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An ATP-dependent supercoiling topoisomerase of *Chlamydomonas reinhardtii* affects accumulation of specific chloroplast transcripts

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ABSTRACT

We have found that Chlamydomonas reinhardtii cells contain an ATP-dependent topoisomerase activity that supercoils circular DNA in vitro. Subsequent addition of a type I topoisomerase eliminates the supercoils. Like bacterial gyrase, this activity is inhibited by low concentrations of novobiocin (<0.1 μM) and by nalidixic acid (<0.1 mM). We have examined the effects of these topoisomerase inhibitors on accumulation of various chloroplast transcripts in vivo. Novobiocin differentially affected such transcripts; some transcripts became more abundant while many others were reduced in the presence of this drug. Nalidixic acid on the other hand caused many transcripts to become more abundant albeit to varying degrees.

Inhibitors of this algal topoisomerase specifically stimulate a family of related transcripts which we have previously shown to be under light-dark control. We discuss how the inhibitors of this topoisomerase might exert their in vivo effects.

INTRODUCTION

Photosynthesis requires precise interactions, in time and space, of many chloroplast proteins and membrane components (reviewed in ref. 1). In part, such interactions must depend upon regulation of the corresponding genes. Therefore, regulation of chloroplast genes, including effects of nuclear genes and of environmental stimuli on this regulation, is of great interest. Here we present evidence that an ATP-dependent supercoiling topoisomerase plays an important role in this regulation.

Bacterial DNA gyrase is inhibited by novobiocin (2) and can be purified by affinity chromatography employing a novobiocin-sepharose column (3). Using such a column, we have partially purified an ATP-dependent topoisomerase activity from whole Chlamydomonas reinhardtii cells. This activity supercoils DNA in vitro, and such supercoiled DNA is relaxed upon subsequent addition of a type I topoisomerase. The supercoiling activity is inhibited by novobiocin and by nalidixic acid, a drug which also inhibits bacterial gyrase (2).

In order to test for functioning of this topoisomerase in chloroplast

transcription, we have studied the effects of inhibitors of this enzyme (i.e. novobiocin and nalidixic acid) on accumulation of various chloroplast transcripts. In bacteria and their phages, these inhibitors and also mutations that alter gyrase activity have profound effects on transcription (reviewed in refs. 2 and 4). Novobiocin (or coumermycin) and nalidixic acid (or oxilinic acid) specifically inhibit the ATPase and nicking-closing activities, respectively, of DNA gyrase (2) thus causing a more relaxed state of the DNA (5,6). This change in DNA conformation, in turn, is thought to be one means by which these drugs affect transcription (2,7). Nalidixic acid, but not novobiocin, also damages the DNA and induces the SOS response (8). Consequently, the effects of these drugs on transcription are not identical.

Chloroplast transcripts were studied by probing Northern blots of total cellular RNA with cloned chloroplast DNA fragments (Fig. 1), by probing Southern blots of restriction digests of total chloroplast DNA with in vitro labeled RNA, or by staining agarose gels with ethidium bromide. Of particular interest is transcript A which we have previously shown to be under light-dark control (11). Our results show that accumulation of this and various other chloroplast transcripts is affected by both novobiocin and nalidixic acid.

MATERIALS AND METHODS

Materials

Algal strains CC-278 and CC-406 have been described elsewhere (11). Novobiocin (sodium salt) and nalidixic acid (free acid) were from Sigma. Epoxy-activated sepharose (6B) was from Pharmacia. T4 DNA polymerase was purified by Dr. L. Rowen in our laboratory and T4 polynucleotide kinase was purified by Dr. B. Walker. Topoisomerase I (calf thymus) was from Bethesda Research Laboratories and was used as prescribed by this supplier unless indicated otherwise. DNAase (RNAase free) was from Miles Laboratories. Restriction endonucleases were from Bethesda Research Laboratories and Boehringer Mannheim (Eco RI). Deoxynucleotide triphosphates were from PL Biochemicals, 5'-[α-^{32}P]dCT (~800 Ci mmol^{-1}) was from Amersham and 5'-[γ-^{32}P]ATP (~3000 Ci mmol^{-1}) was from New England Nuclear.

Plasmids pCP43, containing restriction fragment Eco RI 14, and pCP55, containing restriction fragment Eco RI 27 of the Chlamydomonas chloroplast chromosome (see Fig. 1 below), were obtained from J.D. Rochaix, University of Geneva, Geneva, Switzerland. Plasmid pRT1 has been described (11). It contains a 0.8 kb subfragment of restriction fragment Hpa II 5 (~8.0 kb) of the chloroplast chromosome.

