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# Alterations of the Rate of Movement of Deoxyribonucleic Acid Replication Forks

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Antibiotics that inhibit ribonucleic acid (RNA) or protein synthesis are often used in studies of deoxyribonucleic acid (DNA) synthesis. The experiments presented here demonstrate that the rate of movement of DNA replication forks can be influenced by such antibiotics. Addition of either chloramphenicol, which inhibits movement of ribosomes along messenger RNA, or streptolydigin, which inhibits movement of RNA polymerase, leads to a decrease in the rate of fork movement. Rifampin, which inhibits initiation of RNA synthesis, reverses the effects of chloramphenicol or streptolydigin. The slowed movement of DNA replication forks is discussed in terms of obstruction of fork movement by transcription complexes temporarily immobilized on the DNA template.

One of the unifying features of molecular biology is the concept that alteration of the rate of macromolecular synthesis occurs primarily at the level of initiation of synthesis of new molecules, whether deoxyribonucleic acid (DNA), ribonucleic acid (RNA) or protein (18). The rate of propagation of synthesis of macromolecules, i.e., the rate of addition of monomers to a growing molecule, is essentially constant over a wide range of growth rates, though deviations may occur at low growth rates (8, 11, 13, 19).

Numerous studies have verified that replication of DNA is regulated at initiation. Alteration of growth rate is accompanied by alteration of the frequency of initiation of new rounds of replication (13); inhibition of protein or RNA synthesis prevents initiation of new rounds of replication of DNA while allowing synthesis of nascent DNA molecules to continue (12, 14, 16, 17, 20, 24). Antibiotics that inhibit protein or RNA synthesis are often used to inhibit initiation of DNA replication; the purpose of this communication is to demonstrate that such antibiotics can also affect the rate of propagation of DNA replication.

## MATERIALS AND METHODS

**Bacterial strains and growth conditions.** Most experiments were performed with *Escherichia coli* 15 TAU bar. Other strains used were *E. coli* 15 TAMT (16), B/r thy$^-$, and K12-PC2 (5). The organisms were grown at 37 C in a minimal medium (7) with 0.2% glucose as carbon source. Required amino acids were supplied at 50 μg/ml. A combination of 2 μg of thymidine/ml, 200 μg of deoxyadenosine/ml, and 200 μg of uridine/ml was used to supply the thymine and uracil requirements, as this resulted in maximal levels of intracellular thymidine triphosphate (TTP) (M. L. Pato, manuscript in preparation). The TTP pool in *E. coli* 15 TAU bar was approximately 120 pmol/absorbance at 450 nm (1 ml of a culture with optical density of 450 nm = 1). Samples from cultures in exponential growth were frozen at −70 C in 15% glycerol, and fresh cultures started from these frozen inocula were grown for at least an additional 4 generations before use. The optical density at 450 nm of cultures at the start of experiments was less than 0.400.

**Labeling procedures.** Labeling of cultures with [$^3$H]thymidine (5 to 10 μCi/ml) was carried out either for a minimum of five generations of growth or for 5 min before initiating experiments. The former procedure yields cells with fully labeled chromosomes. Both procedures yield cells in which the specific activity of the TTP pool is approximately equivalent to that of the exogenous thymidine; therefore, the rate of DNA replication in the culture is directly related to the rate of uptake of labeled thymidine.

Samples of 50 μl were removed from labeled cultures into chilled 5% trichloroacetic acid containing 100 μg of thymidine/ml, collected on membrane filters, and washed with cold 5% trichloroacetic acid containing 100 μg of thymidine/ml and then with hot water. The hot water wash removes rifampin (RIF) from the filters. Radioactivity on dried filters was determined in a toluene-based scintillation mixture.

**Chemicals.** [$^3$H]thymidine (6.7 Ci/mmol) was purchased from New England Nuclear Corp., and chloramphenicol (CAM) was purchased from Sigma Chemical Co. RIF and streptolydigin (STL) were generous gifts from Ciba-Geigy A/S, Copenhagen, and G. Whitfield, the Upjohn Company, Kalamazoo, Mich., respectively.

