

## Regulation of Membrane Phospholipid Synthesis in *Escherichia coli* During Temperature Up-Shift

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The synthesis of membrane phospholipids and that of stable ribonucleic acid were inhibited during temperature up-shift of both *rel*<sup>+</sup> and *relA* strains of *Escherichia coli*. The kinetics of the inhibition of the synthesis of both molecules were correlated with the kinetics of guanosine 5'-diphosphate-3'-diphosphate synthesis. Metabolic down-shift experiments gave similar results.

The synthesis of membrane phospholipids and the synthesis of stable RNA species are regulated by the *relA* locus of *Escherichia coli* (14, 16, 17, 19-21). Amino acid starvation of stringent (*rel*<sup>+</sup>) strains results in a two- to four-fold decrease in the rate of phospholipid synthesis, whereas relaxed (*relA*) strains synthesize phospholipids normally during amino acid starvation.

In vivo experiments have shown that the control of synthesis is exerted at two levels, fatty acid synthesis (16, 17, 22) and phospholipid synthesis (15). The molecular mechanism causing the decreased rate of lipid synthesis is thought to involve the inhibition of fatty acid and phospholipid biosynthetic enzymes by guanosine 5'-diphosphate-3'-diphosphate (ppGpp). Cashel and Gallant (3) showed that this unusual nucleotide accumulates during amino acid starvation of *rel*<sup>+</sup> (but not *relA*) strains (for a review, see reference 20). However, the data obtained in vitro on the inhibition of various lipid biosynthetic enzymes are sufficiently ambiguous (for a review, see reference 6) that further in vivo experiments are indicated.

By amino acid starvation of mutants (*spoT*) blocked in the degradation of ppGpp (11), Nunn and Cronan (17) showed a quantitative correlation between the inhibition of phospholipid synthesis and the intracellular level of ppGpp. However, Gallant and co-workers (9) recently reported that shift of a *rel*<sup>+</sup> strain, NF859, from 23 to 40°C resulted in a rapid accumulation of ppGpp, whereas the accumulation in a *relA* strain, NF1035, was about 20-fold less. Surprisingly, these authors found that the accumulation of ppGpp in the *rel*<sup>+</sup> strain had no effect on the rate of stable RNA synthesis. In direct conflict

with these results (9) are the data reported by Chaloner-Larsson and Yamazaki (5) who found that stable RNA synthesis was inhibited after the shift of strain NF859 (*rel*<sup>+</sup>) from 23 to 40°C. These workers also observed an accumulation of ppGpp after temperature shift, but the magnitude of this accumulation was only about 25% of that reported by Gallant and co-workers (9). The work of Gallant et al. (9) led Raetz (20) to conclude that ppGpp is not a direct inhibitor of phospholipid synthesis, although, as he pointed out, no direct experiments on the effects of temperature up-shift on phospholipid synthesis in *E. coli* had been reported. In this paper we report the effects of temperature up-shift and metabolic down-shift on phospholipid synthesis.

The accumulation of ppGpp and the rates of synthesis of both phospholipid and stable RNA were measured after temperature shift in strain NF859 (*rel*<sup>+</sup>) and strain NF1035 (*relA*) (Fig. 1). As shown in Fig. 1 (left panel), upon shift of strain NF859 (*rel*<sup>+</sup>) from 23 to 40°C we observed the accumulation of ppGpp reported previously (5, 9). The maximal accumulation of ppGpp was about 1.1 nmol/10<sup>9</sup> cells (no accumulation of guanosine 5'-triphosphate-3'-diphosphate [ppp-Gpp] was found), a value midway between the values of Gallant and co-workers (9) and Chaloner-Larsson and Yamazaki (5). In agreement with the latter workers, we found that the rate of stable RNA synthesis was curtailed during the time interval when high levels of ppGpp were present. During that time interval the rate of phospholipid synthesis was also inhibited. The rates of both stable RNA and phospholipid began to increase as the levels of ppGpp returned to normal (Fig. 1, left panel).

In addition to our finding of an inhibition of RNA synthesis, our results also disagree with the report of Gallant and co-workers (9) concerning the magnitude of the ppGpp accumulation which occurs in the *relA* strain NF1035