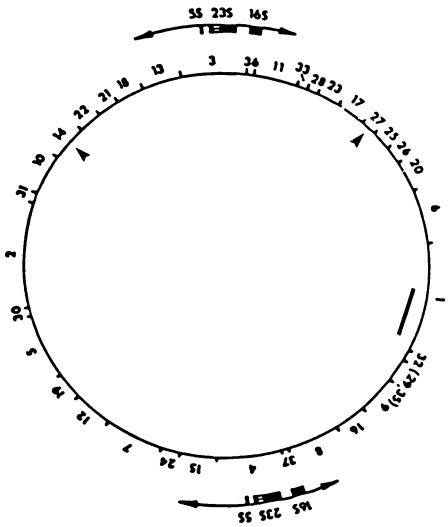

Fig. 1. Cloned chloroplast DNA fragments used as probes for Northern blots. Eco RI sites on the chloroplast chromosome (~190 Kb) are shown (9). Probes Eco RI 14 (~5.6 Kb) and Eco RI 27 (~2.7 Kb) (arrows) containing the genes rbcL (LS) and psbD (D2), respectively, were obtained from plasmids pCP43 and pCP55, respectively. Note that other workers (10) have called Eco RI fragments 14 and 27, 15 and 3, respectively. A third probe is a 0.8 Kb subfragment of restriction fragment Hpa II 5 (~8.0 Kb) and was obtained from plasmid pRT1 (11). Hpa II 5 is contained completely within an ~8.5 Kb Bam HI/Bgl II subfragment (bar in figure) of Eco RI 1 (~17.4 Kb) (11). The transcripts which hybridize to these various probes are described in the Results.

Preparation Of A Crude Protein Fraction Containing An ATP-Dependent Topoisomerase

Light-grown cells (4 l) in the logarithmic growth phase (~5 x 10^6 cells/ml) were pelleted, washed once with 50 ml 100 mM KCl, 25 mM Hepes, pH 8.0, and resuspended in 5 ml ice-cold buffer A (25 mM Hepes, pH 8.0, 200 mM KCl, 1 mM EDTA, 1 mM DTT, 0.5 mM PMSF, 10% ethylene glycol) containing 20 mM $MgCl_2$ and 10 mM PMSF (12). This suspension was frozen in dry ice-ethanol, thawed at 4°C and then made 0.7% in Triton X-100. After incubation for 15 min, the lysate was spun in an Eppendorf centrifuge for 10 min. The supernatant was collected by pouring and centrifuged again for 90 min at 30,000 rpm in a Ti 65 rotor. Two phases resulted from this centrifugation, a lower green phase and an upper yellowish phase. These crude protein fractions could not be directly tested for DNA supercoiling activity because they contained large amounts of nucleic acids which interfered with the

supercoiling assay (described below). The upper phase was collected and loaded directly onto a novobiocin affinity column (see below).

Affinity Chromatography

Novobiocin-sepharose was prepared as described in ref. 3. A column (1 ml bed volume) was equilibrated with buffer A and loaded with the crude lysate prepared as described above. After washing the column with 5-6 ml of buffer A, proteins were eluted with 5 M urea in buffer A. About 300 µl of eluate were collected and discarded and then one 1 ml fraction was collected and dialyzed against buffer A with KCl reduced to 50 mM. All operations were performed at 4°C. The dialyzed column eluate was assayed for supercoiling activity as described below.

Supercoiling Assay

Reactions in a final volume of 10 µl contained 5 µl enzyme extract, 25 mM KCl, 7.5 mM $MgCl_2$, 1 mM DTT, 2 mM ATP, 2 mM spermidine and 0.5 µg plasmid pRT1 that had been relaxed with calf thymus topoisomerase I. Incubation was at 25°C for 10 hrs. Samples were then loaded onto a 1% agarose (SeaKem, FMC Corporation) gel and electrophoresed in TBE buffer (90 mM Tris base, 90 mM Boric acid, 2.5 mM EDTA) for 12-18 hrs at 40 mV. Gels were stained with ethidium bromide (5 µg/ml) and photographed under UV light. In some experiments, gels were used to prepare Southern blots (13) which were probed with ^{32}P-labeled linearized plasmid DNA. This greatly increased the sensitivity of detection of supercoiled DNA.

Novobiocin And Nalidixic Acid Treatment

Medium (11) containing varying amounts of novobiocin or nalidixic acid was inoculated to 1.5×10^5 cells/ml with fresh light-grown cells. Novobiocin was added directly as the powder or from a 50 mg/ml stock solution in H_2O. Nalidixic acid was added from a 10 mg/ml stock solution in 0.1 N NaOH. Stock solutions were prepared immediately prior to use. Cultures were grown in the light (~2000 lux; cool-white fluorescent light) at 25°C for 60-70 hrs and were then harvested by centrifugation (2000 x g for 3 min).

Northern Blot Analyses

Northern blots (14) of total cellular RNA were prepared and probed as described previously (11) except that 50% formamide was used in all hybridizations and all washes were done at 42°C. Probes were ^{32}P-labeled by the replacement synthesis method (15).