## RESULTS

After initiation of DNA replication has been blocked by the inhibition of protein or RNA synthesis, synthesis of DNA should continue for a time equal to the replication time of the chromosome; this is estimated to be approximately 40 to 50 min (4, 13, 25). However, since transcription and concomitant translation occur on the chromosome simultaneously with replication, interference with these processes could influence the rate of progression of replication forks and extend the period of residual replication.

**Effect of CAM and RIF on DNA replication.** The effect of addition of CAM and RIF on DNA synthesis in *E. coli* 15 TAU bar is shown in Fig. 1; in Fig. 1A inhibitors were added to cells containing fully labeled chromosomes, and in Fig. 1B the cells were labeled for 5 min before addition of inhibitors to allow equilibration of the nucleotide pools with exogenous thymidine. When protein synthesis was inhibited with CAM, the rate of DNA replication as measured by thymidine incorporation decreased rapidly by approximately 40%, and replication was not completed until approximately 80 min after CAM addition. Addition of RIF, which inhibits initiation of synthesis of RNA, allowed an initial rapid rate of DNA replication, and synthesis was completed within 40 to 50 min. The increase in the amount of DNA above that present at the time of addition of CAM or RIF was about 50 to 55%, as measured by thymidine incorporation in cells with fully labeled chromosomes.

In addition to blocking initiation of DNA replication, inhibition of protein synthesis could create an impediment to the normal movement of the DNA replication fork, e.g., by allowing messenger RNA (mRNA) synthesis without concomitant movement of ribosomes along nascent mRNA. In this case, removal of all transcribing RNA polymerases from the DNA with RIF should nullify the slowing effect of CAM. When RIF and CAM were added simultaneously, the residual synthesis of DNA followed the pattern seen with RIF alone; indeed, RIF addition at any time after CAM reverses the slowing effect of CAM, as seen in Fig. 2 where RIF was added 10 min after CAM.

**Effect of amino acid starvation on DNA replication.** To determine if inhibition of protein synthesis, per se, or some side effect of CAM was responsible for the decreased rate of DNA replication, experiments were performed in which the bacteria were starved for required amino acids. A similar, though possibly less severe, retardation of DNA replication was obtained (Fig. 3). This effect of amino acid starvation was reversed by addition of RIF (data not shown).

**Effect of STL on DNA replication.** Unlike RIF, which inhibits the initiation of transcription, STL inhibits the propagation of RNA synthesis. Since *E. coli* 15 TAU bar is relatively

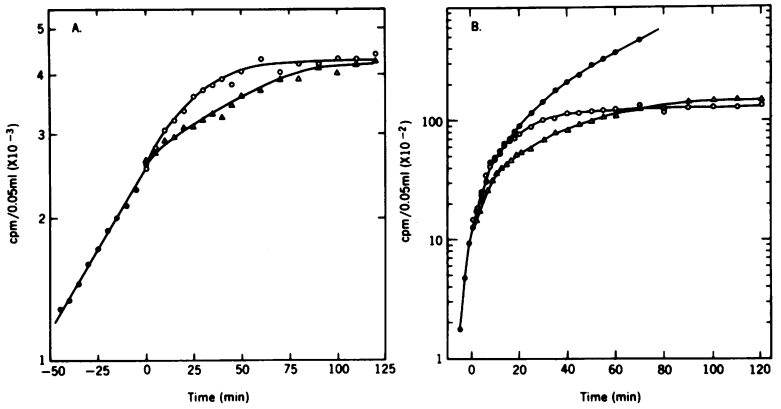


FIG. 1. *Residual DNA synthesis in the presence of CAM or RIF. E. coli 15 TAU bar was grown with a generation time of 42 min at 37 C in a glucose minimal medium supplemented with 2 μg of thymidine/ml, 200 μg of uridine/ml, 200 μg of deoxyadenosine/ml, and 50 μg of required amino acids/ml. (A) [³H]thymidine (5 μCi/ml) was added approximately five generations before additions of antibiotics. (B) [³H]thymidine (10 μCi/ml) was added 5 min before antibiotics. CAM (Δ, 200 μg/ml) or RIF (O, 200 μg/ml) were added at zero time; the control culture received no antibiotics (●). Samples of 0.05 ml were removed as indicated into 5% trichloroacetic acid. Samples were filtered, washed with trichloroacetic acid and hot water, and counted in a toluene-based scintillation mixture.*

