by chilling in ice. In experiments in which λ *cI* protein ("λ repressor") was used, RNA polymerase addition followed a 5-min incubation of DNA and *cI* protein at 0°.

RESULTS

Effect of Superhelical Turns on the Magnitude of Transcription. Fig. 1*A* depicts the total amount of RNA synthesized as a function of RNA polymerase concentration for four covalently-closed λb2 DNA samples containing *0*, *−50*, *−110*, and *−160* superhelices. The magnitude of transcription is related to the degree of superhelicity; the total amount of

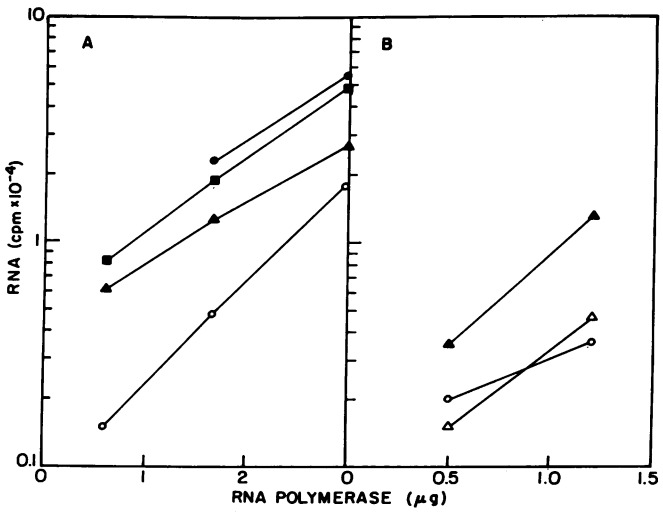

FIG. 1. Transcription of superhelical and linear DNA at different RNA polymerase concentrations. To the reaction mixture described in *Methods* was added: (*A*) 0.6 μg of closed-circular DNA of varying superhelicity or; (*B*) 0.4 μg of circular or linear DNA. RNA polymerase was added and the mixture was incubated at 17° for 5 min. The reaction was begun by addition of 0.33 mM ATP, GTP, and UTP and 0.08 mM [^{3}H]CTP (1 Ci/mmol). The reaction was terminated after 5 min by chilling in ice. RNA was precipitated with 5% cold trichloroacetic acid plus 0.1% sodium pyrophosphate and filtered through Whatman GF/C glass-fiber filters. The precipitate was washed with 50 ml of 5% trichloroacetic acid plus 0.1% sodium pyrophosphate, followed by 10 ml of anhydrous methanol. The dried filters were counted in 5 ml of toluene–Omnifluour in a Packard Scintillation Counter. ○, *0* superhelical turns; ▲, *−50* superhelical turns; ●, *−110* superhelical turns; ■, *−160* superhelical turns; △, linear.

RNA synthesis increases as the number of negative superhelical turns increases from *0* to *−110*. Total transcription reaches a maximum from the template with *−110* superhelical turns. Fig. 1*B* compares the transcription of linear and circular templates. The total amount of RNA from the linear template is similar to that from the circular DNA with no superhelical turns.

Effect of Superhelical Turns on Initiation and Termination. There are three possible explanations for the increased transcription from DNA with more negative superhelical turns: (*i*) more RNA chains are initiated; (*ii*) the RNA chains grow faster; and (*iii*) fewer RNA chains are terminated during the transcription assay. In an effort to distinguish among these possibilities, we have measured the growth rate of the RNA chains. For this purpose we estimated chain size by zone sedimentation in sucrose gradients containing formaldehyde to eliminate effects of secondary structure (9, 10).

Fig. 2*A* compares the sedimentation profile of RNA transcribed for 1 or 2 min from DNA with no superhelical turns. The 1-min [^{3}H]RNA was mixed with the 2-min [^{32}P]RNA before sedimentation. Most of the RNA chains continue to grow between 1 and 2 min. From the sedimentation position of ribosomal RNA markers, we estimate an approximate molecular weight of 3×10^5 and 6×10^5 for the peak 1-min and 2-min RNA samples, respectively. A similar constant growth rate was observed for transcription of DNA with *−160* superhelical turns. This similarity in growth rate of most of the RNA chains from the *0* and *−160* DNA is shown in Fig. 2*B* by a direct comparison of [^{3}H]RNA and [^{32}P]RNA from the two templates. Thus, no major differences in growth rate of RNA chains or in termination are apparent during the first 2 min of RNA synthesis.

