INVOLVEMENT OF THE RC LOCUS IN RNA SYNTHESIS INHIBITION BY LEVALLORPHAN IN *E. COLI*

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1. Introduction

The inhibition of protein synthesis in auxotrophic RC^{st} (*rel*$^+$) bacteria by starvation in one essential amino acid or by impairment of aminoacyl-tRNA synthetase activity, leads to a halt of net RNA synthesis [1–5] and to a large increase in pp5′G2′(3′) pp synthesis formation [6–8].

None of these phenomena are observed in RC^{rel} (*rel*$^-$) mutants [6, 7, 9]. The link between the alteration of some specific step(s) of protein synthesis and the control of RNA synthesis is very likely ppGpp as suggested by Cashel [7]. Thus, it seemed appropriate to evaluate the concentration of ppGpp in conditions where the balance between protein and RNA synthesis had been disrupted by levallorphan. Levallorphan is a morphinane derivative, which reduces the growth rate of *E. coli*, causes an immediate inhibition of ribosomal RNA synthesis while affecting protein synthesis much less [10, 11]. The present report indicates that the transitory RNA synthesis inhibition induced by levallorphan is accompanied by an over-production of ppGpp in *rel*$^+$ but not in *rel*$^-$ alleles.

2. Materials and methods

The phototrophic *E. coli* $K_{12}SF^-$ and the following mutants: 15 TAU (*thy*$^-$, *arg*$^-$, *ura*$^-$, *rel*$^+$), 15 TAU (*thy*$^-$, *arg*$^-$, *ura*$^-$, *rel*$^-$); CP 78 (*his*$^-$, *arg*$^-$, *thr*$^-$, *leu*$^-$, B_1^-, *rel*$^+$) and CP 79 (*his*$^-$, *arg*$^-$, *thr*$^-$, *leu*$^-$, B_1^-, *rel*$^-$) were grown in the medium previously described [10] and modified to reduce phosphate concentration to 2.5×10^{-4} M. Glucose or succinate were the carbon sources. The synthesis of RNA was followed by incorporation of ^{3}H-uracil in the material insoluble in cold 1 M formic acid. ppGpp was labelled, isolated and measured as described by Cashel [6, 7].

3. Results

The concentration of ppGpp increased in *rel*$^+$ but not in *rel*$^-$ mutant after levallorphan addition (fig. 1) Similar results were observed on the CP 78/CP79 allelic pair. In all cases, however, Levallorphan did affect the growth rate of *rel*$^+$ and *rel*$^-$ mutants by the same factor.

For comparison the influence of levallorphan on ppGpp and RNA synthesis in the *phototrophic* strain $K_{12}S$ growing on glucose mineral medium is shown in fig. 2. The growth rate was permanently reduced by 60% while the synthesis of RNA first inhibited by 90% progressively accelerated to fit the requirements of the new growth rate. ppGpp synthesis was transiently enhanced. Chloromycetin (100 μg/ml), when added 15 min after levallorphan, caused an immediate fall in the level of ppGpp and stimulated the accumulation of large amounts of RNA.

Thus, the pattern of RNA synthesis after levallorphan addition (i.e.: a transitory and selective inhibition of ribosomal RNA synthesis) clearly resembles that observed after a shift from a rich to a poor me-

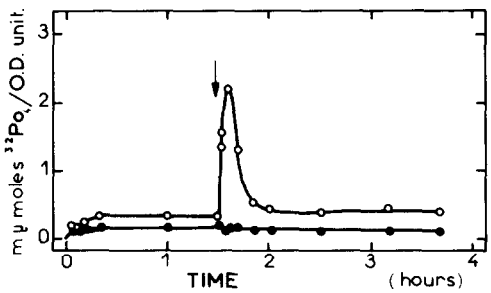

Fig. 1. Concentrations of ppGpp in *rel*$^+$ and *rel*$^-$ *E. coli* 15 TAU after levallorphan addition. *E. coli* 15 TAU (*rel*$^+$) (○—○) and 15 TAU (*rel*$^-$) (●—●) were grown exponentially in a glucose minimal medium and received ^{32}Pi (20 μCi/ml) at time zero. After 90 min levallorphan 1.5 mM was added (arrow). In both cases the growth rate was reduced from 0.95 to 0.25 g/hr.

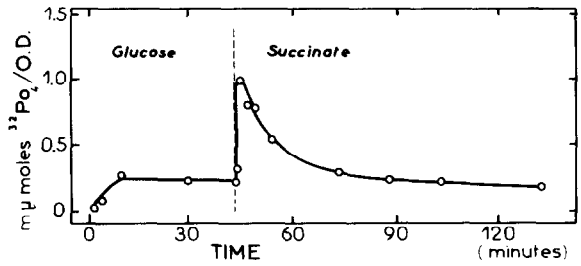

Fig. 3. Over production of ppGpp after a carbon source induced down shift. A culture of *E. coli* K_{12}S F$^-$ in a glucose-mineral medium (μ = 0.42 g/hr) received ^{32}Pi (18 μCi/ml) at time zero. 45 min later the bacteria were filtered and resuspended in the succinate-mineral medium containing also ^{32}Pi (18 μCi/ml).

