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Inhibition of Deoxyribonucleic Acid Replication in *Bacillus brevis* by Ribonucleic Acid Polymerase Inhibitors

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The incorporation of [^{3}H]thymidine into deoxyribonucleic acid by exponentially growing cells of *Bacillus brevis* was inhibited by streptolydigin and rifampin in the same concentration range in which these drugs inhibit ribonucleic acid synthesis. Complete inhibition occurred within one-third generation time after drug addition, suggesting an effect on deoxyribonucleic acid chain elongation.

The replication of DNA is an extremely complex process that is linked to many other cellular processes, especially in its initiation phase (9). However, even the elongation of DNA chains must involve steps other than DNA synthesis, since it is known to be a discontinuous process (10), yet no known DNA polymerase is capable of initiating the synthesis of new DNA chains (4). In the course of studies on macromolecular synthesis in growing cultures of *Bacillus brevis*, we had observed that inhibitors of RNA polymerase also inhibit DNA synthesis (12), suggesting a possible linkage between transcription and replication. In this communication, we present a more detailed analysis of this phenomenon.

The effects of various concentrations of streptolydigin and rifampin on the incorporation of [^{3}H]thymidine into DNA and of [^{3}H]uridine into RNA are shown in Fig. 1. The rates of DNA and RNA syntheses were affected by the drugs in similar concentration ranges, 50% inhibition being produced by 0.3- and 0.15-μg/ml concentrations, respectively, of streptolydigin and by 4- and 10-ng/ml concentrations, respectively, of rifampin. To ascertain whether the incorporation of [^{3}H]thymidine indeed reflected DNA replication, we examined its sensitivity to various replication inhibitors. Nearly complete inhibition of [^{3}H]thymidine incorporation into DNA was produced by 100 μg of the DNA gyrase inhibitors (3) nalidixic acid and novobiocin per ml and by 12 μg of the DNA polymerase III inhibitor (8) 6-(*p*-hydroxyphenylazo)uracil per ml, indicating that this reaction involved DNA replication.

The possibility had to be considered that rifampin and streptolydigin inhibited the incorporation of [^{3}H]thymidine into DNA by interfering with thymidine metabolism. The specific activity of intracellular [^{3}H]dTTP was measured before and after treatment with streptolydigin or rifampin and was found to vary not more than 15%, showing that the antibiotics had no significant effect on the labeling of the thymidine nucleotide pool. A specific effect on thymidine metabolism was also excluded by the observation that streptolydigin and rifampin inhibited the incorporation of [^{3}H]deoxyguanosine into DNA in a similar manner (data not shown).

To determine whether the inhibition of DNA synthesis by rifampin directly involved RNA polymerase, we made use of a rifampin-resistant mutant of *B. brevis*. Strain 15a was isolated from *B. brevis* 8185 by its ability to grow on nutrient agar plates containing rifampin (5 μg/ml). RNA synthesis in cells of *B. brevis* 15a that had been made permeable to small molecules by treatment with toluene (13) was unaffected by concentrations of rifampin that completely inhibited RNA synthesis in permeable cells of *B. brevis* 8185, indicating that rifampin resistance was due to an altered RNA polymerase and not to impermeability to the drug. When the effect of rifampin on growing cultures of *B. brevis* 15a was examined, it was found that even high concentrations of the drug (50 μg/ml) had no effect on the incorporation of [^{3}H]thymidine into DNA and produced only a slight inhibition (28%) of RNA synthesis. It appears, therefore, that inhibition of RNA polymerase is responsible for the inhibition of DNA synthesis by rifampin. Further evidence for an essential involvement of RNA polymerase in DNA replication was provided by the observation that lipiarmycin, another specific inhibitor of RNA polymerase (14), also inhibited the incorporation of [^{3}H]thymidine into DNA (data not shown).