In contrast, there is a large difference in the amount of RNA transcribed from the *0* and *−160* DNA during the first 2 min. This is shown by a comparison of [^{3}H]RNA from the two templates sedimentated in parallel gradients (Fig. 2*C*). From the large differences in total RNA transcribed at this early time after initiation, when most of the RNA chains are still growing at a constant rate, we conclude that the increased transcription from highly superhelical DNA results mainly from new initiation events.

The origin of the very rapidly sedimenting RNA found after transcription of superhelical templates (Fig. 2*B* and *C*) is obscure. The rapid sedimentation might reflect a residual association with the DNA template or an artifact of the formaldehyde reaction.

Changes in Initiation Pattern as a Function of Superhelicity. The additional initiation events observed on the more superhelical DNA could occur from the same sites used on less superhelical DNA or, alternatively, from the activation of new or infrequently used sites. A related and perhaps more interesting question is which template approximates most closely the initiation pattern found *in vivo*. After infection of *E. coli* by phage λ, the host RNA polymerase initiates transcription mainly at two sites on λ DNA, leading to the production of a class of "immediate-early" RNA. Initiations from these sites are subject to inhibition by the λ *c*I protein ("λ repressor") (Fig. 3).

To determine how the different templates affect the use of initiation sites, we estimated the fraction of RNA from each template that comes from the early gene region. The amount of this early gene RNA should give an estimate of the frequency of initiation from the immediate-early promoter sites. Two criteria were used to estimate the early gene RNA: hybridization of RNA to DNA homologous to the template only in the early gene region (φ80-λ early DNA, Fig. 3), and inhibition of transcription by purified λ *c*I protein.

By comparison of the extent of hybridization to φ80-λ early DNA and to λ DNA, we can show which regions of λ DNA are predominantly transcribed and relate the tran-

TABLE 1. *Percent of total RNA complementary to λ early genes as a function of superhelicity*

DNA	Incubation time (min)	Percent early gene RNA
0	5	81
−50	5	63
−110	5	48
−160	5	41
Linear	5	53
−110	10	44
−110	1	42

Reactions were done as in Fig. 1 (with 0.6 μg of polymerase). The DNA templates used are denoted here by the number of superhelical turns in each sample. RNA was synthesized, purified, and hybridized as in *Methods*. The percent early gene RNA is defined as [hybrid with φ80-λ early (cpm)/hybrid with λ (cpm)] × 100.

scription pattern to that initially present after λ infection. The results of such hybridization assays are shown in Table 1. RNA transcribed from each of the templates noted was purified and added to hybridization mixtures containing either λ or φ80-λ early DNA. The percent early gene RNA increases as the template becomes less superhelical. These results indicate that at least some of the additional initiation events on highly superhelical DNA involve new or less frequently used sites outside the early gene region. It should also be noted here that linear DNA has a lower percent of early gene RNA than either DNA with *0* or *−50* superhelical turns.

