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THE EFFECT OF RIFAMPICIN ON THE REPLICATION OF THE REPLICATIVE FORM OF BACTERIOPHAGE ΦX174 DNA

D. VAN DER MEI, J. Th. BRONS AND H. S. JANSZ
Laboratory for Physiological Chemistry, State University, Utrecht (The Netherlands)
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SUMMARY

The effect of inhibition of RNA synthesis by the antibiotic rifampicin on the replication of the double-stranded replicative form (RF) of ΦX174 DNA has been studied. Inhibition of RNA synthesis shortly after the onset of RF replication is severely inhibitory; inhibition of RNA synthesis at later times allows RF replication to proceed at a normal rate for a considerable time.

INTRODUCTION

Upon infection of the bacterial host the single-stranded DNA of bacteriophage ΦX174 is converted to a double-stranded DNA, the replicative form (RF). This process occurs under the influence of pre-existing host enzymes since it can take place under conditions of complete inhibition of phage- or host-directed protein synthesis[1,2].

However, the further replication of the parental into progeny RF requires the synthesis of a phage specific protein, coded for by cistron A (according to the nomenclature of Sinsheimer and Hayashi[3]) of the ΦX phage[2].

Blocking of cistron A protein synthesis either by a raise in temperature of cells infected with temperature sensitive mutants of ΦX174 that map in cistron A or by the addition of 150 μg chloramphenicol per ml leads to the arrest of RF replication.

This indicates that for the continuation of RF replication the continuation of cistron A protein synthesis is required. This, in turn, could be accomplished through a stable messenger RNA, formed early after infection, which allows for the synthesis of several copies of cistron A protein and consequently for several rounds of RF replication. Alternatively the continuation of cistron A protein synthesis may require the continuation of messenger RNA synthesis. In order to distinguish between these mechanisms the effect of rifampicin on RF replication was studied. This antibiotic specifically inhibits messenger RNA synthesis by preventing initiations of transcription[4,5].

MATERIALS AND METHODS

In order to measure RF synthesis the incorporation of [^{3}H]thymidine into DNA was determined using *Escherichia coli* H502, a hcr$^-$ thy$^-$ strain, which was

Abbreviation: RF, double-stranded replicative form DNA.

pretreated with mitomycin C. This treatment suppresses host-cell DNA synthesis almost completely whereas RF replication remains unaffected (ref. 6 and Fig. 2). Furthermore the experiments were carried out in the presence of 35 μg/ml chloramphenicol, which allows RF replication to continue in the absence of single-stranded DNA synthesis[1,7].

Cells were grown to a cell density of $2 \cdot 10^8$/ml in TPG medium[8] *plus* thymidine (20 μg/ml), after which the culture was incubated for 10 min in the dark with 25 μg/ml of mitomycin C. Cells were then spun down and washed once in a buffer that was identical to the growth medium except for the carbon- and nitrogen-containing compounds. The cells were resuspended in one tenth of the original volume of buffer *plus* 35 μg/ml chloramphenicol and infected with ΦX174 at a multiplicity of infection of 5. After 10 min at 37° without aeration to adsorb the phage to the cells, TPG medium *plus* thymidine (2 μg/ml) and 35 μg/ml chloramphenicol was added to restore the original cell density and aeration was resumed. In all experiments this moment is taken as the onset of the process of infection.

RNA and DNA synthesis were measured by the incorporation of [^{3}H]uridine and [^{3}H]thymidine, respectively into trichloroacetic acid-precipitable material. Precipitation was accomplished by adding 100 μg of bovine serum albumin as a carrier and 1 vol. of 20 % cold trichloroacetic acid. After 10 min in the cold the precipitate was spun down.

For the determination of labeled RNA, the precipitate was washed three times with cold 5 % trichloroacetic acid, solubilized in a small volume of 0.5 M KOH and counted. When DNA was the labeled compound, the precipitate was solubilized in 0.5 M KOH between each of the trichloroacetic acid washes.

RESULTS AND DISCUSSION

The uptake of [^{3}H]uridine into RNA was determined in the presence of various concentrations of rifampicin. In infected as well as uninfected cells 100 μg of rifampicin per ml effectively inhibited RNA synthesis. Fig. 1 shows that for an exponentially growing culture of *E. coli* H502 total inhibition of RNA synthesis is achieved within 5 min after addition of the drug.

The effect of 100 μg/ml of rifampicin on the incorporation of [^{3}H]thymidine in DNA is shown in Fig. 2. Uninfected cells do not incorporate label to any extent into DNA. In cells infected with ΦX174 a nearly linear incorporation of [^{3}H]thymidine in DNA up to 50 min after infection is observed. That this incorporation represents RF replication was shown by the fact that the ^{3}H-labeled material isolated at 60 min after infection by the lysis procedure of Clewell and Helinski[9] cosediments in a linear sucrose gradient (15–50 %) with added ^{14}C-labeled marker RF. The addition of rifampicin 10 min prior to infection gives only a small amount of incorporation of [^{3}H]thymidine in DNA, possibly corresponding to the conversion of the single-stranded DNA of the invading bacteriophage to the parental RF (see also ref. 10).