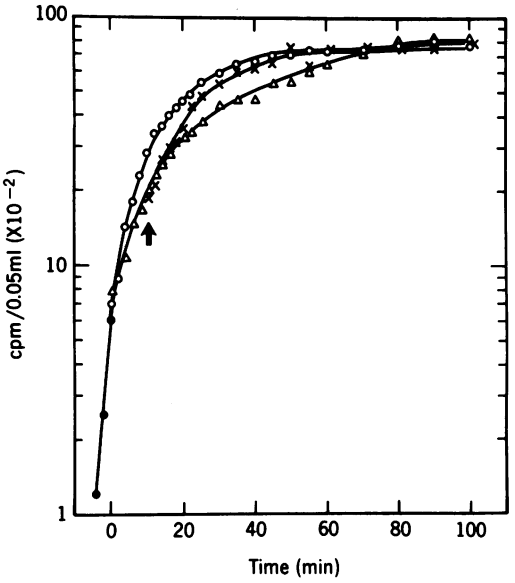


FIG. 2. *Residual DNA synthesis in the presence of both CAM and RIF. E. coli 15 TAU bar was treated as described in the legend to Fig. 1B, except the concentration of CAM (Δ) and RIF (O) was 100 μg/ml, and RIF was added (×) at 10 min to a portion of the culture which had received CAM at zero min.*

impermeable to STL, mutants sensitive to STL were selected using a penicillin selection technique similar to that previously described (22). The permeable mutant used in these experiments showed little or no residual incorporation of [$^3$H]uridine at STL concentrations of 50 μg/ml or higher, and yielded results with [$^3$H]-thymidine incorporation strikingly different from those obtained with RIF. Addition of STL to this mutant resulted in a decrease in the rate of DNA replication equivalent to or slightly more severe than that seen with CAM (Fig. 4). To determine whether this effect on DNA replication is exerted at a level other than inhibition of transcription, cells were pretreated with RIF for 5 min prior to STL addition to remove transcription complexes from the DNA. The subsequent incorporation of [$^3$H]thymidine was identical to that for RIF alone (Fig. 5).

**Strain differences.** Various strains of *E. coli* have yielded differing results with regard to several parameters related to DNA replication; e.g., the time interval between CAM addition and inhibition of initiation of DNA synthesis (12, 16, 24). Therefore, several strains of *E. coli* were examined for the relative effects of CAM and RIF on the rate of DNA replication. *E. coli* strains 15 TAU bar, 15 TAMT, and PC2 (K-12) showed severe retardation of DNA replication in the presence of CAM relative to the replication observed in the RIF, whereas *E. coli* B/r thy$^-$ showed only a slight decrease in the rate of replication in the presence of CAM as compared to the results with RIF (Fig. 6), and a slight decrease in the presence of STL.

## DISCUSSION

If only the initiation of DNA replication is inhibited, the rate of replication in a population of cells should fall in a manner consistent with the termination of rounds of replications in cells of different ages. The data presented here show that certain inhibitors of protein or RNA synthesis cause a more severe retardation of the rate of DNA replication than would be expected from an inhibition exclusively at the level of initiation of new rounds of replication. For example, addition of CAM results in a rapid decrease of the rate of DNA replication by about 40% in *E. coli* 15 TAU bar (Fig. 1).

A plausible explanation accounting for the observations reported here can be offered. Transcription complexes transiently immobilized on the chromosome may present a temporary obstacle to the DNA replication machinery, resulting in a decrease in the rate of movement of the DNA replication forks. This is perhaps most readily visualized in the case of STL, an inhibitor of RNA synthesis at the level of propagation. If STL immobilizes RNA polymerases on the