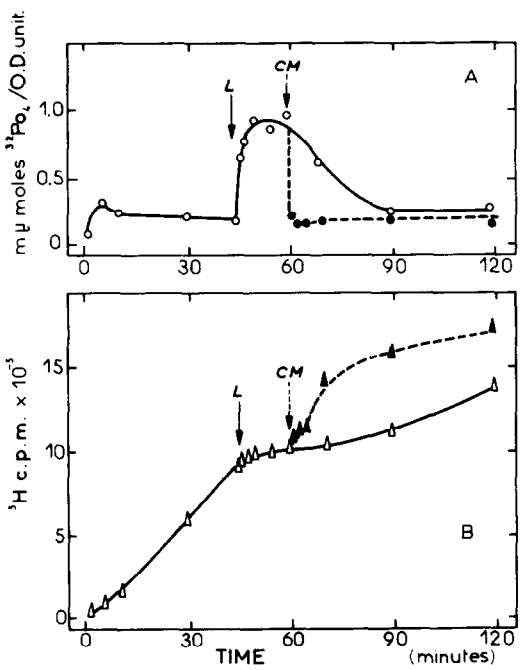

Fig. 2. Influence of levallorphan on ppGpp and RNA synthesis in *E. coli* K_{12}S. A culture of *E. coli* K_{12}S F$^-$ in glucose mineral medium was split in two parts. One part received ^{32}Pi (18 μCi/ml) and the other ^{3}H-uracil (20 μg/ml). Levallorphan 1.2 mM was added (L) after 45 min in both parts. 15 min later, chloramphenicol (CM) (100 μg/ml) was added to half of each part (●----●, ▲----▲). The growth rates were 0.42 g/hr without and 0.20 g/hr with levallorphan added. (A) ppGpp concentrations; (B) uptake of ^{3}H-uracil.

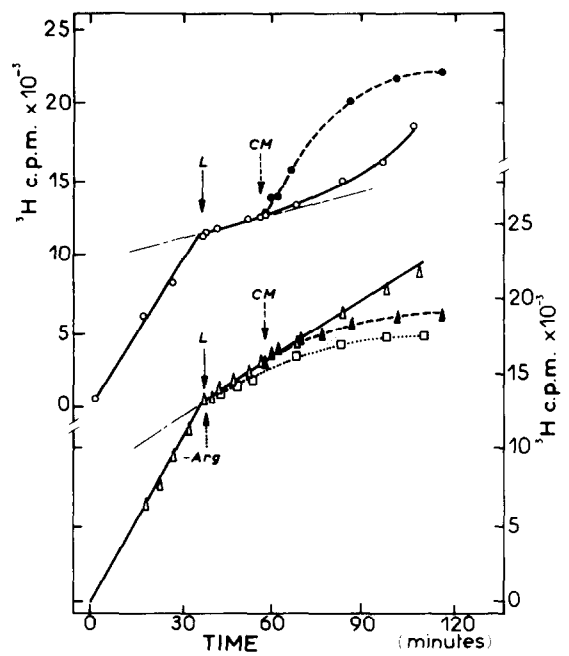

Fig. 4. Influence of levallorphan on the uptake of uracil in *rel*$^+$ and *rel*$^-$ bacteria. ^{3}H-uracil (20 μg/ml) was added to log-phase cultures of *E. coli* 15 TAU (*rel*$^+$) and 15 TAU (*rel*$^-$) in glucose minimal medium. Levallorphan 1.2 mM was added (L) after 40 min (○—○, △—△). After 60 min half of each culture received 100 μg/ml chloromycetin (CM) (●----●, ▲----▲). As control, a part of the *rel*$^-$ culture was filtered at 40 min and resuspended in arginine free medium (□----□).

dium. The fact that the level of ppGpp was simultaneously enhanced suggests that the product of the RC locus is normally involved in this type of regulation. A very similar response of ppGpp synthesis was observed in the same prototrophic strain after a shift-down involving the carbon source (fig. 3). The final growth rate, in this case, was about the same as after the addition of 1.5 mmole/l of levallorphan in the glucose-containing medium (i.e. conditions of the experiments of fig. 2).

If the transitory inhibition of rRNA synthesis induced by levallorphan is directly connected to the extra-synthesis of ppGpp, one can postulate that bacteria bearing the *rel*$^-$ allele should be unable to regulate specifically RNA synthesis after levallorphan addition. Comparison of RNA synthesis in *rel*$^+$ and *rel*$^-$ pairs supports this contention (fig. 4).

In the *rel*$^-$ mutant, the synthesis of RNA was not inhibited more than the rate of growth, and did not respond to the addition of chloromycetin. As control, the removal of arginine yielded a lower initial rate of RNA synthesis.

4. Discussion

Since the growth rate of the *rel*$^+$ and *rel*$^-$ allelic strains tested is equally sensitive to levallorphan, whereas RNA synthesis is specifically depressed in *rel*$^+$ mutants, it appears that the primary site of action of levallorphan is not RNA synthesis itself. This specific and transitory RNA inhibition associated with an increased ppGpp synthesis suggests that levallorphan triggers a regulation process mediated by the product of the RC gene.

From a number of experiments, it is more likely that levallorphan acts at the level of the bacterial membrane [12–16]. Morphinane derivatives were shown by Gale et al. [17] and Simon et al. [18] to alter the permeation of some amino acids. The limitation of amino acid availability inside the cell could explain: i) the limitation of the growth rate of auxotrophic strains of *E. coli* in an amino acid containing medium; and ii) the shift down pattern and the ppGpp overproduction observed. However, reducing the growth rate of a prototrophic strain in a medium devoid of amino acids either by levallorphan addition or by changing the carbon source raised the level of ppGpp in both cases. This could support the interpretation either that both treatments induce a leakage of endogenous amino acids or that the regulatory function of the RC gene can be triggered in several ways.

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