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Effect of DNA Gyrase Inactivation on RNA Synthesis in *Escherichia coli*

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The average chain growth rates of rRNA and of total RNA were not affected by a thermal inactivation of DNA gyrase in a temperature-sensitive *gyrB* mutant of *Escherichia coli*. The fact that total RNA synthesis decreased under these conditions suggests that transcription is primarily affected at the step of chain initiation. The fraction of rRNA in total pulse-labeled RNA was not altered by inactivation of the enzyme, indicating that the latter is not required to actively maintain a high rate of synthesis of this RNA species.

It has been demonstrated that drug inactivation of DNA gyrase (5, 25), as well as thermal inactivation of the enzyme in conditional lethal mutants of *Escherichia* coli (9, 16), affect RNA synthesis. Likewise, relaxed DNA is a poorer template than negatively supercoiled DNA for RNA polymerase activity in vitro (1, 6, 20, 28). In light of these observations, we speculated that gyrase activity or DNA supercoiling might be one of the conditions required for efficient rRNA synthesis.

It has been reported (14, 30) that rRNA synthesis is preferentially inhibited by drugs inhibiting DNA gyrase activity. In contrast, Wahle and Mueller (26), performing a very thorough investigation, could not find any evidence for preferential inhibition of rRNA synthesis by this drug or any other gyrase inhibitors.

Considerable evidence suggesting that inhibitors of DNA gyrase do not merely inactivate the enzyme but have additional effects on nucleic acid metabolism has since been accumulated (3, 9, 10, 16, 27). We have, therefore, studied the synthesis of rRNA in a temperature-sensitive *gyrB* mutant (16, 18), thus avoiding possible artifacts caused by use of the antibiotics.

Both the chromosomal and extrachromosomal DNAs become less supercoiled in the *gyrB* mutant LE316 a few minutes after a temperature shift to the restrictive temperature (10, 17, 29; M. Gellert, unpublished data). The rate of RNA accumulation in the mutant is slightly reduced during the first 40 min after the temperature shift but drops faster thereafter (E. Wahle, unpublished data), in agreement with the linear rate of RNA synthesis observed after a temperature shift of the *gyrB* mutant (16).

To test for a possible preferential effect of gyrase inactivation on rRNA synthesis, we estimated the fraction of rRNA in total pulse-labeled RNA synthesized after a temperature shift. We used two different methods: liquid hybridization to *Proteus* DNA enriched with rRNA coding sequences (15) and hybridization to filter-bound DNA of λ*daroE* (7). The results of the former experiments are shown in Fig. 1. No significant reduction in the relative rate of rRNA synthesis could be detected in the mutant strain. Very similar results were obtained by hybridization to λ*daroE* (data not shown). The decrease in the relative rate of rRNA synthesis observed approximately 5 min after the temperature shift in both strains is probably due to the transient accumulation of ppGpp caused by a shortage of amino acids (22).

The activity of the *rrn* promoters can also be studied with a λ phage carrying an *rrnE* promoter fused to the *lacZ* gene (12). At both the permissive and restrictive temperatures,

