Proc. Natl. Acad. Sci. USA
Vol. 76, No. 7, pp. 3304–3308, July 1979
Biochemistry

Differential sensitivity of gene expression *in vitro* to inhibitors of DNA gyrase

(*Escherichia coli*/plasmid/transcription/novobiocin/cell-free system)

HUEY-LANG YANG*, KAREN HELLER*, MARTIN GELLERT†, AND GEOFFREY ZUBAY*

*Department of Biological Sciences, Columbia University, New York, New York 10027; and †Laboratory of Molecular Biology, National Institute of Arthritis, Metabolism and Digestive Diseases, National Institutes of Health, Bethesda, Maryland 20205

Communicated by Charles Yanofsky, May 7, 1979

ABSTRACT **We have used the antibiotics coumermycin A_1, novobiocin, and oxolinic acid, which are specific inhibitors of DNA gyrase, to study the coupled transcription and translation of several bacterial and plasmid genes in a DNA-directed cell-free system. The expression of different genes is reduced to different extents by inhibition of DNA gyrase activity. Among the genes tested, the *lac* operon, an rRNA gene, and the colicin gene of colicin E1 plasmid were found to be most sensitive, while the *trp* operon and some other genes in colicin E1 plasmid were relatively unaffected by the inhibitors. These results, together with earlier work on the transcription of circular DNA templates, indicate that DNA supercoiling can significantly enhance transcription from certain promoters.**

A number of *in vitro* studies in purified transcription systems have shown that negatively supercoiled DNA is a more efficient template than the same type of DNA in a relaxed form. The DNAs studied in this way include those obtained from the bacteriophages λ (1) and PM2 (2), the replicative forms of φX174 (3) and fd (4), and the plasmid colicin E1 (ColE1) (5). The conclusion from these studies is that supercoiling increases the amount of transcription both by raising the rate of transcription from existing initiation sites and by making new transcription sites available. The stimulation is promoter specific and varies over a wide range; it also depends upon the conditions (temperature, ionic strength, polymerase-to-DNA ratio, etc.) under which transcription is carried out. Seeburg *et al.* (4) have shown with the replicative form of fd that the rate of formation and the stability of the polymerase–DNA initiation complex are enhanced by supercoiling. From structural considerations alone it is clear that negative supercoiling favors unwinding of the double helix. Because the formation of the initiation complex is believed to require a partial unwinding of the double helix (6, 7), it seems likely that the stimulatory effect of supercoiling on transcription results mainly from the reduced activation energy required to form the initiation complex.

In view of the common occurrence of supercoiling in viral, plasmid, and chromosomal DNA (8), a thorough understanding of transcription and the regulation of transcription requires that the effect of supercoiling be carefully considered.

Investigations on the effects of supercoiling have been greatly facilitated by the discovery of the enzyme DNA gyrase, which catalyzes supercoiling (9), and of a number of antibiotics (nalidixic acid, oxolinic acid, novobiocin, and coumermycin) that specifically inhibit this enzyme (10–12). Before the target site of these drugs was known, observations had been made indicating a direct inhibition of transcription both *in vivo* (13–15) and *in vitro* (16). Most notable was a study by Shuman and Schwartz (15) which showed that nalidixic acid inhibited gene expression in a selective manner. Recently, the expression of the tryptophan operon in cells infected by the bacteriophage φ80p*trp* was measured (17). It was found that transcription of the *trp* operon in the phage DNA can originate at either the λP_L promoter or the *trp* promoter. By manipulating growth conditions it was possible to show that *trp* operon expression showed greater inhibition by coumermycin, novobiocin, or nalidixic acid when initiation of transcription was from the phage promoter. This has been the only report indicating a promoter-specific effect after the blockage of DNA gyrase activity by antibiotics. We have taken advantage of the existence of gyrase-specific antibiotics and of mutants refractory to coumermycin to investigate the effects of gyrase on the expression of a number of genes in a cell-free system.

