ADJUSTMENT OF RNA CONTENT DURING TEMPERATURE UPSHIFT IN ESCHERICHIA COLI

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SUMMARY

An Escherichia coli (relA$^+$ spoT$^+$) strain growing at 40^{o}C contains a lower RNA content (per protein) than the same strain growing at 23^{o}C. When the cells are transferred from 23^{o}C to 40^{o}C, the cell adjusts the RNA/protein ratio as follows. Immediately upon temperature upshift protein accumulation attains the normal rate for the cell growing at 40^{o}C but RNA accumulation for about 20 min proceeds at a rate substantially slower than the normal rate for 40^{o}C. During this adjustment period, ppGpp accumulates to a peak level which is about 30% of that accumulated when the cells growing at 40^{o}C are starved for isoleucine. Thus, ppGpp appears to regulate RNA accumulation during this adjustment period.

INTRODUCTION

When uncharged tRNA accumulates in Escherichia coli (as during amino acid starvation), a drastic reduction of stable RNA synthesis follows (1, 2). This "stringent" control is mediated by the relA$^+$ gene product which catalyzes the formation of two guanosine 3', 5'-polyphosphates, pppGpp and ppGpp. A great many whole cell studies have demonstrated a close correlation between the accumulation of ppGpp and stringent control of RNA synthesis (1, 2). Thus ppGpp has been considered as a negative effector of stable RNA synthesis. However, Gallant et al. (3) recently reported that when wild-type E. coli growing at 23^{o}C are shifted to 40^{o}C, transient accumulation of ppGpp ensues but RNA accumulation is unaffected. Since ppGpp accumulation during this temperature upshift is eliminated by the simultaneous addition of a mixture of certain amino acids (3), it appears that the supply of these amino acids cannot meet the demand by increased protein synthesis, thus raising the level of uncharged tRNA. Uncharged tRNA is known to stimulate ribosome-dependent ppGpp synthesis (4, 5). Gallant et al. (3) have thus suggested that ppGpp may not directly exert the stringent control of stable RNA, and that the real effector of stringent control is either derived from ppGpp or is another compound formed in parallel by the

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relA$^+$ gene product.

Results presented here suggest that the modest increase in the ppGpp level may be required for the adjustment of the RNA content to a lower level for *E. coli* growing at 40°C.

METHODS

An *E. coli* strain NF 859 (*relA*$^+$ *spoT*$^+$ *argA*$^-$ *metB*$^-$) was provided by Dr. J. Gallant. Cells were grown overnight by shaking at 23°C or 40°C in Tris-maleate minimal medium (TMM) (6) containing 0.4% glucose and 1 mM phosphate. Log phase cultures were prepared by diluting the overnight cultures with fresh medium and continuing to grow at the respective temperature. Cell mass density was determined by absorbance at 500 nm (A_{500}). One absorbance unit (ml x A_{500}) of a culture contains about 5 x 10^8 cells.

RNA or protein accumulation was measured by labeling with [^{14}C]uracil (1 μCi/12μg/ml) in the presence of cytosine (100 μg/ml) (3), or with [^{14}C] arginine (1 μCi/50μg/ml), respectively. The labeled RNA and protein were assayed by the paper disc method of Bollum (7) and also by the membrane filter method as used by Gallant *et al.* (3).

RNA was assayed by the orcinol method as previously described (8) and protein was assayed by the Lowry's method (9). For ppGpp, the cells were labeled for at least one doubling time with [^{32}P]orthophosphate (400 μCi/1 μmole/ml) and [^{32}P]ppGpp was assayed as previously described (10).

RESULTS AND DISCUSSION

Since the recently reported lack of correlation between ppGpp accumulation and RNA accumulation was observed in *E. coli* NF 859 (*relA*$^+$ *spoT*$^+$) (3), we have used NF 859 throughout the present work. Gallant *et al.* (3) reported that when this strain is transferred from 23°C to 40°C, ppGpp accumulates to a peak level substantially higher than that accumulated during isoleucine starvation at 23°C and it takes 60 min for ppGpp to return to the basal level for the cells growing at 40°C. Our results (Fig. 1) are quantitatively different from their observations: when the cells were transferred from 23°C to 40°C, the ppGpp level rose to a peak level which was only about 40% of that accumulated during isoleucine starvation at 23°C, and the level then declined to the basal level for 40°C within 25 min of the temperature shift.

Gallant *et al.* (3) did not determine the level of ppGpp which accumulates when the cells growing at 40°C are starved for amino acid. Fig. 1 shows that the level of ppGpp accumulated during temperature shift even at its peak