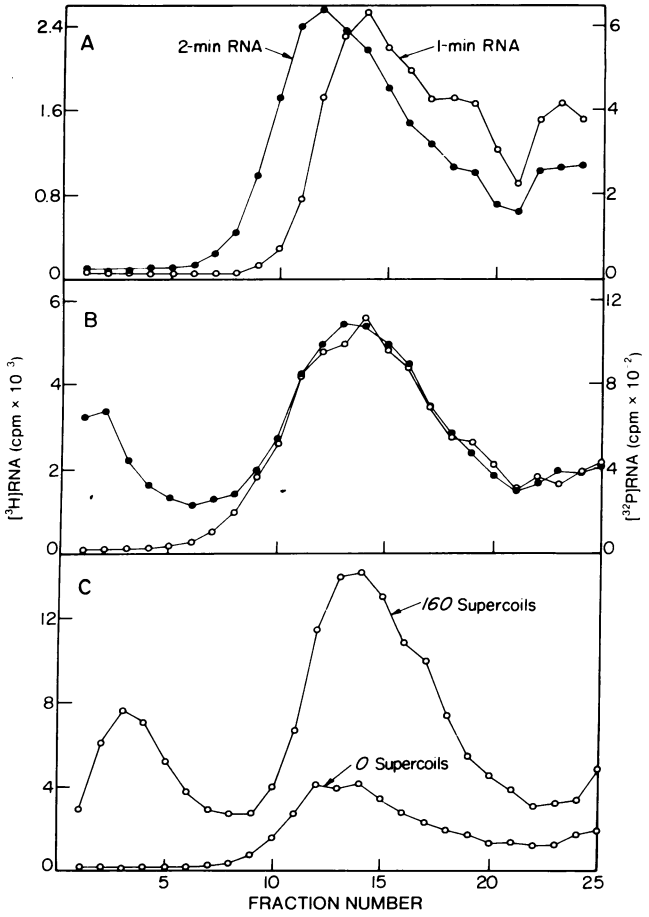

FIG. 2. Sedimentation analysis of RNA transcribed from closed, circular DNA with *0* or *−160* superhelical turns. To the reaction mixtures used for Fig. 1 were added 0.75 μg of DNA and 1.8 μg of RNA polymerase. The reaction of the RNA with formaldehyde followed the procedure of Boedtker (9). The reaction mixtures were made 0.1% in sodium dodecyl sulfate and heated for 2 min at 75–80°. Then formaldehyde was added to 1.1 M and phosphate buffer (pH 7.7) to 0.1 M. For cosedimentation of ^{3}H- and ^{32}P-labeled RNA, reaction mixtures were mixed before treatment with formaldehyde. The transcription times and templates used were: (*A*) 1-min [^{3}H]RNA and 2-min [^{32}P]RNA, *0* supercoils, cosedimented; (*B*) 2-min RNA, *0* supercoils [^{3}H]RNA, *−160* supercoils [^{32}P]RNA, cosedimented; (*C*) 2-min RNA, *0* supercoils [^{3}H]RNA, *−160* supercoils [^{3}H]RNA, sedimented in parallel gradients. RNA was sedimented for $6^{1}/_{2}$ hr at 50,000 rpm. The gradient tubes contained 5–20% sucrose, 1.1 M formaldehyde, and 0.1 M phosphate (pH 7.7). Samples were collected by pumping from the bottom of the tube. RNA assays were done as for Fig. 1 except that 100 μg of carrier DNA was added to each fraction before precipitation. (○) [^{3}H]RNA; (●) [^{32}P]-RNA. Sedimentation was from right to left on the figure.

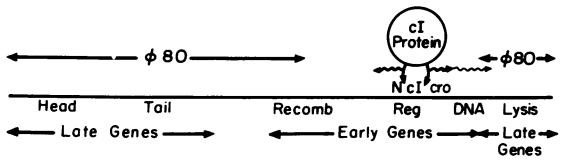

FIG. 3. λ genes and their regulation. The *horizontal line* represents a linear λ DNA molecule. Most λ genes are shown generically by the designations below the line: genes that specify phage head and phage tail structures and lysis proteins (*late genes*) or genes that specify recombination, regulation, or DNA replication proteins (*early genes*). The approximate regions of φ80 DNA in the φ80-λ *early* hybrid used for RNA analysis are shown above the line. The *cI* gene codes for the repressor that maintains lysogeny (11, 12); the *N* gene codes for a regulatory protein that activates most other λ genes (13, 14). The early genes are transcribed in two stages *in vivo*: the immediate-early stage includes leftward transcription from gene *N* and rightward transcription which extends through the DNA genes but probably represents mainly the *cro* gene; the delayed-early stage (dependent on *N* protein) includes leftward transcription through the recombination genes and enhanced rightward transcription from the replication genes (13). The immediate-early stage of RNA synthesis is represented on the figure by the *wavy arrows* (⇝). *In vitro*, in the absence of a termination factor, the distinction between immediate-early and delayed-early stages apparently does not occur (15). The *cI* protein acts at the sites indicated by *arrows* to block the immediate-early stage of RNA synthesis *in vivo* (11, 12) [and properly initiated early gene RNA synthesis *in vitro* (7, 16)].