MATERIALS AND METHODS

Chemicals. Coumermycin A_1 and oxolinic acid were gifts of W. F. Minor obtained from the Bristol Labs (Syracuse, NY) and the Warner-Lambert Co. (Hillside, NJ), respectively. Novobiocin and nalidixic acid were purchased from Sigma. [^{3}H]Leucine and [^{3}H]UTP were obtained from New England Nuclear. Restriction endonuclease *Eco*RI was obtained from New England Biolab.

Bacterial Strains and DNA Isolation. *Escherichia coli* K-12 strain 2089 (*lac*Δ514 *trp str*^{r}), which contains a deletion of the *lac* region including the *i* gene, has been described elsewhere (18). Strain N4182 is a derivative of 2089 that contains a *cou*^{r} mutation. It was prepared by P1 transduction, with phage grown on strain NI741 (9). Transductants were isolated by replica plating from LB agar plates (19) onto similar plates containing 25 μg of coumermycin per ml. λ DNAs were prepared from lysogens carrying λp*lac5* (18), λd*trpO*^{c}*w1* (18), and λ*rif*^{d}*18* (20). ColE1 DNA was prepared from strain 514 carrying the ColE1 plasmid. This strain was grown in L broth to late exponential phase followed by chloramphenicol induction for 16 hr. DNA was extracted from the cells by the clear lysate method (21) and further purified on a CsCl gradient with the addition of ethidium bromide (21). The supercoiled DNA was contained in the lower band from the CsCl gradient; its purity was estimated by gel analysis as greater than 90%. Relaxed closed-circular ColE1 DNA was made by a procedure described elsewhere (9). Linear ColE1 DNA was prepared by *Eco*RI treatment of the supercoiled DNA (22). *E. coli* DNA was made by a modification of the method of Clewell and Helinski (21).

Conditions for Cell-Free Synthesis. The DNA-directed coupled system for transcription and translation contains DNA, a crude S-30 extract of *E. coli*, and all the salts and substrates necessary for transcription and translation. Details of the composition and procedures used to make the S-30 extracts and

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U. S. C. §1734 solely to indicate this fact.

Abbreviation: ColE1, plasmid colicin E1.

assay procedures for β-galactosidase have been reported elsewhere (18, 23). DNA was added at 50 μg/ml of synthesis mixture. All reactions were carried out at 37°C for 20 min for rRNA synthesis and 60 min in all other cases.

Gel Analyses. ^{3}H-Labeled proteins were analyzed by electrophoresis on a 10–20% linear gradient polyacrylamide gel (24). ^{3}H-Labeled RNAs were analyzed by electrophoresis on a 2% agarose/0.5% polyacrylamide slab gel (25). Further details of these gel procedures, fluorography, and densitometry have been reported elsewhere (23). Supercoiled, relaxed, and linear forms of ColE1 DNA were analyzed by electrophoresis on a 1.5% agarose gel (25).

Colicin activity synthesized in the cell-free system was assayed by using the sensitive 514 strain according to the procedure of Hershman and Helinski (26). An end-point method of titration was used. Bacterial lawns were treated with colicin-containing extract over a wide range of dilutions. The dilution at which some clearing of the lawn was detectable after 6 hr was noted. In this way the relative amounts of colicin from different preparations could be quantitated within a factor of 2. As a control it was shown that strain 514 carrying the ColE1 plasmid was insensitive to the colicin-containing extracts.

RESULTS

The effect of gyrase inhibitors on coupled transcription–translation of several genetic loci was examined, in some cases, with the same locus contained in different molecular forms of DNA. Synthesis of proteins encoded by ColE1 plasmid DNA was studied by using supercoiled, relaxed circular, and linear forms of the DNA. The gene assignments for most of the protein products are not certain except for colicin E1, which is the 56,000-dalton protein (Fig. 1). In the absence of novobiocin (Fig. 1 *left*) the supercoiled and relaxed closed-circular DNAs were equally effective as templates. Novobiocin at 2.5 μg/ml (Fig. 1 *right*) had no effect on the quantity of the various protein products obtained when supercoiled template was used. By contrast, when relaxed closed-circular DNA was used as template, novobiocin caused a substantial reduction in the yield of some of the protein products. Thus, the yields of the 56,000- and the 27,500-dalton proteins, two of the more abundant proteins made in the DNA-directed S-30 system, were reduced to about one-third and one-half, respectively, of the amounts made in the system without novobiocin. Inspection of a number of the remaining protein peaks showed little or no sensitivity to DNA gyrase activity. Controls using an S-30 system from the coumermycin-resistant strain showed that novobiocin had little effect on the yield of any of the proteins (Fig. 1).