Southern Blot Analyses

Chloroplast DNA was extracted (16) and purified by two rounds of cesium chloride density centrifugation. Twelve µg of DNA were cut with Hpa II,

fractionated in a 0.7% agarose gel and blotted onto nitrocellulose (Schleicher and Schull) (13). The nitrocellulose sheet was cut into 5 mm wide strips. Cellular RNA (11) was ^{32}P-labeled as follows. Twenty µg of each sample were first treated with DNAase (20 µg/ml) for 15 min at 37°C. Following phenol/chloroform extraction, RNA was precipitated, washed and resuspended in 30 µl water. One µg of RNA was diluted into 25 µl of 100 mM Tris, pH 9.5, and heated at 90°C for 40 min. The volume was adjusted to 50 µl with 5 µl 50% glycerol, 5 µl $MgCl_2$ (0.1 M), 5 µl DTT (50 mM), 10 µCi 5'-[γ-^{32}P]ATP and an appropriate amount of water. Two µl T4 polynucleotide kinase (~20 units) were added and after 1 hr at 37°C the reactions were stopped and further processed as described in ref. 17. Sister strips of the Southern blot of chloroplast DNA were probed (17) with algal RNA prepared from control cells and from novobiocin or nalidixic acid treated cells.

RESULTS

Chlamydomonas Contains An ATP-Dependent Supercoiling Topoisomerase

For several reasons (refs. 18 and 19, R.J. Thompson, Ph.D. Thesis, Vanderbilt University, 1982, and the chloroplast transcription results described below) we suspected that the chloroplast of C. reinhardtii might contain a DNA topoisomerase which, like bacterial gyrase, is sensitive to novobiocin and to nalidixic acid. Novobiocin-sepharose affinity chromatography has been used to purify DNA gyrase from bacteria (3) and to purify a novobiocin binding protein from mouse cells that is required for DNA topoisomerase II activity (20). Using this technique (see Methods for details), we have obtained a protein fraction from whole cells which exhibited DNA supercoiling activity in vitro (Fig. 2). This protein fraction had low activity, however, and for this reason the topoisomerase reactions for the assays shown in Figure 2 were incubated for 10 hr. Qualitatively similar results were observed after shorter incubation times (e.g. 2 hrs) with this and other topoisomerase preparations. Note that all the reactions assayed in Figure 2 were incubated under similar conditions. Thus, the higher order supercoiling seen in the lane labeled "+ enzyme + ATP", as compared with the "substrate" or "+ enzyme - ATP" lanes, cannot be attributed to effects of changing temperature or ionic strength. The supercoiling activity required ATP (Fig. 2) and, like bacterial gyrase, was inhibited by low doses (<0.1 µM) of novobiocin and by significantly higher doses (<0.1 mM) of nalidixic acid (Fig. 3). Other eukaryotic ATP-dependent topoisomerases require much higher concentrations of novobiocin (>0.1 mM) to be inhibited (2 and 21) and, to our knowledge, are affected little, if at

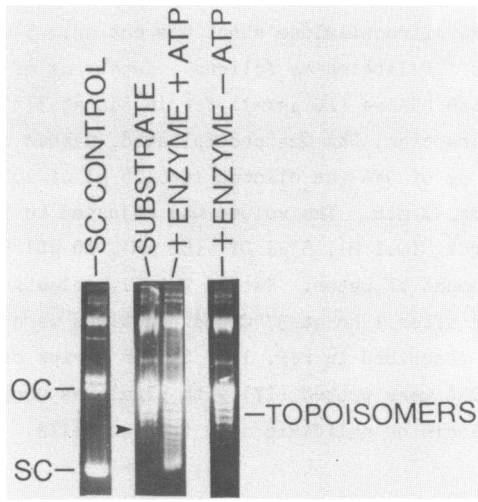

Fig. 2. Chlamydomonas contains an ATP-dependent topoisomerase. A protein fraction eluted from a novobiocin-sepharose column was tested for its ability to supercoil relaxed closed circular DNA. Details are described in Methods. OC, relaxed closed circular DNA plus nicked circles; SC, supercoiled DNA. The arrowhead is discussed in the legend to Figure 3.

all, by nalidixic acid. The algal enzyme also supercoiled relaxed pBR322 DNA (data not shown).

The experiment in Figure 3 employed the same topoisomerase preparation that was used in Figure 2. However, because adequate storage conditions had not been worked out, the supercoiling activity decayed rapidly (i.e., within 24 hrs). For this reason, the reaction products that were more supercoiled than the substrate were blotted onto nitrocellulose and hybridized to ^{32}P-labeled DNA (Fig. 3). This greatly increased the sensitivity of product detection.