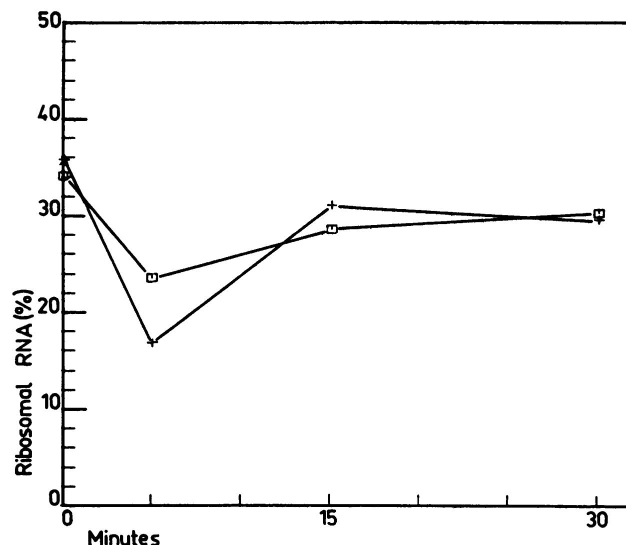

FIG. 1. Relative synthesis rate of rRNA. LE234 (*gyrB*$^+$) (+) and LE316 (*gyrB ts*) (□) were grown at 30°C in minimal medium (supplemented with Casamino Acids and the required amino acids) to an A_{460} of 0.2 before the temperature was shifted to 42°C (0 min). At the times indicated, 0.5-ml samples were taken to 50 μCi [5-^{3}H]uridine (30 Ci/mmol) and incubated at 42°C for 20 s. RNA was extracted and hybridized to *Proteus* DNA for the determination of rRNA content (15). Methods for the isolation of competitor rRNA, ^{32}P-labeled RNA for use as internal standard, and pulse-labeled RNA have been described previously (26). Labeled rRNA was competed out by a large excess of unlabeled rRNA in two of four parallel incubations. The fraction of rRNA was calculated from the amount of pulse-labeled RNA that did not hybridize in the presence of competitor and the total incorporation of radioactivity into RNA as determined by precipitation (13). The data were corrected for the hybridization efficiency of the internal standard, for differences in counting efficiency of hybridized and precipitated RNA, and for the molar fraction of radioactive bases in rRNA and mRNA (2).

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the rates of β-galactosidase synthesis under the control of the *rrnE* promoter are identical in the *gyrB* mutant and the wild-type strain (Wahle, unpublished data).

We reported previously that novobiocin and oxolinic acid reduce the chain elongation rate of rRNA (26). The same conclusion was reached independently by Jorstad and Morris (8) for the *lac* mRNA.

To compare the chain growth rate of rRNA in the mutant and the wild-type cells, we labeled cultures with [^{14}C]uracil for two doubling times. Twenty minutes after a temperature shift-up, rifampin and [^{3}H]uridine were added and the increase in the ^{3}H/^{14}C ratio of 4S RNA (synthesized from genes at the ends of rRNA transcription units) was measured as described previously (21, 24). No significant differences between the interpolated runoff times of strains LE234 and LE316 could be detected (Fig. 2). The fact that the ^{3}H/^{14}C ratios did not reach a constant final value probably reflects a partial resistance to rifampin in the strains we used. Although this resistance might modify the determination of the absolute rate of RNA chain growth, it is not likely to affect the comparison of the two isogenic strains.

The rate of chain growth of total RNA in the two strains at the restrictive temperature was monitored by the reduction in the [^{3}H]uridine incorporation rate after the addition of rifampin (24). Again, no substantial differences between the mutant and the wild-type strain were observed (Fig. 3).