The expression of linear ColE1 DNA was also quite sensitive to novobiocin (Fig. 1). ColE1 DNA, converted to a linear form by *Eco*RI treatment, was only about one-seventh as efficient as the untreated DNA in directing colicin synthesis. This was due primarily to the fact that a large fraction of this small linear DNA was rapidly degraded in the S-30 system. About 80% of the linear DNA was converted to fragments soluble in 5% trichloroacetic acid after 20 min of incubation whereas less than 3% of the covalently circular DNA was solubilized during this time. In the presence of novobiocin, the yields of the 56,000- and 27,500-dalton proteins were reduced to about one-fourth and one-half, respectively, of the amounts made in the system without novobiocin, again only when the S-30 was prepared from the sensitive strain.

The bacteriocin activity of colicin E1 synthesized in the S-30 system was estimated by its effect on the growth of *E. coli* strain 514. The ratio of bacteriocin activity (measured to an accuracy of about a factor of 2; see *Materials and Methods*) to newly synthesized 56,000-dalton protein (determined by gel analysis)

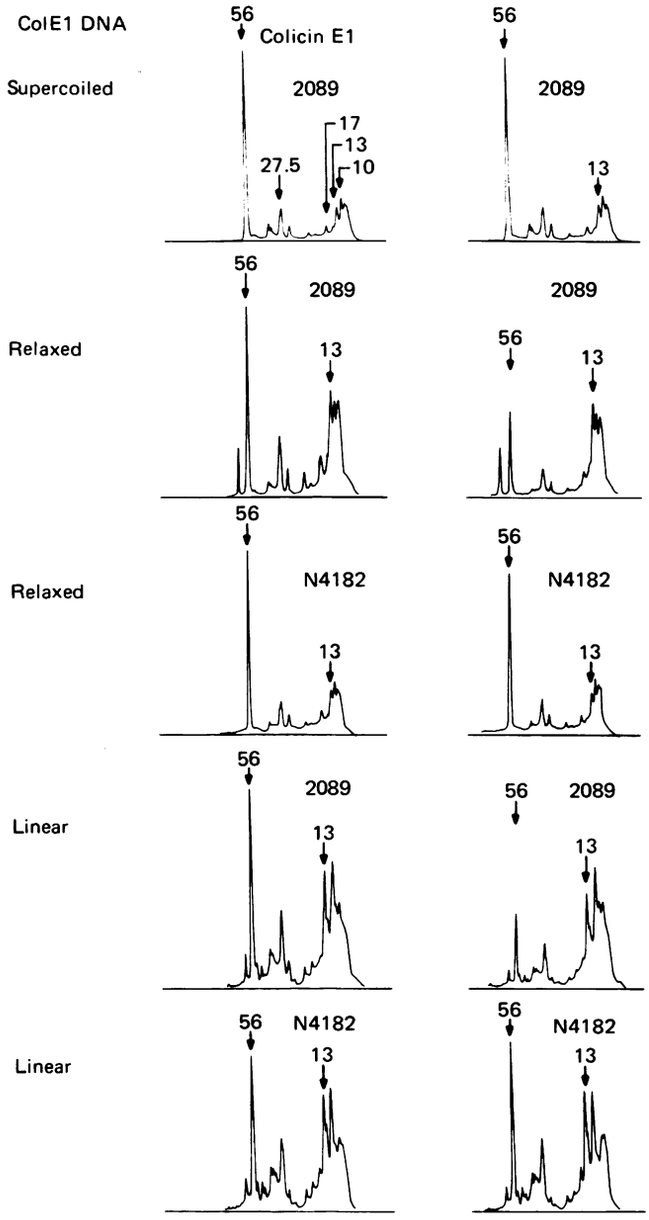

FIG. 1. Gel analysis of [^{3}H]leucine-labeled proteins obtained in the DNA-directed system by using ColE1 DNA in the supercoiled, relaxed, or linear form and S-30 derived from either strain 2089 or its novobiocin-resistant derivative N4182. (*Left*) No novobiocin present. (*Right*) Novobiocin at a concentration of 2.5 μg/ml was present during synthesis. Approximate sizes (in kilodaltons) of some proteins are given in the upper left frame. Pairs of gels, horizontally arranged, were subjected to fluorography and densitometry under identical conditions so that they could be quantitatively compared. See text for further discussion.

was the same when either supercoiled or relaxed circle was used as template in the S-30 system. Visible clearings were produced after 6 hr on a lawn of strain 514 by 10 μl of the incubation mixture. When linear ColE1 template was used to direct synthesis, this ratio was reduced to about one-eighth of the original, indicating that the ≈56,000-dalton protein derived from the latter mixture had much less biologic activity. The *Eco*RI-cleaved ColE1 DNA is cut near the COOH-terminal portion of colicin gene (see, e.g., ref. 27), which eliminates the bacteriocin activity of the resulting gene product (28). From this result it is tentatively concluded that the ≈56,000-dalton protein obtained from the linear ColE1 DNA-directed cell-free system