Binding of proteins to the circular DNA cannot be responsible for the appearance of the more supercoiled bands since deproteinization by SDS/proteinase K treatment (Fig. 4) or by phenol extraction (data not shown) did not alter the mobility of these bands. Figure 4 also shows the results of an experiment in which relaxed circular DNA supercoiled by the algal topoisomerase was subsequently treated with calf-thymus topoisomerase I (a DNA relaxing enzyme). If the algal supercoiling activity results from a relaxing enzyme which works in concert with a DNA binding protein that wraps the DNA around itself, then addition of topoisomerase I should not

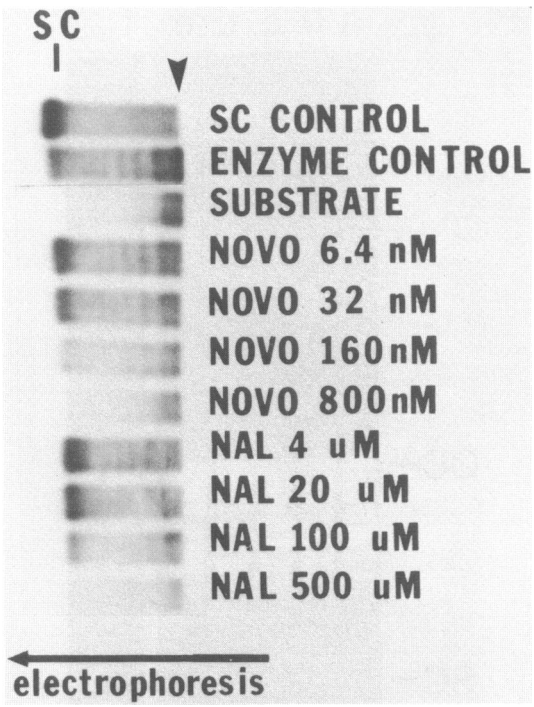

Fig. 3. Sensitivity of the ATP-dependent topoisomerase to novobiocin and nalidixic acid. Reactions were carried out as described in Methods in the presence of the indicated drug concentrations. An agarose gel similar to the one in Figure 2 was cut roughly one half of the way between the OC and SC markers. The arrowhead corresponds to the position of the gel indicated by the arrowhead in Figure 2. The portion of the gel containing product (SC) was used to make a Southern blot which was then probed with linearized plasmid. Note that the band seen in the lane labeled "substrate" is the most supercoiled topoisomer of the substrate and not fully relaxed DNA.

inhibit formation of the supercoiled product or relax the supercoils which were formed (22,23). Clearly, the topoisomerase I caused disappearance of the supercoiled product (Fig. 4). We thus believe that the algal supercoiling activity involves an enzyme which functions similarly to bacterial DNA gyrase.

In addition to the ATP-dependent supercoiling topoisomerase, Chlamydomonas cells also yielded a DNA-nicking-closing activity which did not require ATP, did not supercoil DNA in vitro and was not inhibited by novobiocin or nalidixic acid (data not shown). This activity was obtained from a novobiocin-sepharose column when crude extracts, which were not

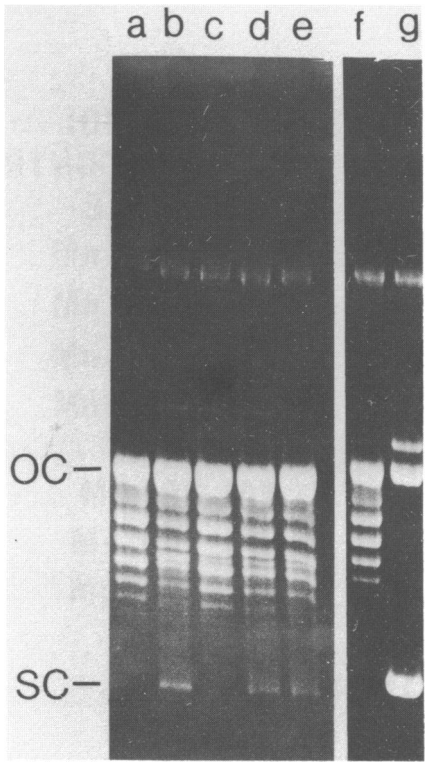

Fig. 4. The ATP-dependent topoisomerase is a supercoiling enzyme. A standard reaction mixture containing the algal topoisomerase (see Methods) was scaled up five-fold, incubated for 10 hr and then four 10 µl aliquots were removed. One sample received nothing (lane e), one received topoisomerase I reaction buffer (lane d) and a third was treated with 1 U of calf-thymus topoisomerase I for 1 hr (lane c). SDS and proteinase K were added to the fourth sample (final concentrations 0.2% and 100 µg/ml, respectively) and incubation was continued for 1 hr (lane b). Supercoiled DNA (0.5 µg) was incubated for 10 hr in the absence of algal topoisomerase (lane g) and subsequently with 1 U of topoisomerase I under the same conditions as in lane c (lane f). The relaxed substrate is shown in lane a.

first separated into two phases by ultracentrifugation (see Methods) were loaded onto the column. Since trace amounts of this activity may have been present in the ATP-dependent topoisomerase preparation and could have partially relaxed the supercoiled product formed, we cannot, at this time, say if the supercoiling occurs in steps of two.