Some insight into the role of RNA polymerase in DNA replication was provided by studies of the time course of inhibition of DNA synthesis by streptolydigin and rifampin. RNA synthesis was arrested almost immediately upon the addition of the antibiotics (Fig. 2). The onset of inhibition of DNA synthesis was less rapid, 50%

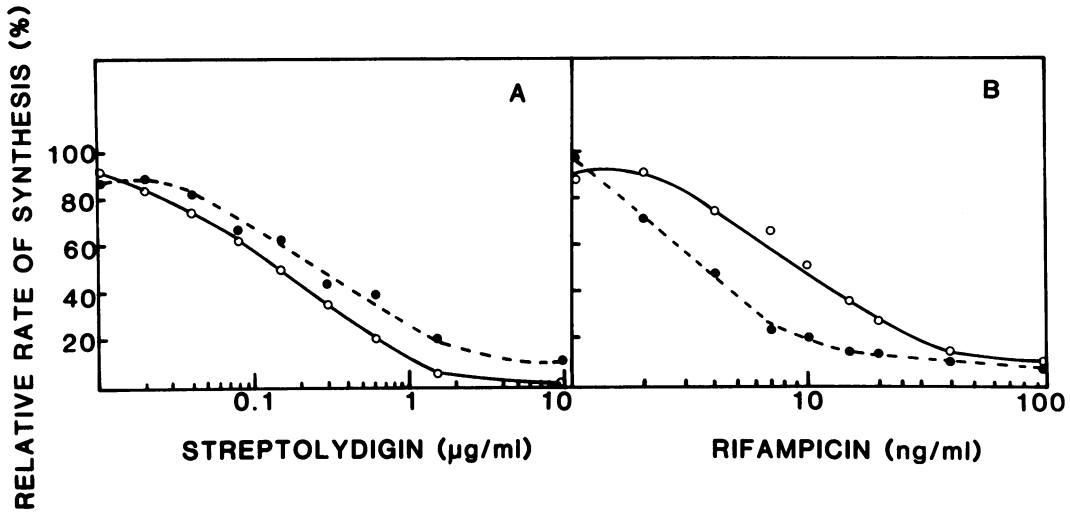

FIG. 1. *Effect of various concentrations of streptolydigin and rifampin on DNA and RNA syntheses. B. brevis ATCC 8185 was grown in a rotary shaker at 37°C in nutrient broth (Difco Laboratories) supplemented with (per liter): KCl, 1 g; $CaCl_2$, 0.112 g; $MgCl_2 \cdot 6H_2O$, 0.4 g; $MnCl_2 \cdot 4H_2O$, 2 mg; $FeSO_4 \cdot 7H_2O$, 0.28 mg; glucose 1 g. Culture samples (1 ml) were removed during mid-exponential growth (100 Klett units; filter no. 42) and incubated at 37°C with shaking in the presence of streptolydigin or rifampin at the indicated concentrations. The samples were supplemented with 1 μCi of [methyl-^{3}H]thymidine (20 Ci/mmol) after 12 min or with 1 μCi of [5-^{3}H]uridine after 1.5 min. After 30 s of further incubation, the labeling was terminated by the addition of 1 ml of cold 0.6 N trichloroacetic acid containing 10 mM sodium pyrophosphate. Acid-insoluble radioactivity was measured as described previously (12). (A) Effect of streptolydigin and (B) effect of rifampin on the incorporation of (●) [^{3}H]thymidine into DNA and (○) [^{3}H]uridine into RNA.*