was a mixture containing mostly defective colicin missing a COOH-terminal portion of its polypeptide chain and a small fraction of completed colicin. This suggests that only a very small fraction of the linear DNA was ligated during cell-free synthesis.

After cell-free synthesis, the condition of the ColE1 DNAs was examined after phenol and pancreatic RNase A treatments. Gel analysis of the isolates showed that the fully supercoiled template was maintained during synthesis whereas about 80% of the relaxed circular form was converted to the fully supercoiled form in the absence of novobiocin. No discrete forms of DNA were detected when the linear DNA was used as template. Evidently not enough of this DNA was converted to the fully supercoiled form to be detectable, and much of the remainder was either wholly or partially degraded. These results indicate an effect of novobiocin that is both gene-specific and dependent on the molecular conformation of the DNA.

Larger effects of gyrase inhibitors were seen in some other systems. Expression of the *lac* operon of *E. coli* was studied with DNA from the virus λp*lac5*. In this system, cyclic AMP was used because it acts in conjunction with the activator protein CAP for optimal expression of the *lac* operon. The S-30 extracts used were prepared from strain 2089, which contains a deletion of the *lac* region including the *i* gene (for repressor), and strain N4182, a derivative of strain 2089, which carried a *cou*^{r} mutation (10). Under standard *in vitro* conditions, 1 hr at 37°C was allowed for synthesis. Gene expression was estimated by the amount of β-galactosidase synthesized, by use of a simple colorimetric assay. Synthesis was carried out at various levels of novobiocin from 0 to 7 μg/ml; the results are shown in Fig. 2. β-Galactosidase synthesis was 85% inhibited at 1 μg of antibiotic per ml. This residual β-galactosidase synthesis, which was refractory to inhibition by higher concentrations of antibiotic, may have been due to read-through from a λ initiation site, as explained below. Conclusive evidence for the involvement of gyrase in this inhibition comes from parallel experiments using S-30 extract made from the coumermycin-resistant strain (N4182). In this strain the β-galactosidase level was inhibited by only about 12% at 1 μg of novobiocin per ml, and even at 5 μg/ml only a 40% reduction was observed. Some inhibition in the coumermycin-resistant strain is to be expected from experiments that show that DNA gyrase from the *cou*^{r} strain is partly inhibited by high levels of novobiocin (unpublished results).