Novobiocin Differentially Affects Chloroplast Transcripts

Since chloroplast genomes have many similarities with bacterial genomes (24), since bacterial gyrase is important in transcription (7), and since

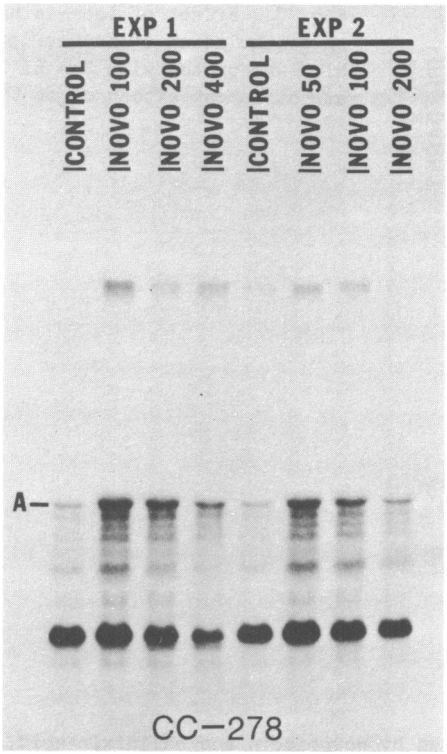

Fig. 5. Effects of increasing doses of novobiocin on accumulation of transcript A. Northern blots of total cellular RNA (10 μg per lane) were probed with the 0.8 Kb subfragment of Hpa II 5 contained within plasmid pRT1 (see Materials). Different RNA samples were used in experiments 1 and 2.

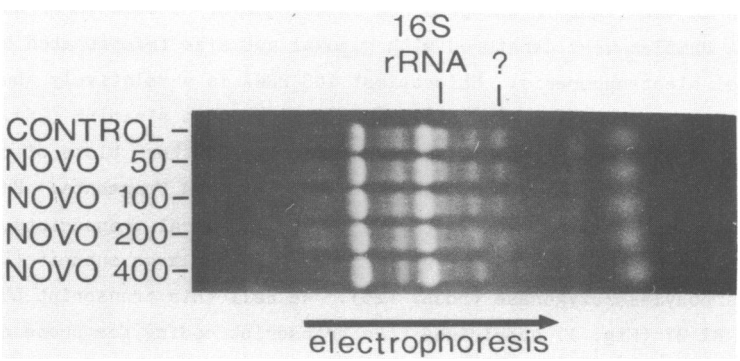

Fig. 6. Effect of increasing doses of novobiocin on accumulation of 16S rRNA. Denatured total cellular RNA (30 μg per lane) was electrophoresed in a 1% agarose gel at 120 V for 6 hrs and then stained with ethidium bromide (5 μg/ml for 1 hr). The identity of transcript ? is unknown.

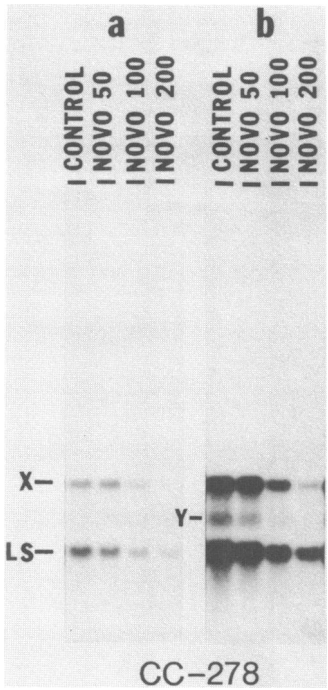

Fig. 7. Effect of increasing doses of novobiocin on accumulation of transcript LS. A Northern blot was probed with Eco RI 14. Panels a and b are different exposures of the same blot.

this enzyme is inhibited by novobiocin and nalidixic acid, we asked whether these drugs affect accumulation of specific chloroplast transcripts. We have measured the abundance of specific chloroplast transcripts by Northern blot hybridizations to specific labeled chloroplast DNA fragments. Total cellular RNA was prepared from CC-278 cells (or from CC-406 cells) which were grown in the light in the presence or absence of novobiocin or nalidixic acid. RNA samples were denatured with glyoxal and size fractionated by agarose gel electrophoresis. Chloroplast 16S rRNA is a relatively abundant species and was visualized directly by ethidium bromide staining (25). Less abundant transcripts were analyzed by probing Northern blots of the RNA fractionated by size with different chloroplast DNA fragments. Probe Eco RI 14 (Fig. 1) has been shown to hybridize to several transcripts, the smallest and most abundant of which codes for the large subunit (LS) of RuBP carboxylase/oxygenase (rbcL) (25). We call this transcript LS. Probe Eco RI 27 (Fig. 1) hybridizes to a transcript coding for protein D2 (psbD), a PS II associated protein (26). We call this transcript D2. Other transcripts which hybridize to the Eco RI 3 and 14 probes are described below. A third probe derived from restriction fragment Hpa II 5 is described