An alternative explanation for the changes in percent of early gene RNA invokes differences in the termination patterns for the various templates. Thus, for example, one could say that the same fraction of RNA chains initiate at the immediate-early promoter sites on DNA with *−110* superhelical turns as on DNA with *0* superhelical turns; however, since the RNA chains elongate further on the DNA with *−110* superhelical turns, the percent of early gene RNA decreases because the RNA chains extend into regions inhomologous with the φ80-λ early DNA used in the hybridization assay. If this is the case, a significant change in percent of early gene RNA should be found for an incubation time short enough to prevent any possible chain growth into the regions distal

TABLE 2. *Percent repression as a function of superhelicity*

DNA	Percent early RNA repressed	Percent total RNA repressed
0	50	25
−50	53	50
−160	9	0
Linear	33	3
Linear *imm*434	0	0

The procedures are the same as in Table 1 except that one binding unit (7) of λ *cI* protein was prebound for 5 min at 0° before addition of RNA polymerase to one reaction mixture. For each template an additional reaction mixture was used without repressor. The percent repression is defined as [hybrid (non-repressed reaction) − hybrid (repressed reaction)/hybrid (non-repressed reaction)] × 100.

to early genes. Table 1 shows the results of an experiment in which transcription from DNA with *−110* superhelical turns was allowed to proceed for 1 min or for 10 min. The 1-min RNA sample shows no increase in the percent of early gene RNA compared to the 10-min RNA sample. The 1-min RNA sample should have an average molecular weight no greater than 3×10^5 (Fig. 2), and the λ region of φ80-λ early DNA probably extends for nearly 4×10^6 daltons from the immediate-early promoter sites (17). Thus the differences in percent of early gene RNA probably reflect differences in the pattern of initiation.

Repressor Activity as a Function of Superhelicity. The degree of inhibition of transcription (repression) obtained with the λ *c*I protein is another probe for the number of chains started at the initation sites used by RNA polymerase *in vivo*. The *c*I protein binds to two sites near the initiation sites for immediate-early RNA synthesis, and inhibits the synthesis of RNA chains from these sites (see Fig. 3). Table 2 presents the percent of total and of early gene RNA that can be repressed in our *in vitro* system. DNA with *−160* superhelical turns shows little repression, as expected from the small fraction of early gene RNA found for this template in the data of Table 1. DNA with *0* and *−50* superhelical turns shows much greater repression, also as expected from the data of Table 1. Linear DNA is less subject to repression than a closed-circular template with *0* and *−50* superhelical turns. Linear λ*imm*434 DNA, which is insensitive to λ *c*I protein (11, 12), exhibits no repression. The loss of repression by superhelical DNA does not result from a failure of superhelical DNA to bind the *c*I protein (18).

The extent of repression of RNA synthesis is less than that expected if all of the early gene RNA is initiated at the immediate-early promoter sites shown in Fig. 3. This may reflect initiation at other sites within the early gene region or an inability to achieve complete repression under the *in vitro* conditions used in these experiments (7).

From the results of Tables 1 and 2, we conclude that the spectrum of initiation sites changes with the superhelical density of the DNA template and that the initiation pattern from closed-circular DNA with few or no superhelical turns approximates most closely that observed *in vivo*.

DISCUSSION

Possible necessity for RNA polymerase to unwind the double helix

We find that total RNA synthesis from λ DNA increases as the number of negative superhelical turns increases from *0* to *−110;* transcription reaches a maximum for the template with *−110* turns and does not increase further for a template with *−160* turns. The increase in the magnitude of transcription probably results from more frequent initiation of RNA chains. The additional initiation events appear to occur predominantly from promoter sites not used by the host RNA polymerase *in vivo* during the earliest stage of λ infection.

We believe that the physical basis for the increased initiation of RNA chains from superhelical DNA is the fact that unwinding events are energetically favored on a DNA molecule with negative superhelical turns (19, 20). Therefore, any event that involves unwinding of the DNA duplex should occur more readily with DNA carrying negative superhelical turns. We suggest two general mechanisms by which the increased initiation from superhelical DNA might arise: (*a*) RNA polymerase must unwind the DNA duplex as a prelude to initiation; or (*b*) the DNA must assume a new conformation at the promoter site which requires unwinding of the DNA duplex.

The critical difference between the two proposed mechanisms is that the conformational change in *b* is independent of the binding by RNA polymerase. An example of such a conformational change is the generation of branched, "hairpin" structures with a few unpaired bases (21). Such a conformational change might be required for either proper site recognition by RNA polymerase at the binding stage or for other processes necessary for initiation.