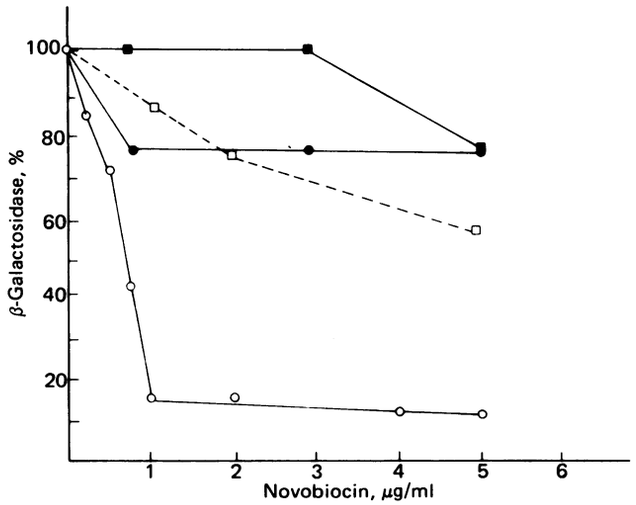

FIG. 2. DNA-directed β-galactosidase synthesis as a function of novobiocin concentration. All syntheses were 60 min in duration. Strains 2089 (circles) and its novobiocin-resistant derivative N4182 (squares) were used as sources of S-30 extracts. λp*lac5* (○, □) and λd*trpO*^{c}*W1* DNA (●, ■) were used to test the activities of the *lac* operon and the *trp* operon, respectively.

The effect of coumermycin on λp*lac5* DNA-directed β-galactosidase synthesis was also measured. When the S-30 extract was made from strain 2089, β-galactosidase synthesis was 50% inhibited by 0.5 μg of novobiocin or 0.1 μg of coumermycin per ml (Table 1).

The antibiotics oxolinic acid and nalidixic acid affect a second protein component necessary for DNA gyrase activity (11, 12). Of these two drugs, only oxolinic acid was studied here because it is substantially more potent. High levels of oxolinic acid (40 μg/ml) were required to bring about a 50% inhibition of β-galactosidase synthesis; this level is comparable to that required to inhibit the activity of purified DNA gyrase (11, 12). The effect of oxolinic acid was also tested with S-30 extracts from strain N4182 carrying the *cou*^{r} mutation. *A priori* it was expected that these extracts would be resistant to coumermycin but not to oxolinic acid. The anticipated resistance to oxolinic acid was also seen (see Table 1). Although the mechanism of this cross resistance is not understood in detail, we have found that the *cou*^{r} mutation enhances oxolinic acid resistance of the growth of strain N4182 (unpublished observations).

It has been shown elsewhere that when λp*lac5* DNA is used to direct β-galactosidase synthesis some of the synthesis results from initiation of transcription at the *lac* promoter and some from a promoter to the left, probably in the b2 region of λ (29). The latter synthesis is insensitive to cyclic AMP whereas that synthesis which originates from the *lac* promoter is almost totally dependent upon the presence of cyclic AMP. The amount of β-galactosidase synthesis was reduced to about 20% when cyclic AMP was omitted. We found that this "residual" β-galactosidase synthesis was insensitive to novobiocin when an S-30 extract from either strain 2089 or N4182 was used, suggesting that transcription originating in the b2 region of λ was not stimulated by gyrase activity.

The evidence just presented suggested that interactions at the initiation point of transcription may be most susceptible to the presence of novobiocin. To show this more directly we operationally separated the processes of transcription and translation by initiating RNA synthesis in the absence of amino acids. Novobiocin (2.5 μg/ml) was added either at zero time or at 12 min, at which time initiation of RNA synthesis was also halted by rifampicin and translation was allowed to begin by amino acid addition. In the former case full-scale inhibition was seen, whereas in the latter case no inhibition was detected. Such studies showed that the inhibiting effect of novobiocin on β-galactosidase synthesis was confined to the period prior to rifampicin addition. This observation supports the notion that the main inhibitory effect of novobiocin relates to its effect on the initiation of transcription.