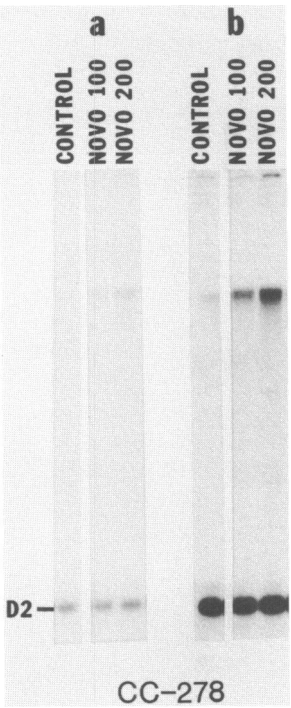

Fig. 8. Effect of increasing doses of novobiocin on accumulation of transcript D2. A Northern blot was probed with Eco RI 27. Panels a and b are different exposures of the same blot.

in Figure 1. This probe hybridizes to transcript A whose function is unknown (11). This transcript is actually the largest member of a family of overlapping transcripts which originate from Hpa II 5 (11). We have previously shown (11) that transcript A, as well as the other members of this family, overaccumulates when certain strains (i.e. CC-278) are grown in the dark versus in the light. We emphasize the results for transcript A because it is the largest and most strongly affected transcript of the family that overaccumulates in the dark.

Certain doses of novobiocin, within a rather narrow range, clearly stimulated accumulation of transcript A (Fig. 5). Maximum accumulation occurred at 100-200 μg/ml. At higher concentrations (400 mg/ml), the abundance of transcript A declined and became similar to that in control cells. In contrast, at these same doses, novobiocin clearly inhibited accumulation of chloroplast 16S rRNA (Fig. 6) and of transcripts LS, X and Y (Fig. 7). The functions of transcripts X and Y are unknown. Novobiocin had little if any effect on the D2 transcript (Fig. 8). Together, these results clearly show that novobiocin differentially affected accumulation of certain chloroplast transcripts, specifically it enhanced transcript A. Novobiocin did

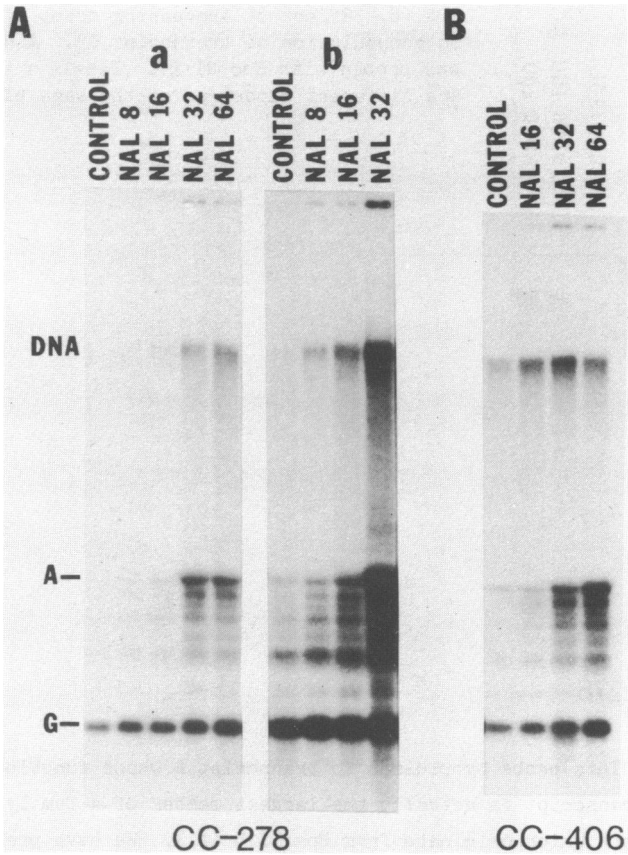

Fig. 9. Effect of increasing doses of nalidixic acid on accumulation of transcript A. Northern blots of RNA from strains CC-278(A) and CC-406(B) were probed with the 0.8 Kb insert of plasmid pRT1. Panels a and b are different exposures of the same blot. Transcript G overlaps with and may be derived from transcript A (11).

not cause overaccumulation of transcript A solely by inhibiting processing or degradation to discrete RNA species of smaller size. If this were the case, then conditions which cause overaccumulation of transcript A should concomitantly cause reduced accumulation of the species which are presumably derived from transcript A. This was clearly not the case (Fig. 5).

Nalidixic Acid Affects Chloroplast Transcripts

Nalidixic acid stimulated transcript A to an even greater extent than novobiocin (Fig. 9). In contrast to novobiocin, however, nalidixic acid at the concentrations used here also stimulated accumulation of transcripts