If RNA polymerase has the capacity to separate the strands of the DNA duplex (mechanism *a* above), the enzyme should have an increased affinity for binding sites on the superhelical molecule because the affinity of an unwinding substance for closed-circular DNA increases as the DNA acquires more negative superhelical turns (19). There are two ways in which this increased affinity might result in more frequent initiation from superhelical DNA: (*i*) an increase in the fraction of enzymes bound to the template; or (*ii*) a reduction of an energy barrier between site recognition and actual chain initiation.

Case *i* implies that a fraction of the RNA polymerase molecules are not bound to nonsuperhelical DNA or bound so loosely that initiation is unlikely for kinetic reasons. Thus, the affinity increase for superhelical DNA might serve to increase the number of bound enzyme molecules or the dissociation time of an already bound enzyme. Case *ii* implies that the existence of a tight-binding (high affinity) site is not a sufficient condition for initiation, although probably a necessary condition (22). For such a situation, the interaction of RNA polymerase with DNA may be envisioned as a complex process involving: (*a*) site recognition, (*b*) strand separation, and (*c*) subsequent steps such as enzyme migration from a binding site to a distinct initiation site or a conformation change at the binding site (23–25)†. Either stage *b* or *c* involves a possible energy barrier between site recognition and initiation. The additional unwinding contribution of the superhelical DNA to the binding energy of RNA polymerase might serve to convert a "nonproductive" binding site to one productive for initiation. The fact that RNA synthesis fails to increase between *−110* and *−160* superhelical turns may reflect a saturation of available productive sites or a loss of enzyme molecules at tight-binding sites incapable of initiation.

Recently Saucier and Wang (26) showed by direct physical chemical measurements that the binding of an RNA polymerase molecule to λ DNA causes a slight unwinding of the DNA helix. The unwinding angle per bound polymerase is about 120° with an estimated uncertainty of a factor of two. While the magnitude of this unwinding is small, it can nevertheless cause a large difference in the binding affinity of RNA polymerase for superhelical DNA (20). If we define K_τ and K_0 as the binding constants of RNA polymerase to DNA with τ superhelical turns and zero superhelical turns, respectively, $K_{\tau=-50}/K_0$ is estimated to be about 25 (calculated from ethidium bromide binding data, Wang, unpublished results). Thus, the presence of 50 negative superhelical turns in λ DNA should increase the affinity of any binding site for RNA polymerase by about 25-fold.

† Mangel, W. F. & Chamberlin, M. (1973) *J. Biol. Chem.*, submitted for publication.

Transcription from superhelical DNA involves a change in the distribution of initiation events as well as an increase in frequency. This change in distribution might ensue from either of the two general mechanisms suggested at the beginning of the *Discussion* because either mechanism provides for potential initiation at sites not used on nonsuperhelical DNA. The apparent difference in transcription specificity between linear DNA and closed-circular DNA with no superhelical turns is not obvious from the discussion so far. We can think of two possible explanations for the difference between linear and circular templates: (*i*) under the conditions of our experiments some initiation occurs at the free ends of the linear DNA; or (*ii*) prior binding by some RNA polymerase molecules introduces enough positive turns into the circular molecule to restrict subsequent unwinding events.

Possible regulatory role for superhelicity

Our results indicate that structural alternations that affect DNA unwinding might play a significant role in cellular regulation. We can consider two types of mechanism: (*a*) structural alterations at specific sites which increase or decrease the energy needed for local strand separation or conformation change of the DNA (for example, binding or DNA modification by specific protein "factors"); or (*b*) general structural alterations which change the difficulty of strand separation or of conformation changes at all potential sites on the DNA (for example, the winding or unwinding of a superhelix by DNA-binding proteins). In the latter case, both the magnitude and specificity of transcription from a large region of DNA can be altered without the regulatory protein acting at each potential initiation site for transcription ("action at a distance" regulation). One can also imagine similar effects on DNA replication. In this context, we find particularly interesting the recent reports that DNA of the *E. coli* "chromosome" and perhaps nucleohistone exist in a superhelical state (27, 28).