In the reaction directed by λp*lac5* DNA, it seems likely that a large fraction of the DNA becomes covalently circular during the reaction and, thus, the involvement of DNA gyrase is readily

Table 1. λp*lac5* DNA-directed synthesis of β-galactosidase by use of S-30 extract derived from normal (2089) and coumermycin-resistant (N4182) cells and various drug inhibitors*

Strain	Coumermycin, 0.3 μg/ml	Novobiocin, 2.5 μg/ml	Oxolinic acid, 100 μg/ml
2089	22	18	13
N4182	95	96	41

* Results are reported as percent of β-galactosidase synthesis in the absence of drug. Control is considered to have 100% synthesis.

explainable. However, effects of gyrase inhibitors are seen even when the *lac* operon is added in the form of a DNA that cannot readily be circularized. The DNA used in these experiments was total *E. coli* DNA prepared by a method which gives a fragmented DNA with an average size of about 10^7 daltons (see *Materials and Methods*). The β-galactosidase yield with this DNA under standard conditions was about 0.4% of that obtained when an equivalent amount of λp*lac*5 DNA was used. This was consistent with the lower content of *lac* operon in *E. coli* DNA. When 2089 S-30 was used, novobiocin (2.5 μg/ml) reduced the β-galactosidase yield by 62%; this is a smaller reduction than was seen with λp*lac*5 DNA (see Table 1). If N4182 S-30 was used, only a 10% reduction in β-galactosidase yield was seen. Thus, as with λp*lac*5 DNA, the inhibiting effect of novobiocin in 2089 S-30 appears to be due to its effect on gyrase. As in the case of linear ColE1 DNA, discussed above, it appears from this result that transcription from a linear template is sensitive to inhibition of DNA gyrase.

The effect of gyrase inhibitors on transcription of some other genes was also studied to investigate further the differential sensitivity of different promoters. *A priori* it seemed likely that the importance of gyrase and supercoiling to gene expression might vary appreciably from one gene to another due to the varied structures of different promoters (see, e.g., ref. 30). The *trp* operon was examined with the aid of λd*trpO*^{c}*W1* DNA, which contains the *trp* promoter fused to the *z* gene for β-galactosidase. Previous studies had shown that expression of the *z* gene in this fusion behaves as if it were under normal *trp* operon control (31). This DNA also has a constitutive mutation in the *trp* operator (O^c) so that it is not sensitive to *trp* repressor. In experiments parallel to those described above, the effects of novobiocin on normal *lac* expression and *trp–lac* expression were compared in normal and novobiocin-resistant cells (see Fig. 2). The results show that *trp* expression has a much lower sensitivity to novobiocin. Indeed, the inhibition seen (15–25%) at 5 μg of novobiocin per ml is almost the same in normal and mutant extracts.

The influence of gyrase inhibition on transcription of ribosomal RNA genes was also examined by using λ*rif*^{d}*18*, which contains one of the seven rRNA cistrons (*rrnB*) of *E. coli* (32). In this case, gene activity was estimated from the amounts of mature 16S and 23S radioactive rRNA made in the standard cell-free system with [^{3}H]UTP. After synthesis and deproteinization, the crude nucleic acid-containing extract was analyzed by gel electrophoresis and fluorography. Novobiocin at 2.5 μg/ml caused a reduction in synthesis of 16S and 23S RNAs of one-sixth to one-seventh that of the system without novobiocin (compare Fig. 3 *A* and *B*) when the standard S-30 extract was used. When the S-30 extract was prepared from the coumermycin-resistant strain, no reduction (<10%) in rRNA synthesis was seen (results not shown).

Whereas gyrase has been demonstrated to be the target of the drugs in question, it could be argued that DNA stability and not DNA structure might be the primary cause of lowered gene expression in the presence of the gyrase-inhibiting drugs. To test this possibility, we estimated the extent of degradation of the DNA at various times after starting synthesis by measuring the trichloroacetic acid-soluble radioactivity obtained from [^{3}H]thymidine-labeled DNA. By this assay all the λ and circular ColE1 DNAs used were found to be greater than 96% stable after 1 hr. Linear ColE1 DNA and linear fragments of *E. coli* chromosomal DNA were 50% solubilized after 10 and 20 min, respectively. In no cases did novobiocin influence the degradation rates. Thus, it does not appear that DNA stability is significantly influenced by novobiocin.

