PROTEIN SYNTHESIS AND FORMATION OF GUANOSINETETRAPHOSPHATE

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1. Introduction

Guanosinetetraphosphate (ppGpp) accumulates in the nucleotide pool of *rel*$^+$ strains of *E. coli* during a number of conditions where the normal course of protein synthesis is affected [1, 2]. The accumulated ppGpp decays after treatment of the cell with several antibiotics which are strong inhibitors of protein synthesis [1, 2]. In a previous paper we suggested that an initiation process of protein synthesis was necessary for ppGpp synthesis [2]. Rifampicin induces a decay of the accumulated ppGpp [2–4] and it has been suggested that the effect of rifampicin is a consequence of the breakdown of the mRNA in the cells [2, 4].

Here we report that rifampicin is without effect on the capacity of the cells to form ppGpp if mRNA is present in the cells. This condition was obtained by infection of *E. coli* with either R17, T7 wild type or T7 gene 1 mutant bacteriophages.

2. Materials and methods

2.1. *Bacterial strains and media*

E. coli B, AS19, is a *rel*$^+$, *su*$^-$ wild type strain sensitive and highly permeable to rifampicin [5, 6]. The cells were grown exponentially at 37° in a Tris-glucose medium with 2×10^{-4} M P supplemented with 18 L-amino acids, adenosine and guanosine [2].

All experiments were started at a culture density of about 1.5×10^8 bacteria/ml. Cultures were infected at a multiplicity of 5 with either T7$^+$ or the double mutant in gene 1 T7 am23, ts 342-15 [7] (kindly supplied by Dr. M. Chamberlin).

2.2. *Measurements of capacity for ppGpp formation*

The nucleotide pool was prelabelled with $^{32}PO_4^{3-}$ for at least 10 min before the capacity for ppGpp formation was tested by treatment of the cultures with 10^{-2} M hydroxylamine for 10 min [2].

The amount of ppGpp was determined by thin-layer chromatography on polyethyleneimine cellulose sheets [1].

2.3. *Measurement of protein and RNA synthesis*

Incorporation of [^{14}C]methionine (10 μCi/μmole) and [^{14}C]uracil (5 μCi/μmole) into cold 5% TCA precipitable material was used to follow protein and RNA synthesis. The samples were filtered on glass fibre paper discs (Whatman GF/C), washed with 1% TCA and ethanol, dried and counted with 5 ml toluene containing 0.25% PPO in a Beckman scintillation counter.

3. Results

3.1. *Decay of capacity for ppGpp formation after rifampicin treatment*

Rifampicin induces a breakdown of the ppGpp accumulated in the nucleotide pool [2]. To test if this reflects a change in the capacity of the cells to form ppGpp we challenged a culture of AS19 with hydroxylamine at different times after the addition of rifampicin. Fig. 1A and B show that the capacity of the cells to form ppGpp drops in parallel with decrease in rate of protein synthesis.

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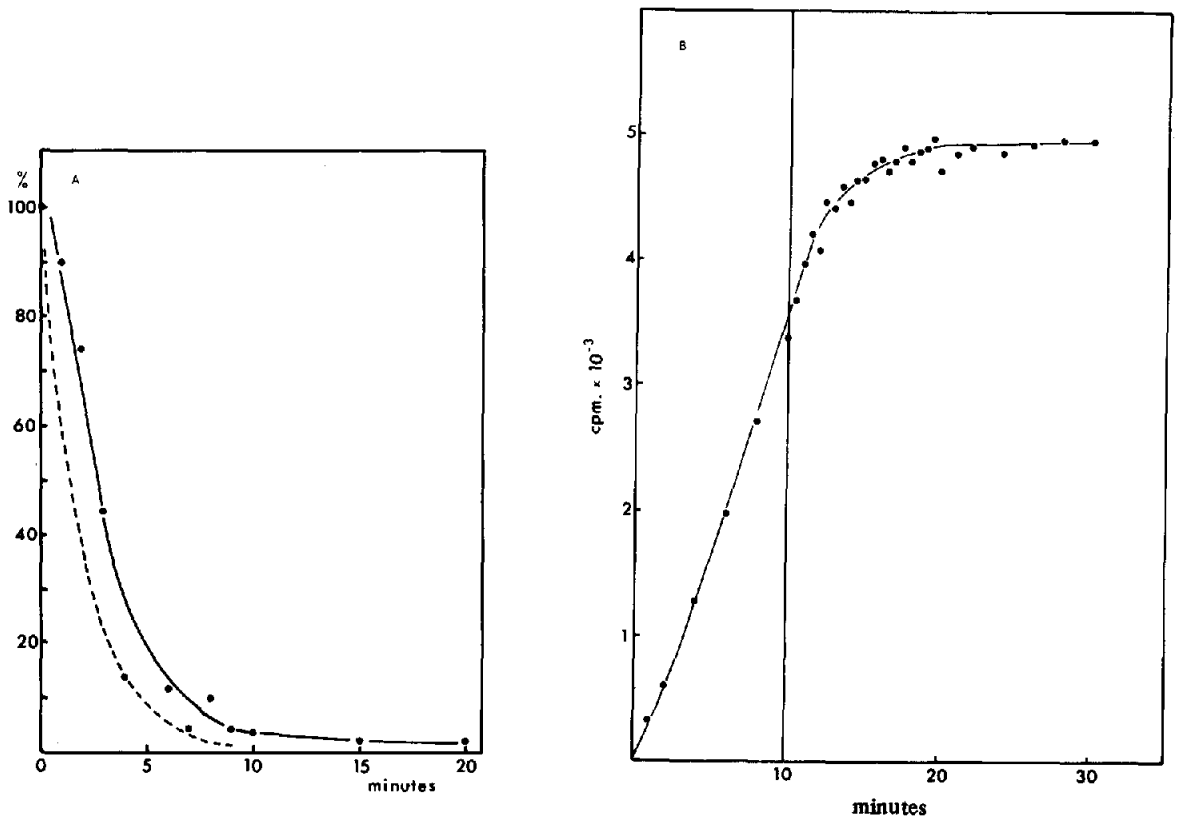