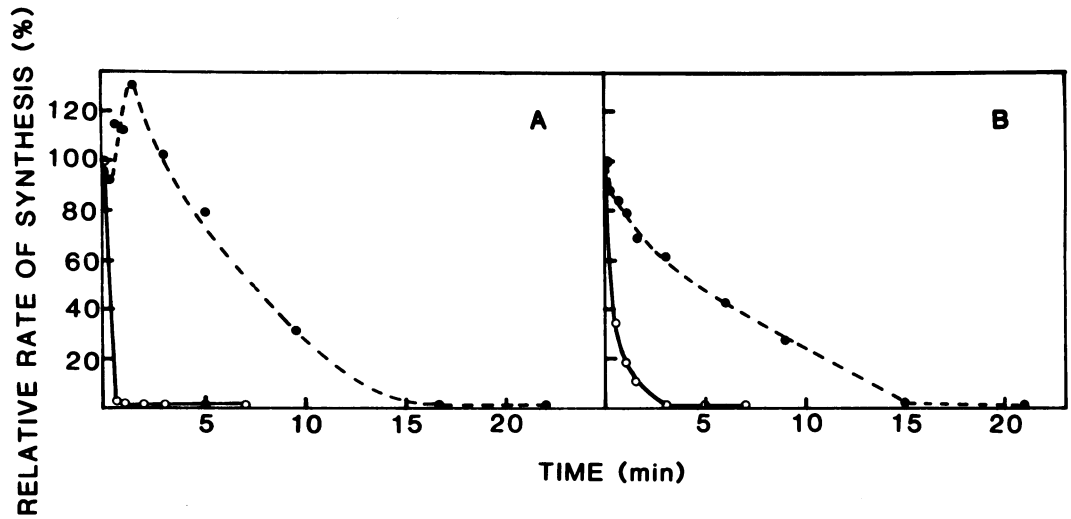

FIG. 2. *Time course of inhibition of DNA and RNA syntheses by streptolydigin and rifampin. Culture samples (1 ml) were removed during mid-exponential growth and incubated at 37°C with shaking in the presence of (A) 20 μg of streptolydigin per ml or (B) 2 μg of rifampin per ml. At the indicated times after antibiotic addition, the samples were supplemented with (●) 1 μCi of [methyl-^{3}H]thymidine or (○) 1 μCi of [5-^{3}H]uridine and then incubated for another 30 s. The incorporation of radioactivity into acid-insoluble material was measured as described in the legend to Fig. 1. The values at 0 min represent incubations to which no antibiotic had been added.*

inhibition being attained 5 to 7 min after the addition of streptolydigin or rifampin and complete inhibition being attained after 15 min. Nevertheless, the time required for inhibition was much shorter than the generation time of *B. brevis* (40 min), indicating an effect on DNA chain elongation rather than just on the initiation of chromosome replication, a step already known to involve RNA polymerase (5, 7). A possible mechanism for the involvement of RNA polymerase in DNA chain elongation is the synthesis of RNA primers for discontinuous DNA synthesis (4). Such a situation is seen in the replication of filamentous bacteriophages M13 and fd, in which primers are provided by the rifampin-sensitive host RNA polymerase (2). On the other hand, the replication of the *Escherichia coli* chromosome as well as that of some bacteriophages is rifampin resistant and depends on the priming activity of the *dnaG* product (1, 6). Thus, it is of interest that the replication of the *B. brevis* chromosome is rifampin sensitive, resembling that of the filamentous phages rather than that of the *E. coli* chromosome. If this interpretation of our results were correct, one would expect a slight delay in the inhibition of DNA replication upon inhibition of RNA primer synthesis, because of the completion of the synthesis of the discontinuous DNA fragment. However, the time required for the synthesis of a DNA molecule the size of an Okazaki fragment, i.e., 8 to 10S, is about 2 s (10), much less than 5 min, the half-time for onset of inhibition by streptolydigin and rifampin. On the other hand, if discontinuous synthesis involving RNA primers occurred only with one of the complementary DNA strands, a longer delay in the onset of complete inhibition of DNA synthesis might be expected. Another observation, which we cannot explain at this time, is the stimulation of DNA synthesis that occurs within the first 2 min after streptolydigin addition (Fig. 2A). The resolution of these questions must await the detailed in vitro study of the mechanism of chromosome replication in *Bacillus* species.

In conclusion, we have presented three lines of evidence that implicate RNA polymerase in the replication of the *B. brevis* chromosome: (i) DNA replication is inhibited by streptolydigin, rifampin, and lipiarmycin, which are known to be specific inhibitors of bacterial RNA polymerase; (ii) the concentrations of streptolydigin and rifampin required for the inhibition of DNA and RNA syntheses are very similar; and (iii) DNA synthesis is not inhibited by rifampin in a strain of *B. brevis* with a rifampin-resistant RNA polymerase. The rapid onset of inhibition of DNA synthesis by streptolydigin and rifampin suggests an effect on DNA chain elongation. Our results are consistent with the notion that the rifampin-sensitive RNA polymerase of *B. brevis* may be involved in the synthesis of primers for the initiation of Okazaki fragments, a hypothesis which remains to be confirmed by in vitro studies.

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