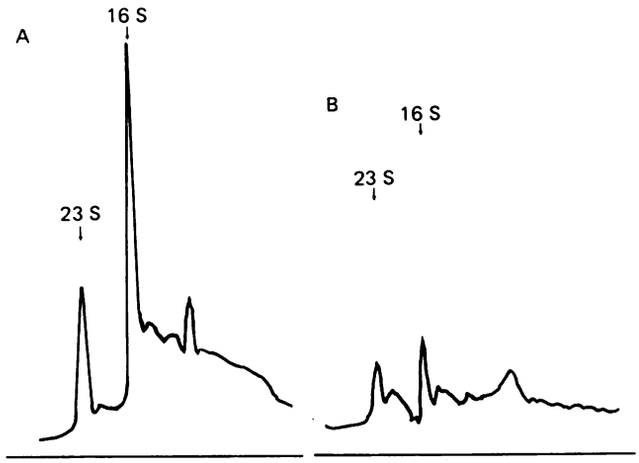

FIG. 3. Gel analysis of ^{3}H-labeled RNA made in the λd*rif*^{d}*18* DNA-directed cell-free system in the presence (*B*) and absence (*A*) of 2.5 μg of novobiocin per ml, with S-30 from strain 2089. Details for obtaining these densitometry tracings are given in *Materials and Methods*.

DISCUSSION

Two aspects of this work deserve particular attention. First, transcription of different genes shows quite different sensitivities to inhibitors of DNA gyrase. DNA gyrase is shown to be the target of the inhibitors by the observation that the action of coumermycin and novobiocin is mostly eliminated when the usual cell-free extract used in RNA and protein synthesis is replaced by an extract prepared from a mutant containing a coumermycin-resistant gyrase. In correlation with similar results of *in vivo* experiments (17), these findings open the possibility that DNA supercoiling might play a regulatory role in transcription. It is possible that the metabolic state of the cell modulates the extent of supercoiling of the template, thereby influencing the transcription activity of genes in a differential manner. To explore this question further, *in vitro* studies with different gene systems might be helpful to see if consistent patterns of sensitivity can be found. For example, do other genes associated with catabolism, like the *lac* operon, show a higher sensitivity than genes associated with anabolism, like the *trp* operon?

Second, the effect of DNA gyrase activity on transcription seems to be expressed through its alteration of DNA structure. This is seen most readily in our results with ColE1 DNA, where transcription of the relaxed form is sensitive to inhibitors of DNA gyrase while transcription of the supercoiled form is unaffected. If one follows this line of reasoning, it is then puzzling that transcription of linear DNA (linear fragments of *E. coli* chromosomal DNA and ColE1 DNA cleaved by endonuclease *Eco*RI) is also sensitive to DNA gyrase inhibitors. It is conceivable that axial rotation even of DNA molecules with free ends is sufficiently hindered in the coupled transcription–translation system so that the equivalent of a superhelical strain can be built up. There is a precedent for a requirement for DNA gyrase activity in the function of a linear DNA molecule; the *in vivo* replication of phage T7 DNA is known to be blocked by coumermycin (33).

Note Added in Proof. Recently we have examined the sensitivity to novobiocin of various genes in the plasmid pBR322. This was done as with ColE1 by using the relaxed circular form of the DNA in the absence or presence of novobiocin. Whereas the yield of β-lactamase was unaffected by novobiocin, the yield of the 34,000-dalton tetracycline resistance protein was reduced to 16%.

We are grateful to Mrs. Catherine Anderson for technical assistance. This work was supported by grants from the National Institutes of Health (GM16648-10 and AI13277-02) to G.Z.