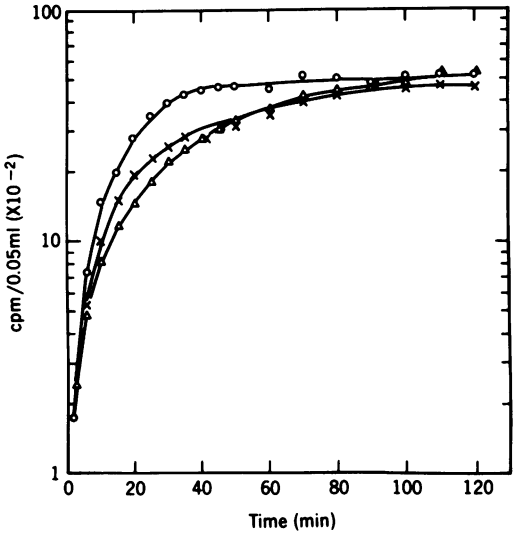


FIG. 3. *Residual DNA synthesis in the absence of required amino acids. A culture of E. coli 15 TAU bar was filtered and resuspended in the presence of [$^3$H]thymidine (10 μCi/ml) minus the amino acids arginine, tryptophan, and proline (×), plus amino acids and 200 μg of RIF/ml (O), and plus amino acids and 200 μg of CAM/ml (Δ). Samples were taken as in the legend to Fig. 1.*

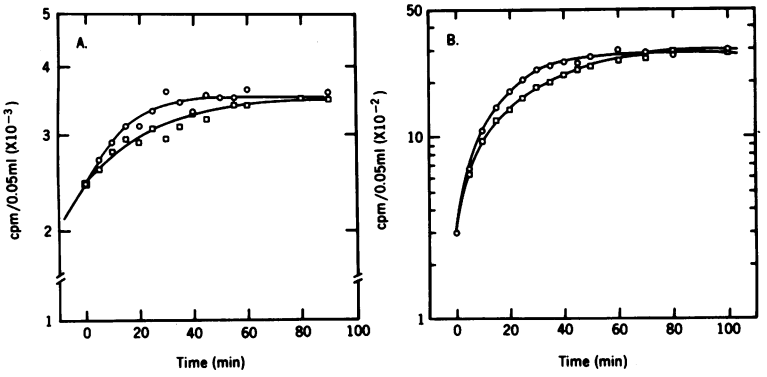


FIG. 4. *Residual DNA synthesis in the presence of STL. Conditions were identical to those described in the legend to Fig. 1 except a STL-permeable mutant of E. coli 15 TAU bar was used. (A) Cells were labeled with [³H]thymidine for 5 generations of growth before addition of 200 μg of RIF/ml (○) or 200 μg of STL/ml at zero min (□). (B) Cells were labeled for 5 min with [³H]thymidine (10 μCi/ml) before addition of antibiotics.*

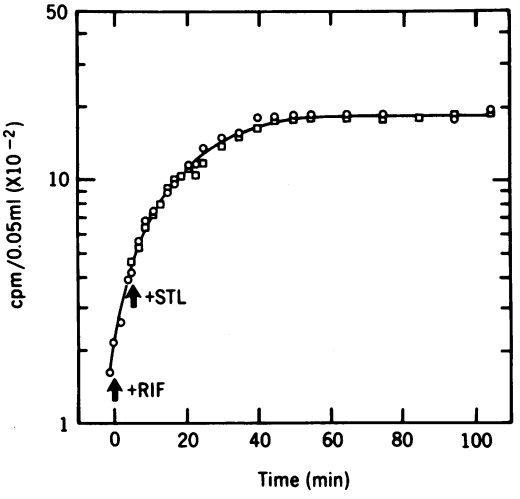


FIG. 5. *Residual DNA synthesis in the presence of RIF and STL. Conditions were identical to those described in the legend to Fig. 4B, except 200 μg of RIF/ml (○) was added at zero min and 200 μg of STL/ml was added to a portion of the culture with RIF at 5 min (□).*

DNA template in vivo as has been suggested from in vitro evidence (6), immobilized transcription complexes may present an obstacle which must be removed before the DNA replication fork can proceed. The decreased rate of DNA replication in the presence of STL, interpreted here as a decrease in the rate of replication fork movement, is not due to a direct effect on the enzymes of DNA replication as demonstrated by the observation that removal of transcription complexes from the DNA template with RIF abolishes the subsequent effects of STL.