Addition of rifampicin after infection does not stop RF replication immediately. The extent to which replication of RF can proceed in the presence of rifampicin depends upon the time of addition of the drug. Addition of rifampicin 13 min after

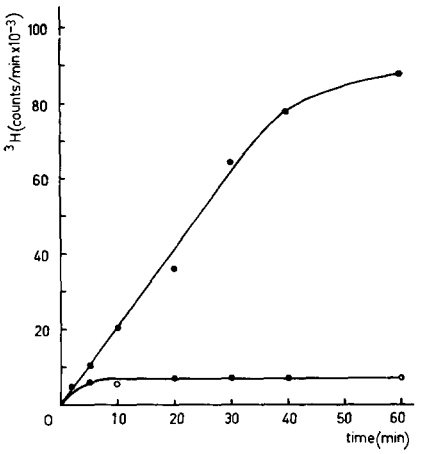

Fig. 1. Effect of 100 μg/ml of rifampicin on RNA synthesis. A culture of *E. coli* H502 was grown to a cell density of 2 · 10^8/ml, washed once, and devided in two portions. Growth medium *plus* [^{3}H]uridine (5 μC/ml) was added, and one of the portions received in addition rifampicin (100 μg/ml). Incorporation of label in RNA was determined by measuring acid-insoluble material in samples withdrawn as indicated in the figure. ●–●, no rifampicin; ○–○, 100 μg/ml of rifampicin.

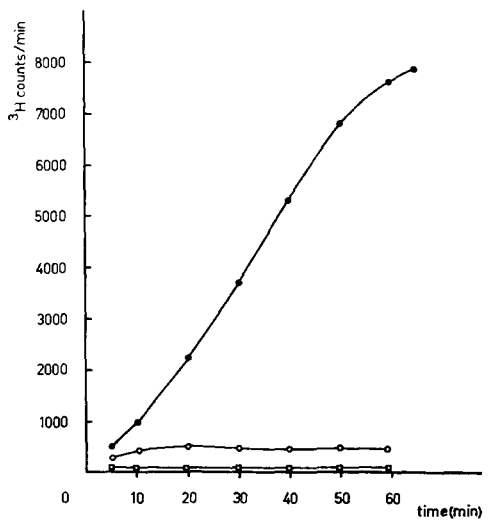

Fig. 2. Time course of DNA synthesis in mitomycin C treated *E. coli* H502 in the presence of 35 μg/ml chloramphenicol. Two cultures of *E. coli* H502 were treated with mitomycin C and infected as described in the text. [^{3}H]thymidine (2 μg/ml) was added at the onset of the process of infection (0 min). A third culture was treated in the same way but received no ΦX174. Incorporation of label into acid-insoluble alkali-resistant material was determined in samples withdrawn as indicated in the figure. □–□, uninfected culture; ○–○, infected culture, 100 μg/ml of rifampicin added 10 min before onset of the process of infection; ●–●, infected culture, no rifampicin.

infection allows RF replication to proceed for approximately 5 min at the normal rate; thereafter the rate of RF synthesis gradually decreases (Fig. 3). These results indicate that RNA synthesis is required for the initiation of RF replication as well

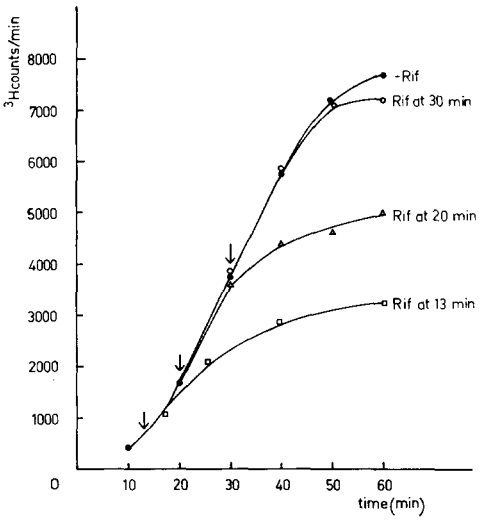

Fig. 3. Effect of rifampicin (Rif) on RF replication. *E. coli* H502 was grown to a cell density of $2 \cdot 10^8$/ml, washed, treated with mitomycin C and infected as described in the text.[^{3}H]Thymidine was added simultaneously with growth medium (0 min). Samples were transferred (arrows) to separate flasks, brought to a final concentration of 100 μg/ml of rifampicin, and incubated further. Aliquots were removed from the cultures and acid-insoluble alkali-resistant counts were determined. ●–●, original culture without rifampicin; □–□, rifampicin added at 13 min; △–△, rifampicin added at 20 min; ○–○, rifampicin added at 30 min.

as for its continuation. However, addition of rifampicin at later times after infection, 20 and 30 min respectively, allows unimpaired RF replication for much longer times, 10 min and 20 min respectively (Fig. 3). This can be explained by assuming that more copies of mRNA for cistron A protein are present late after infection, allowing protein synthesis in the absence of transcription during a longer period than early in infection, or that the cistron A protein accumulates in a pool, which is great enough to permit unimpaired RF replication late in infection for a certain period.

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