1. Botchan, P. (1976) *J. Mol. Biol.* **105,** 161–176.
2. Richardson, J. P. (1975) *J. Mol. Biol.* **91,** 477–487.
3. Hayashi, Y. & Hayashi, M. (1971) *Biochemistry* **10,** 4212–4218.
4. Seeburg, P. H., Nüsslein, C. & Schaller, H. (1977) *Eur. J. Biochem.* **74,** 107–113.
5. Levine, A. & Rupp, W. D. (1978) in *Microbiology,* ed. Schlessinger, D. (Am. Soc. Microbiol., Washington, DC) pp. 163–165.
6. Saucier, J. M. & Wang, J. C. (1972) *Nature (London) New Biol.* **239,** 167–170.
7. Johnsrud, L. (1978) *Proc. Natl. Acad. Sci. USA* **75,** 5314–5318.
8. Champoux, J. J. (1978) *Annu. Rev. Biochem.* **47,** 449–479.
9. Gellert, M., Mizuuchi, K., O'Dea, M. H. & Nash, H. A. (1976) *Proc. Natl. Acad. Sci. USA* **73,** 3872–3876.
10. Gellert, M., O'Dea, M. H., Itoh, T. & Tomizawa, J.-I. (1976) *Proc. Natl. Acad. Sci. USA* **73,** 4474–4478.
11. Gellert, M., Mizuuchi, K., O'Dea, M. H., Itoh, T. & Tomizawa, J. (1977) *Proc. Natl. Acad. Sci. USA* **74,** 4772–4776.
12. Sugino, A., Peebles, C. L., Kreuzer, K. N. & Cozzarelli, N. R. (1977) *Proc. Natl. Acad. Sci. USA* **74,** 4767–4771.
13. Smith, D. H. & Davis, B. D. (1967) *J. Bacteriol.* **93,** 71–79.
14. Puga, A. & Tessman, I. (1973) *J. Mol. Biol.* **75,** 99–108.
15. Shuman, H. & Schwartz, M. (1975) *Biochem. Biophys. Res. Commun.* **64,** 204–209.
16. Staudenbauer, W. L. (1976) *Eur. J. Biochem.* **62,** 491–497.
17. Smith, C. L., Kubo, M. & Imamoto, F. (1978) *Nature (London)* **275,** 420–423.
18. Zubay, G. (1974) *Annu. Rev. Genet.* **7,** 267–287.
19. Rosner, J. L. (1972) *Virology* **48,** 679–689.
20. Kirschbaum, J. B. & Konrad, E. B. (1970) *J. Bacteriol.* **116,** 517–526.
21. Clewell, D. B. & Helinski, D. R. (1969) *Proc. Natl. Acad. Sci. USA* **62,** 1159–1166.
22. Greene, P. J., Betlach, M. C., Goodman, H. M. & Boyer, H. W. (1974) *Methods Mol. Biol.* **7,** 87–111.
23. Yang, H.-L., Zubay, G. & Levy, S. B. (1976) *Proc. Natl. Acad. Sci. USA* **73,** 1509–1512.
24. Studier, W. (1973) *J. Mol. Biol.* **79,** 237–248.
25. Barnes, W. M. (1976) *Science* **195,** 393–394.
26. Hershman, H. R. & Helinski, D. R. (1967) *J. Biol. Chem.* **242,** 5360–5365.
27. Yang, H.-L. & Zubay, G. (1978) in *Microbiology,* ed. Schlessinger, D. (Am. Soc. Microbiol., Washington, DC), pp. 154–155.
28. Hershfield, V., Boyer, H. W., Yanofsky, C., Lovett, M. A. & Helinski, D. R. (1974) *Proc. Natl. Acad. Sci. USA* **71,** 3455–3459.
29. Yang, H.-L. & Zubay, G. (1974) *Biochem. Biophys. Res. Commun.* **56,** 725–731.
30. Chamberlin, M. J. (1976) in *RNA Polymerase,* eds. Losick, R. & Chamberlin, M. J. (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY).
31. Zubay, G., Morse, D. E., Schrenk, W. J. & Miller, J. H. M. (1972) *Proc. Natl. Acad. Sci. USA* **69,** 1100–1103.
32. Lindahl, L., Yamamoto, M. & Nomura, M. (1977) *J. Mol. Biol.* **109,** 23–47.
33. Itoh, T. & Tomizawa, J. (1977) *Nature (London)* **270,** 78–80.