The abrupt decrease in the rate of DNA replication observed after inhibition of translation by CAM or by removal of required amino acids can also be explained by a slowing of fork movement resulting from transient immobilization of transcription complexes on the DNA template. Addition of CAM prevents movement of ribosomes along nascent mRNA but allows continued synthesis of mRNA (10, 21). In the absence of ribosomes on the nascent mRNA, DNA-RNA complexes (e.g., see reference 3) may persist for a time sufficient to impede the progression of DNA replication forks. Again, clearing the DNA of transcription complexes with RIF reverses the anomalous decrease of the rate of DNA replication observed with CAM.

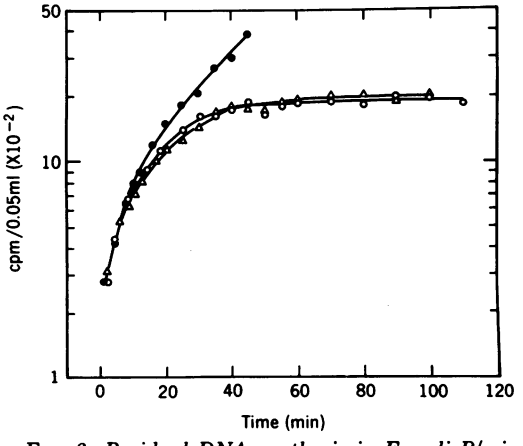


FIG. 6. *Residual DNA synthesis in E. coli B/r in the presence of CAM or RIF. Conditions were identical to those described in the legend to Fig. 1B, except the organism was E. coli B/r thy⁻. [³H]thymidine (10 μCi/ml) was added 5 min before antibiotics. CAM (△, 200 μg/ml) or RIF (0, 200 μg/ml) was added at zero time; the control culture received no antibiotics (●).*

Some other possible explanations can be eliminated. Alterations in the specific activity of the TTP pool are not responsible for the changes in replication rate, as experiments were performed under conditions in which the pool was in equilibrium with the external sources of labeled thymidine. A dependence of the rate of fork movement on the absolute size of the TTP pool has been demonstrated by Zaritsky and Pritchard (25) and Beacham et al. (2); therefore a shrinking of the size of the TTP pool could account for the results. However, addition of CAM, RIF, or STL results in a considerable increase in the size of the TTP pool (M. L. Pato, manuscript in preparation). Furthermore, inhibition of RNA synthesis with RIF or STL, which should affect nucleotide pools in a similar manner, yields very different results on DNA replication fork movement.

The rapid decrease in the rate of replication observed in the presence of CAM might be explained by the complete cessation of replication of a large fraction of the population of chromosomes within a few minutes of CAM addition. The experiment shown in Fig. 2 argues strongly against such an explanation. When RIF is added after CAM, the rate of thymidine incorporation is stimulated, but the final level of thymidine incorporation is unchanged. If RIF were reversing this premature cessation of replication, the plateau level of thymidine incorporation would be greater than that seen with CAM alone.

Condensation of nucleoids in vivo as observed by electron microscopy under certain conditions might be considered as a factor in the rate of movement of replication forks. However, several reported observations argue against such condensation being responsible for the results in this paper. Both CAM and RIF lead to condensation in vivo (26); however, the condensation of nucleoids in the presence of CAM is progressive with time after addition and requires continuing DNA synthesis. The onset of the slowing of replication fork movement suggested here occurs rapidly upon addition of CAM.

I have not stressed the amount of residual DNA synthesis in the presence of CAM or RIF, as this apparently simple value is actually a complex one dependent upon the number of chromosomes with multiple growing points, the length of any lag period that may exist between addition of a particular inhibitor and inhibition of initiation of replication, and the possible occurrence of premature termination of replication. Several recent publications contain discussions relevant to this subject (9, 12, 15). The interpretation of the data presented here is not dependent upon an understanding of the factors contributing to the amount of residual replication. However, the slowing of replication fork movement by CAM addition or amino acid starvation may affect some previously published experiments; e.g., attempts to specifically label terminal regions of the chromosome may yield more labeling of internal regions than expected (4, 15).

The observations that the slowing effect of CAM is strain dependent may prove useful in elucidating the mechanism involved in maintaining correct movement of the replication forks. One interesting possibility is variability in the effectiveness of unwinding proteins (1, 23) which might play a role in removing transcription complexes from the DNA in addition to their postulated role in separating the strands of the DNA helix.

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