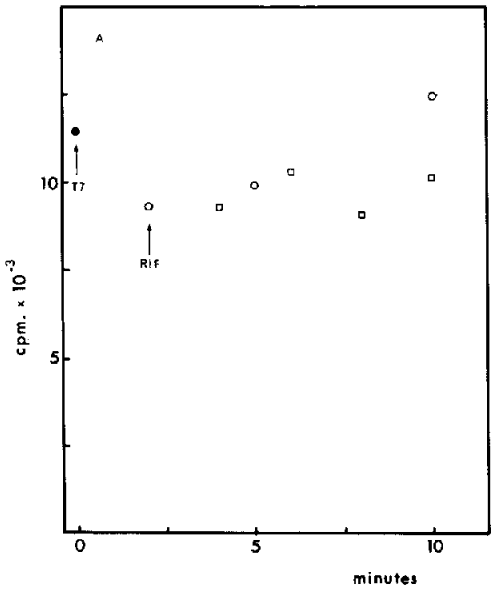
Fig. 1. ppGpp formation and protein synthesis during rifampicin treatment. A) $^{32}PO_4^{3-}$ was added to the culture at −10 min, rifampicin at 0 min. Hydroxylamine was added to aliquots of the culture at the times indicated. The amounts of ppGpp formed after a further 10 min incubation are plotted as percentage of the value before rifampicin addition. The dotted line indicates the residual rate of protein synthesis after rifampicin addition calculated from the curve in B. B) Protein synthesis was followed by incorporation of [^{14}C]methionine into trichloroacetic acid precipitable material. Rifampicin was added at time 0.

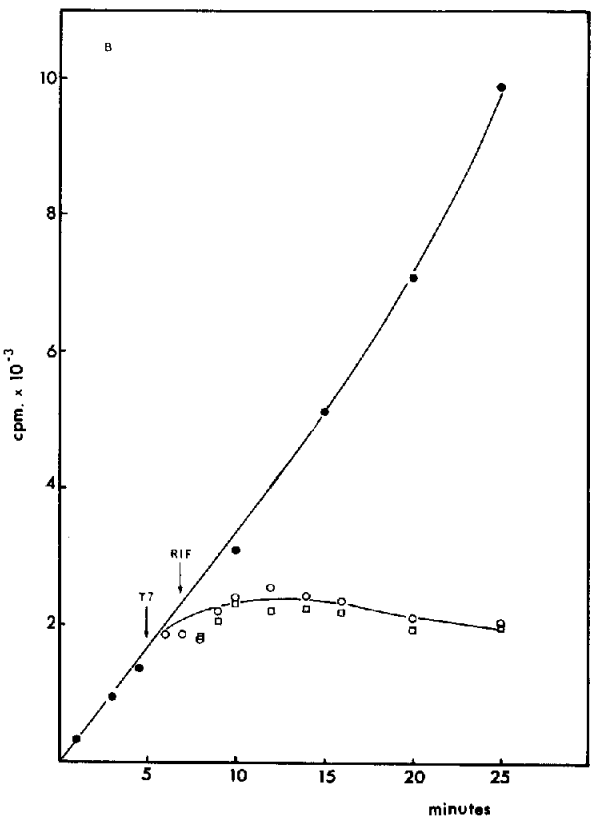
3.2. *ppGpp formation during T7$^+$ infection*

Early during T7 infection of *E. coli* the phage gene 1 coded RNA polymerase is synthesized using the host transcriptional system. The phage polymerase is specific for the transcription of the late T7 mRNA's and is insensitive to rifampicin [7]. In T7 infected cultures it is therefore possible to test if the capacity of ppGpp formation is dependent on host mRNA synthesis.