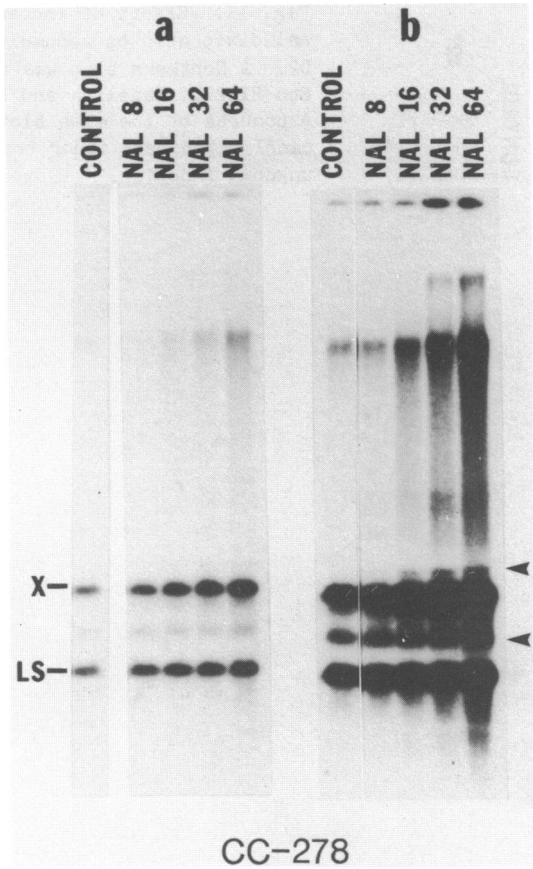

Fig. 10. Effect of increasing doses of nalidixic acid on accumulation of transcript LS. A Northern blot was probed with Eco RI 14. Panels a and b are different exposures of the same blot. Arrows in panel b indicate transcript Y and another larger transcript of unknown function.

LS, X and Y (Fig. 10) and of several other minor transcripts of unknown function which hybridized to the Eco RI 14 and Eco RI 27 probes (Figs. 10 and 11). Nalidixic acid had a minor stimulatory effect on transcript D2 (Fig. 11) and left 16S rRNA virtually unaffected (data not shown). Thus, in contrast to novobiocin, nalidixic acid appeared to have a general stimulatory effect on many chloroplast transcripts. These differences in the effects of nalidixic acid and novobiocin were also seen when total transcripts made in vivo were subsequently labeled in vitro and hybridized to Southern blots of restriction enzyme digests of total chloroplast DNA (Fig. 12).

Fig. 11. Effect of increasing doses of nalidixic acid on accumulation of transcript D2. A Northern blot was probed with Eco RI 27. Panels a and b are different exposures of the same blot. Arrows in panel b indicate minor transcripts of unknown function.

In both the Southern and Northern blot analyses, equal amounts of total cellular RNA from untreated and drug treated cells were compared. Thus, results of both analyses clearly show that novobiocin did not stimulate accumulation of transcript A by overall stimulation of all chloroplast transcripts relative to total cellular transcripts. In fact, novobiocin inhibited accumulation of certain other transcripts (e.g. LS). In contrast to novobiocin, nalidixic acid enhanced to a similar extent the proportion of chloroplast RNAs (relative to total RNA) that hybridized to many different restriction fragments (Fig. 12). The Northern blot results suggest, however, that superimposed on this general stimulation, nalidixic acid increased the proportion of certain chloroplast transcripts (e.g. transcript A) selectively.

DISCUSSION

We have shown that Chlamydomonas reinhardtii contains an ATP-dependent topoisomerase that supercoils relaxed DNA rings (Fig. 2). This enzyme

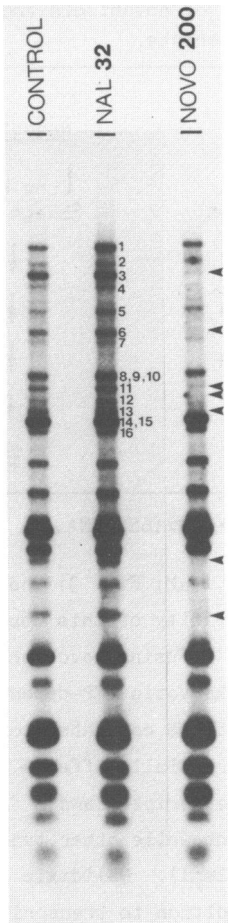

Fig. 12. Hybridization of total RNA from control and from novobiocin (200 μg/ml) or nalidixic acid (32 μg/ml) treated cells to total chloroplast DNA Hpa II restriction fragments. Sister strips (~0.5 μg DNA each) of a Southern blot of the Hpa II fragments were probed with ~1 μg of *in vitro* ^{32}P-labeled cellular RNA (control, 8.48×10^6 cpm; + novobiocin, 8.56×10^6 cpm; + nalidixic acid, 7.35×10^6 cpm). Autoradiography was for 5 hr at -70°C. The Hpa II restriction fragments are numbered as in ref. 27. Arrowheads indicate fragments showing reduced hybridization to RNA from novobiocin treated cells.

appears to function like a bacterial gyrase rather than like a relaxing enzyme which acts in concert with another DNA binding protein whose binding introduces supercoils into DNA (Fig. 4). In either case, our results indicate that this enzyme ultimately can influence torsional stress in DNA. Like DNA gyrase from *E. coli* and other bacteria, this topoisomerase is inhibited

Table I. Summary of effects of novobiocin and nalidixic on accumulation of various chloroplast transcripts.