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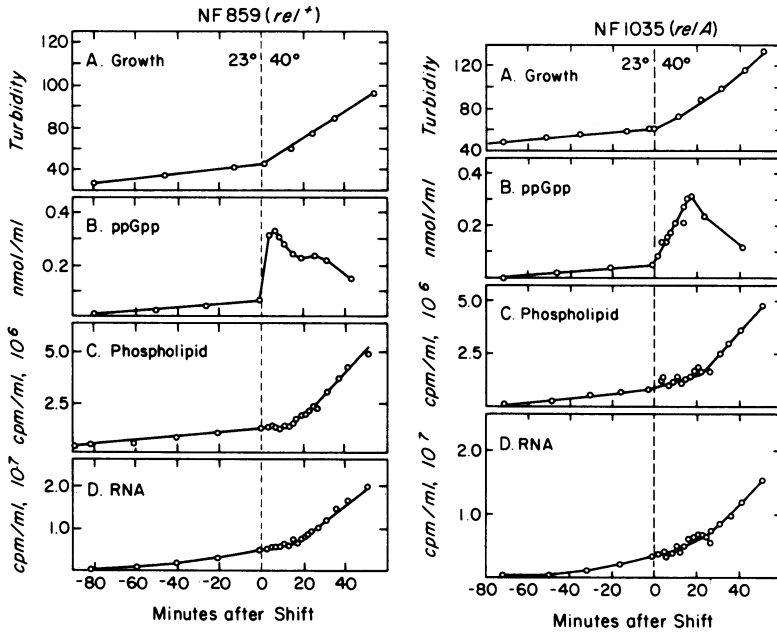


FIG. 1. Temperature up-shift of strains NF859 (*rel*<sup>+</sup>) and NF1035 (*relA*). The strains were grown at 23°C in 20 ml of Tris-buffered minimal medium (12) containing 50 µg of each of the required amino acids per ml, 0.5 mM phosphate, and 0.4% glucose, then shifted to 40°C, and monitored for (A) cell turbidity, (B) ppGpp accumulation, (C) phospholipid accumulation, and (D) RNA accumulation. The data for strains NF859 (*rel*<sup>+</sup>) and NF1035 (*relA*) are shown in the left and right panels, respectively. Turbidity was followed with a Klett colorimeter (green filter; 1 Klett unit equals about  $5 \times 10^8$  cells per ml). Accumulations of ppGpp, phospholipid, and RNA were measured by the incorporation of <sup>32</sup>P<sub>i</sub> into the molecule of interest. Accumulation of ppGpp was measured as described by Cashel et al. (4). Phospholipid synthesis was measured by a modification (17) of the method of Bligh and Dyer (1). RNA accumulation was measured by hydrolysis of the chloroform-methanol-insoluble precipitate (after washing three times with trichloroacetic acid) with 0.2 N NaOH for 20 h at 23°C. After hydrolysis, trichloroacetic acid was added to 5%. After 30 min at 0°C, the mixtures were centrifuged and the radioactivity in the supernatant was measured. Carrier-free <sup>32</sup>P<sub>i</sub> was added to the cultures at a final concentration of 100 µCi/ml at about 80 min before temperature shift. Strains NF859 and NF1035 are isogenic *metB argA* strains that differ only at the *relA* locus (9). Temperature shift was accomplished in a shaking water bath and was complete within 2 min.

after temperature shift. We found that similar amounts of ppGpp were accumulated by strains NF859 (*rel*<sup>+</sup>) and NF1035 (*relA*) (Fig. 1, right panel), whereas Gallant et al. (9) reported a 20-fold-greater accumulation of ppGpp in strain NF859 than in strain NF1035 (Chaloner-Larsson and Yamazaki [5] did not test a *relA* strain). During the time period in which high intracellular levels of ppGpp were present in strain NF1035 (*relA*), the rates of synthesis of both stable RNA and phospholipid were curtailed (Fig. 1, right panel).

It should be noted that the data in Fig. 1 are not sufficiently precise to permit a quantitative correlation between the level of ppGpp and the rate of phospholipid synthesis such as that observed earlier during amino acid starvation (17). This imprecision comes from the lower levels of ppGpp observed upon temperature up-shift and the subjective nature of determining when the

period of transient inhibition begins and ends.

We found that strains CP78 (*rel*<sup>+</sup>) and CP79 (*relA*) accumulated ppGpp in a manner similar to that found in strains NF859 and NF1035. In both strains, temperature shift resulted in a 10- to 15-min lag in the rates of synthesis of both phospholipid and stable RNA (data not shown).

Gallant and co-workers (9) pointed out that the effects of temperature up-shift resemble those of metabolic down-shift in that both perturbations result in the accumulation of only ppGpp, and ppGpp is accumulated by *relA* strains. Our results indicate that the effects of metabolic down-shift (Fig. 2) on phospholipid and RNA synthesis are also similar to those of temperature up-shift (Fig. 1).

Strains CP78 and CP79 were grown on a medium containing a limiting supply of glucose and an excess of succinate. Upon exhaustion of the glucose, growth first ceased and then resumed at