An exponentially growing culture of AS19 was infected with T7$^+$ and 2 min after infection rifampicin (30 μg/ml) was added to the culture. During this period the T7 polymerase was formed in sufficient quantities to allow a continued transcription of the T7 genomes [7]. At different times the capacity to form ppGpp was measured by challenging with hydroxylamine. Fig. 2A shows that the capacity for ppGpp formation was unaffected by the T7$^+$ phage infection and that this capacity was maintained during rifampicin treatment. The low residual RNA synthesis in T7$^+$ infected cultures was insensitive to rifampicin (fig. 2B). Protein synthesis was only slightly affected early during infection by the T7$^+$ infection and no effect of rifampicin addition was observed (fig. 2C).

These experiments indicate that the formation of ppGpp is dependent on the presence of mRNA rather than on a continued host mRNA synthesis.





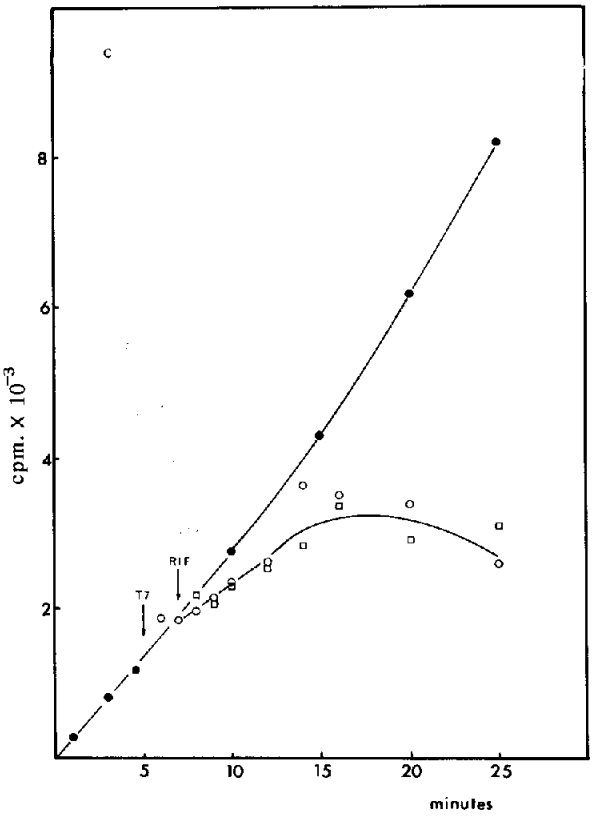


Fig. 2. ppGpp formation, RNA and protein synthesis during $T7^+$ infection. A) Capacity for ppGpp formation as in fig. 1 except that the amounts of ppGpp formed are plotted as radioactivity per 5 μl of culture. B) RNA synthesis was followed by incorporation of [^{14}C]uracil into cold trichloroacetic acid precipitable material. C) Protein synthesis was followed by incorporation of [^{14}C]methionine into trichloroacetic acid precipitable material. In A, B and C: (●—●—●) exponential; (○—○—○) $T7^+$ infected; (□—□—□) $T7^+$ infected + rifampicin.