Transcript	Probe	Effect Of: Novobiocin	Nalidixic Acid[1]
A	Hpa II 5 subfragment	↑ Low doses ≅ High doses	↑↑
LS	Eco RI 14	↓	↑
X	Eco RI 14	↓	↑
Y	Eco RI 14	↓	↑
16S rRNA	X	↓	≅
D2	Eco RI 3	≅	↑

[1]Quantitatively, A > X > LS > Y > D2 > 16S rRNA.

by low doses of novobiocin (<0.1 μM; Fig. 3) and by nalidixic acid (<0.1 mM; Fig. 3). In fact, the high affinity of this topoisomerase for novobiocin has allowed us to partially purify it using novobiocin affinity chromatography. To our knowledge, the known eukaryotic ATP-dependent topoisomerases are not inhibited by these two drugs at the concentrations that we used.

In vivo, novobiocin differentially affects accumulation of chloroplast transcripts. Specifically, transcript A and a family of smaller related transcripts become more abundant while other transcripts are reduced in the presence of this drug (Table I). Nalidixic acid, on the other hand, causes many transcripts, in addition to transcript A, to overaccumulate albeit to varying degrees (Table I). The simplest interpretation of our results is that altering the functions of the ATP-dependent supercoiling topoisomerase ultimately affects accumulation of certain chloroplast transcripts.

We consider the effects of novobiocin and nalidixic acid on chloroplast transcripts by analogy with their effects on transcription in prokaryotes. In bacteria, torsional stress in the DNA depends on the balance of topoisomerase activities which relax or supercoil DNA (28,29), as well as on other proteins that are bound to DNA (30). Novobiocin and nalidixic acid distort this balance by inhibiting DNA gyrase, thus causing a more relaxed state of DNA (5,6). On the other hand, mutations in topoisomerase I cause a more supercoiled state of the DNA (29). Alteration of torsional stress, in turn, is thought to affect the affinity of RNA polymerase for promoters and termination

activity at terminators (2,4,7,31). Whereas it was originally thought that inhibition of DNA gyrase inhibits transcription in general, it is becoming evident that different transcripts respond differently (7). For example, inhibiting the activity of either subunit of E. coli gyrase leads to overproduction in vivo of gyrase itself (32). Similarly, inactivation of the B subunit of this gyrase and of T4 topoisomerase leads to overexpression of some phage T4 genes and to inhibition of others (33).

Nalidixic acid, but not novobiocin, causes accumulation of single- and double-stranded breaks into DNA (2,4). It is thought that this drug causes an allosteric change in prokaryotic type II topoisomerases, thereby uncoupling the ligation reaction from the strand-passage reaction (34). Under certain conditions, these intermediates dissociate to give DNA fragments with covalently linked topoisomerase subunits at their ends. In E. coli, such DNA damages ultimately activate the SOS response which activates many operons (8). An SOS system for Chlamydomonas has not yet been described.

Liu and his coworkers (35) have recently shown that the effects of certain antitumor drugs on eukaryotic type II topoisomerases are similar to the effects of nalidixic acid (or its analogue oxolinic acid) on prokaryotic type II topoisomerases discussed above. It appears that these inhibitors alter the specificity of the topoisomerase cleavage sites in the DNA. Thus, if such sequences overlap promoter sites, competition or interaction between topoisomerase and RNA polymerase could be altered.

Inhibitors of the algal ATP-dependent supercoiling topoisomerase could affect accumulation of chloroplast transcripts directly or indirectly. For example, this topoisomerase might directly alter the conformation of chloroplast DNA, thus affecting chloroplast transcription directly. Alternatively, this topoisomerase might act in the nucleus and specifically affect one or more transcripts whose products are transported into the chloroplast and, in turn, affect synthesis, processing and/or degradation of certain chloroplast transcripts. Experiments are under way to test these possibilities.

During the course of this work, we observed that C. reinhardtii also contains a DNA relaxing activity which does not require ATP and is not inhibited by novobiocin or nalidixic acid (data not shown). We do not yet know whether this activity works in conjunction with the ATP-dependent supercoiling topoisomerase described above. Possibly this relaxing activity is similar to the type I topoisomerase recently isolated from pea chloroplasts (36).

We are particularly interested in the stimulatory effects of novobiocin

and nalidixic acid on transcript A since we have previously shown that, in certain strains, this transcript is under light-dark control (11). Transcript A is the largest member of a family of related transcripts, all of which overaccumulate in the dark. We therefore suspect that this transcript accumulates as a direct consequence of enhanced transcription. Most significantly, in contrast to the other transcripts studied, accumulation of this transcript is stimulated by low doses of novobiocin, but is unaffected by higher doses of novobiocin. In addition, of all the transcripts studied, transcript A is by far the most stimulated by nalidixic acid (Table I). These results suggest a unique regulation of this transcript. Since we suspect this regulation to encompass the light-dark control, we are presently sequencing the target DNA at which the corresponding controlling elements might act. In addition, we are studying this regulation in the mutant 278 grol (11) in which the response of transcript A to both darkness (11) and nalidixic acid (unpublished results) is altered.

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