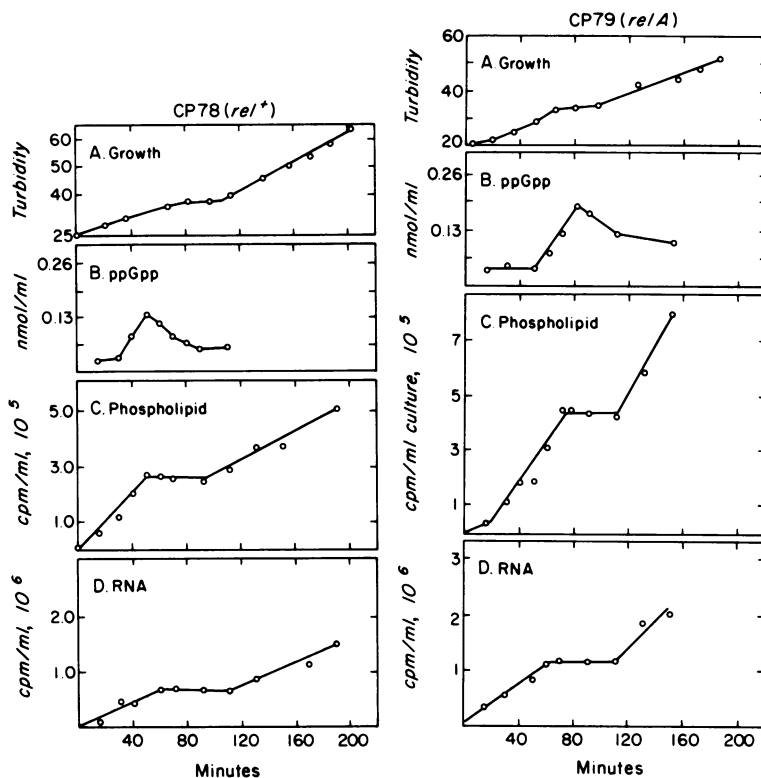


FIG. 2. Metabolic down-shift of strains CP78 (*rel*<sup>+</sup>) and CP79 (*relA*). The strains were grown at 37°C on a Tris medium (17) containing 1 mM phosphate and the required amino acids plus isoleucine (50 µg/ml each) and thiamine (1 µg/ml). The carbon source was a mixture of glucose (0.01%) and sodium succinate (0.25%). <sup>32</sup>P<sub>i</sub> (100 µCi/ml) was added at least 80 min before the break in growth, and (A) turbidity, (B) ppGpp accumulation, (C) phospholipid accumulation, and (D) RNA accumulation were monitored as described in the legend to Fig. 1. The data for strains CP78 (*rel*<sup>+</sup>) and CP79 (*relA*) are given in the left and right panels, respectively. Strains CP78 and CP79 are *arg his leu thr thi* strains isogenic except at the *relA* locus (9). Isoleucine was added to prolong the exponential growth phase of these strains (10). The *relA* gene phenotypes of these strains and those of Fig. 1 were checked by assay of RNA synthesis after amino acid starvation (8) and by their response to one-carbon metabolites (7, 23).

a slower rate when succinate was used as the carbon source (Fig. 2). As shown previously (10, 13), during the transition from glucose to succinate a transient increase in the level of ppGpp (but not pppGpp) occurred and the rate of RNA synthesis was greatly decreased.

The results that we obtained for cell growth, ppGpp synthesis, and RNA synthesis (Fig. 2) were very similar to those reported previously (10, 13). In addition, we found that the rate of phospholipid synthesis also showed a transient inhibition during the glucose-to-succinate transition. The time of the onset of the inhibition of phospholipid synthesis correlated reasonably well with the rise in ppGpp levels. The resumption of phospholipid synthesis began at about the time that the amount of ppGpp had begun to decline (Fig. 2). In agreement with the earlier reports, similar results were obtained for the

*rel*<sup>+</sup> and *relA* strains. Again, the magnitude of the ppGpp accumulation and the gradual attainment of the second phase of growth precluded a quantitative correlation between ppGpp levels and the rate of phospholipid synthesis.

Pao and Gallant (18) recently reported that guanosine 5'-diphosphate-3'-monophosphate (ppGp), a third unusual guanosine nucleotide, accumulates in *rel*<sup>+</sup> strains during amino acid starvation. This nucleotide does not accumulate during temperature up-shift (18) or metabolic down-shift (J. Gallant, personal communication). Using the methods of Pao and Gallant (18), we also were unable to detect the accumulation of ppGp during temperature up-shift; thus, the inhibition of phospholipid synthesis that we observed under these conditions cannot be attributed to an accumulation of this nucleotide.

In conclusion, the rate of phospholipid biosynthesis in *E. coli* was transiently inhibited by temperature up-shift; thus, Raetz's (20) forecast of the opposite result is incorrect. We also found a transient inhibition of the rate of RNA synthesis upon temperature up-shift; thus, our data agree with those of Chaloner-Larsson and Yamazaki (5). The timing of both the onset and the release of the transient inhibition agreed fairly well with the rise and decline of the ppGpp pool (Fig. 1) and thus adds to the case for the involvement of this nucleotide in the regulation of phospholipid synthesis. Furthermore, the inhibition of phospholipid synthesis and the increase in ppGpp occurred in *relA* strains (Fig. 1, right panel). This finding argues that the level of ppGpp, rather than another effect due to a functional *relA* gene, determines the rate of phospholipid synthesis in *E. coli*.

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