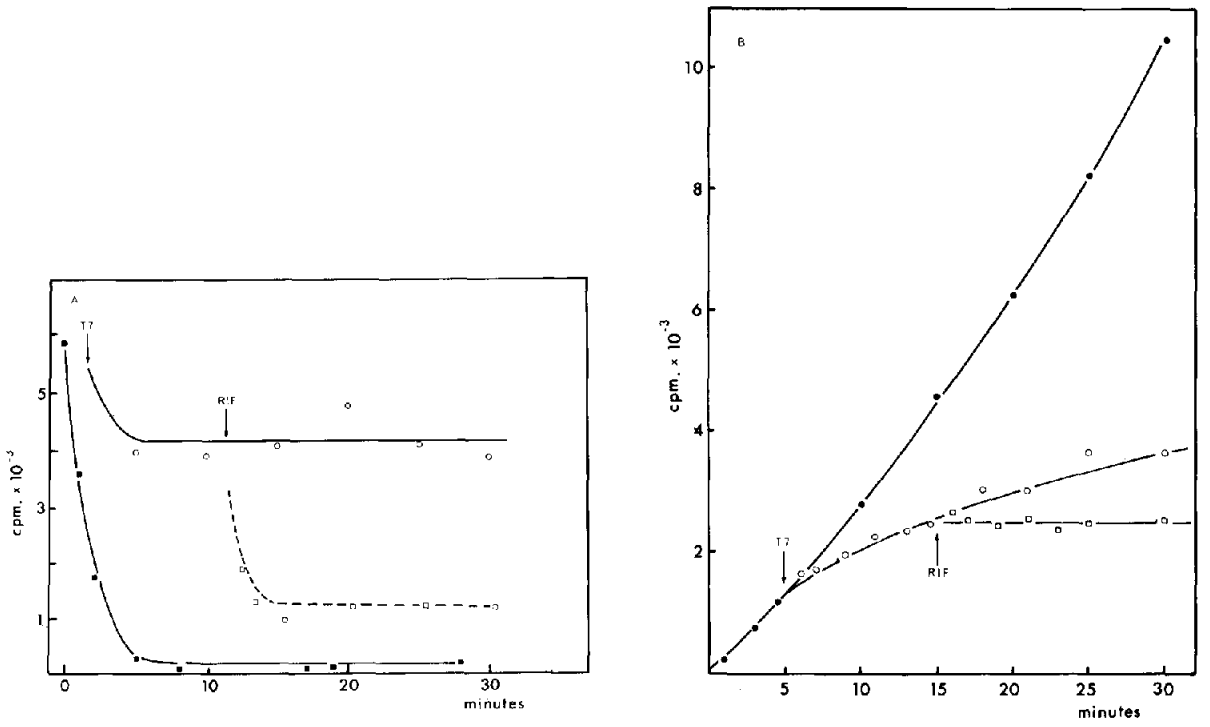


Fig. 3. ppGpp formation and RNA synthesis during T7 am23, ts342-15 infection. A) Capacity for ppGpp formation as in fig. 2A. (●—●—●) Exponential; (■—■—■) exponential + rifampicin; (○—○—○) T7 am23, ts342-15 infected; (□—□—□) T7 am23, ts342-15 infected + rifampicin. B) RNA synthesis as in fig. 2B. (●—●—●) Exponential; (○—○—○) T7 am23, ts342-15 infected; (□—□—□) T7 am23, ts342-15 infected + rifampicin.

3.3. *ppGpp formation during T7 gene 1 mutant infection*

To rule out the participation of the T7 RNA polymerase in the formation of ppGpp we performed similar experiments with a T7 gene 1 mutant bacteriophage. During infection with this phage only the early T7 mRNA's are synthesized due to the lack of a functional T7 RNA polymerase. The early T7 mRNA's corresponding to about 20% of the phage genome are transcribed by the *E. coli* RNA polymerase [8]. Early as well as late mRNA species have been found to be stable with half-lives of more than 20 min [9, 10]. During infection with a T7 gene 1 mutant it is thus possible to block all mRNA synthesis by rifampicin addition while maintaining a low level of mRNA within the cells.

A culture of AS19 growing exponentially at 40° was infected with T7 am23, ts342-15 (gene 1) and 10 min after infection rifampicin was added to the culture. The addition of rifampicin results in an immediate and complete inhibition of RNA synthesis (fig. 3B) and a simultaneous rapid decrease in the capacity to form ppGpp to a level about 20% of the capacity found in the untreated infected culture. In the uninfected culture however rifampicin as usual resulted in a complete loss of capacity for ppGpp formation (fig. 3A).

4. Discussion

From studies of the effect of rifampicin upon the level of ppGpp in a leucine starved *rel*$^+$ strain of *E. coli* Wong and Nazer [3] suggested that the synthesis of ppGpp was dependent on nascent RNA synthesis.

In a similar study Ehrlich et al. [4] suggested that the breakdown of ppGpp during treatment with rifampicin was correlated to the inhibition of RNA synthesis and the subsequent decay of mRNA.

In a previous paper we proposed that the synthesis of ppGpp is coupled to an idling reaction during initiation of protein synthesis [2]. The experiments reported in this paper rule out the participation of the transcription process in the synthesis of ppGpp. Under conditions where all RNA synthesis catalyzed by *E. coli* RNA polymerase was completely inhibited the capacity to form ppGpp is maintained if transcription of phage genomes and thereby the production of mRNA was secured by a rifampicin resistant replicating system. This capacity however was not coupled to the activity of T7 RNA polymerase but is only dependent on the presence of stable mRNA. Similar results were obtained with R17 bacteriophage infected *E. coli* cells. We therefore suggest that the capacity to form ppGpp under all conditions is coupled to an intact protein synthesizing machinery within the cells.

Similar experiments have been carried out by J.D. Friesen and N.P. Fiil (personal communication).

Acknowledgements

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