With respect to λ, the possible importance of such mechanisms is difficult to assess because of a paucity of biochemical data. The only λ regulatory protein for which some biochemical data are available is the *c*I protein. The possibility that the *c*I protein restricts local unwinding has been suggested to explain some complexities in the interaction between *c*I protein and RNA polymerase (7). The *c*I protein does not wind or unwind λ DNA (18; Wang, unpublished data) and thus does not itself exert the general regulatory role postulated in the second mechanism above; conceivably the *c*I protein might influence such regulation indirectly through a capacity to prevent unwinding by RNA polymerase.

We thank Sudhamoy Ghosh for his generous gift of λ *c*I protein; Michael Chamberlin, Tom Maniatis, and Mark Ptashne for the communication of unpublished data, and Michael Chamberlin, Norman Davidson, and Ben Hall for valuable discussion. This investigation was supported by Public Health Service Research Grants GM 17078 (H.E.), GM 14621 (J.W.), and Training Grant GM 01389 from the National Institute of General Medical Sciences. P.B. is a predoctoral trainee.

1. Losick, R. (1972) *Annu. Rev. Biochem.* **41**, 409–446.
2. Helinski, D. R. & Clewell, D. B. (1971) *Annu. Rev. Biochem.* **40**, 899–942.
3. Wang, J. C. (1969) *J. Mol. Biol.* **43**, 263–272.
4. Westphal, H. (1970) *2nd Lepetit Colloquia on Biology and Medicine*, ed. Silvestri, L. (North Holland, Amsterdam), pp. 77–87.
5. Hayashi, Y. & Hayashi, M. (1971) *Biochemistry* **10**, 4212–4218.
6. Burgess, R. R. (1969) *J. Biol. Chem.* **244**, 6160–6167.
7. Wu, A. M., Ghosh, S., Echols, H. & Speigelman, W. G. (1972) *J. Mol. Biol.* **67**, 407–421.
8. Wang, J. C. (1971) *Proc. Nuc. Acid Res.* **2**, 408–416.
9. Boedtker, H. (1968) *J. Mol. Biol.* **35**, 61–70.
10. Richardson, J. P. (1970) *J. Mol. Biol.* **49**, 235–240.
11. Echols, H. (1971) *Annu. Rev. Biochem.* **40**, 827–851.
12. Ptashne, M. (1971) in *The Bacteriophage Lambda*, ed. Hershey, A. D. (Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.), pp. 221–237.
13. Echols, H. (1971) in *The Bacteriophage Lambda*, ed. Hershey, A. D. (Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.), pp. 247–270.
14. Thomas, R. (1971) in *Current Topics in Microbiology and Immunology*, ed. Arber, W. (Springer-Verlag, Berlin), Vol. 56, pp. 13–42.
15. Roberts, J. W. (1970) *Cold Spring Harbor Symp. Quant. Biol.* **35**, 121–126.
16. Steinberg, R. A. & Ptashne, M. (1971) *Nature New Biol.* **230**, 76–80.
17. Davidson, H. & Szybalski, W. (1971) in *The Bacteriophage Lambda*, ed. Hershey, A. D. (Cold Spring Harbor Laboratory Cold Spring Harbor, N.Y.), pp. 45–82.
18. Maniatis, T. & Ptashne, M. (1973) *Proc. Nat. Acad. Sci. USA* **70**, 1531–1535.
19. Bauer, W. & Vinograd, J. (1968) *J. Mol. Biol.* **33**, 141–171.
20. Davidson, N. (1972) *J. Mol. Biol.* **66**, 307–309.
21. Gierer, A. (1966) *Nature* **212**, 1480–1481.
22. Chamberlin, M. (1970) *Cold Spring Harbor Symp. Quant. Biol.* **35**, 851–873.
23. Blattner, F. R. & Dahlberg, J. E. (1972) *Nature New Biol.* **237**, 227–232.
24. Schäfer, R., Zillig, W. & Zechel, K. (1973) *Eur. J. Biochem.* **33**, 207–214.
25. Ramakrishnan, T. & Echols, H. (1973) *J. Mol. Biol.* **78**, 675–686.
26. Saucier, J. -M. & Wang, J. C. (1972) *Nature New Biol.* **239**, 167–170.
27. Worcel, A. & Burgi, E. (1972) *J. Mol. Biol.* **71**, 127–147.
28. Pardon, J. F. & Wilkins, M. H. F. (1972) *J. Mol. Biol.* **68**, 115–124.