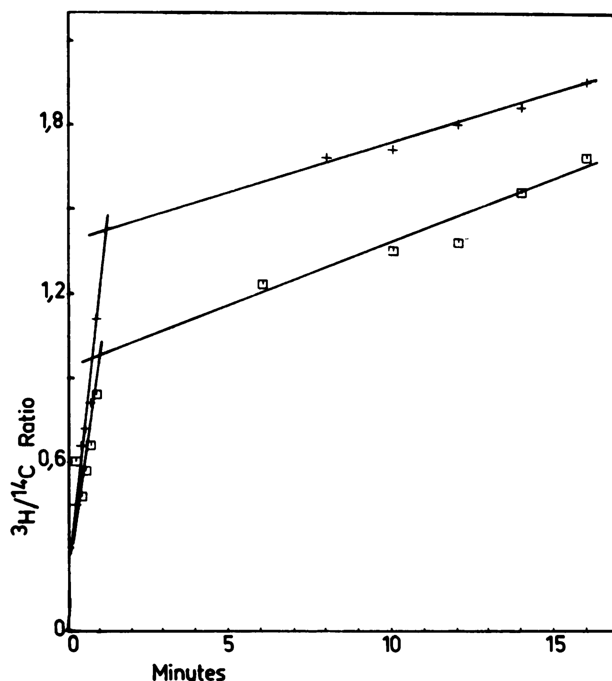

FIG. 2. Kinetics of tRNA synthesis after addition of rifampin. LE234 (*gyrB*$^{+}$) (+) and LE316 (*gyrB ts*) (□) were grown in minimal medium. At an A_{460} of 0.1, a 10-ml portion of each culture was mixed with 2 μCi of [^{14}C]uracil (60 mCi/mmol). The unlabeled cultures were further incubated for control of optical density. The labeled cultures were transferred to 42°C at an A_{460} of about 0.3. At 20 min later, 100 μCi of [5-^{3}H]uridine (30 Ci/mmol) and 250 μg of rifampin per ml (final concentration) were added simultaneously. At the times indicated, samples were taken into stop solution and RNA was extracted and analyzed on polyacrylamide gels. 4S RNA bands were cut out and the ^{3}H/^{14}C ratio was determined (22, 24). The intersection of two straight lines drawn through the two sets of data indicates the time when tRNA synthesis ceases (22).

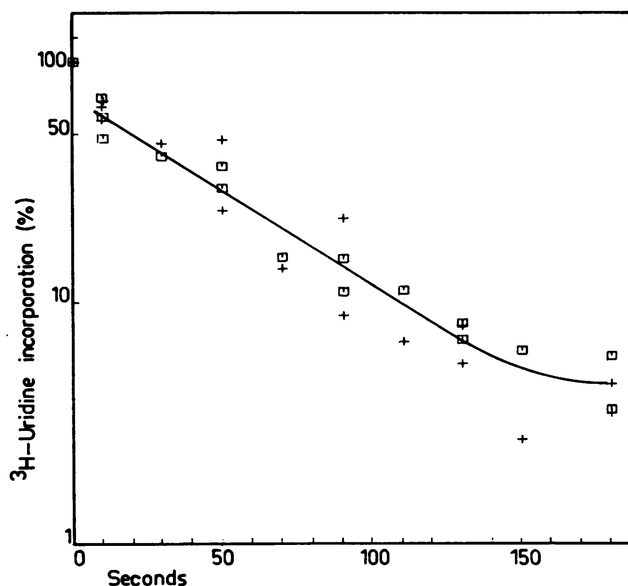

FIG. 3. Rate of total RNA synthesis after addition of rifampin. LE234 *gyrB*$^{+}$ (+) and LE316 *gyrB*(Ts) (□) were grown in minimal medium. At an A_{460} of 0.2, the cultures were divided into 10-ml portions and transferred to 42°C. At 20 min later, 0.5 ml of a culture was incubated with a 5 μCi of [5-^{3}H]uridine (30 Ci/mmol) for 30 s. The incorporation into acid-precipitable material was determined and taken as 100%. The rest of the culture was mixed with rifampin (final concentration, 250 μg/ml), and more samples were taken for pulse-labeling for the determination of acid-precipitable material at later times.

Our data suggest that initiation of RNA synthesis is the most sensitive transcriptional step to gyrase inactivation. This sensitivity might reflect the reduced ability of RNA polymerase to form a productive complex at the promoter region (6, 20, 23). Once initiation has started, however, no activity of DNA gyrase is required to modify local superhelical changes induced by RNA polymerase travelling along the DNA (4).

We further propose that the supercoiling activity of DNA gyrase does not actively modify the partitioning of RNA polymerase between rRNA promoters and non-rRNA promoters. Consequently, the low efficiency of the *rrn* promoter in vitro or under several growth conditions in vivo cannot be accounted for by modulation in DNA gyrase activity. Our results fail to confirm the divergent observations of Oostra et al. (14), and their validity should be called into question, especially as these authors have failed to produce a thorough analysis of the effect of novobiocin on rRNA synthesis. So far, we have been unable to detect any preferential effect of gyrase inactivation on rRNA synthesis in a number of strains under various growth conditions.

The reduction in the rate of RNA chain growth observed after inactivation of DNA gyrase by drugs (26) cannot be reproduced in the *gyrB* mutant. This is the latest addition to the long list of drug effects which are not found in temperature-sensitive gyrase mutants (9, 16, 19, 27). Gyrase inhibitors enhance the formation of drug-enzyme complexes in DNA (11) and induce positive supercoil turns in negatively supercoiled DNA (10). Moreover, drugs such as nalidixic acid bind tightly to DNA (V. Shen, personal communication). It is very likely that any one of these processes alone is sufficient to halt a travelling RNA polymerase molecule on

the DNA. It is evident that, together, they would create chaos.

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