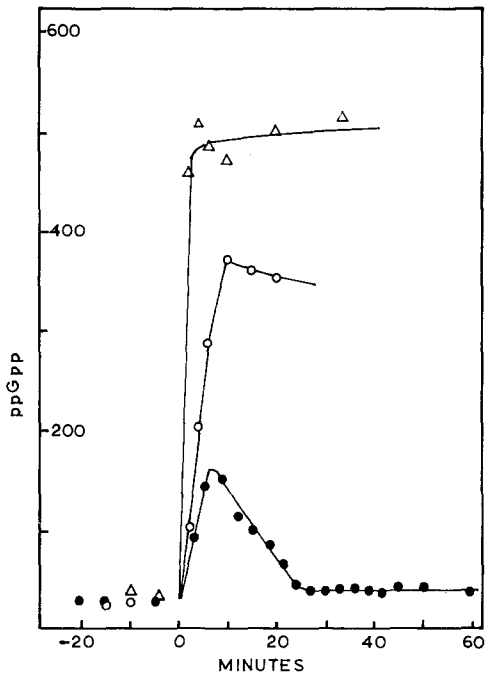

Fig. 1. ppGpp accumulation during temperature shift. A ^{32}P-labeled log phase culture growing at 23°C was transferred to 40°C at zero time. The amounts (in picomole) of ppGpp (●) per absorbance unit were determined before and after shift. As controls, the cells growing at 23°C (○) or 40°C (△) were subjected to isoleucine starvation (without temperature shift) by the addition of valine (500 μg/ml) (12) and ppGpp levels were determined.

corresponds to only 30% of that accumulated during isoleucine starvation at 40°C. RNA accumulation essentially ceased during isoleucine starvation at this temperature (data not shown). Since only partial inhibition of RNA accumulation occurs when ppGpp accumulation is below a maximal level (11), the modest accumulation of ppGpp during temperature shift is expected to cause only a modest inhibition of RNA accumulation. Fig. 2A shows that when the cells were transferred from 23°C to 40°C, RNA accumulation continued but at a rate substantially slower than the normal rate for the cells growing at 40°C. About 20 min after the shift, the cells began to accumulate RNA at the normal rate for 40°C. RNA accumulation was also measured by the membrane filter method as used by Gallant et al. (3) and the results were qualitatively similar. Gallant et al. (3) did not follow RNA accumulation beyond 20 min after the shift nor did they

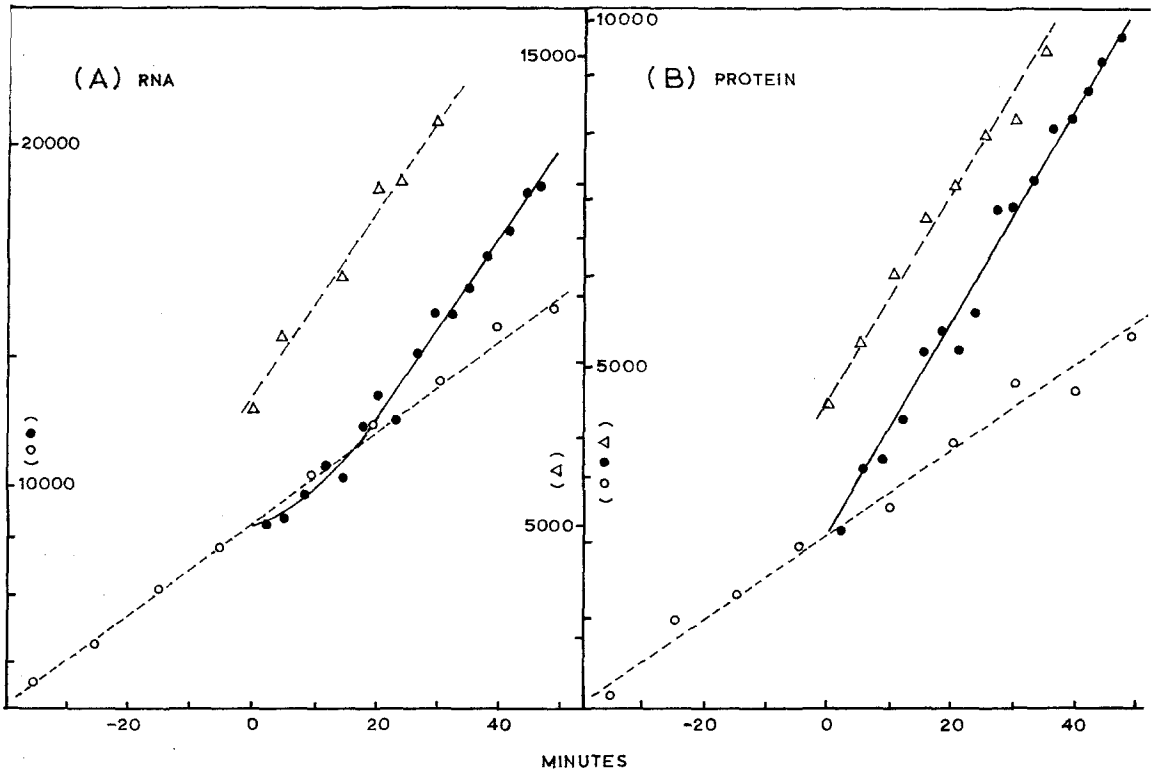

Fig. 2. RNA (part A) and protein (part B) accumulation during temperature shift. A log phase culture, grown for several generations at 23°C, was divided into two portions. One portion was labeled with [^{14}C]uracil and the other with [^{14}C]arginine (see methods). After at least one doubling a portion of each labeled culture was pipetted into prewarmed test tubes at 40°C (zero time). CCl_3COOH-precipitable radioactivities of the labeled RNA or protein were measured by the paper disc method (7) before and after the shift. Symbols: ● , accumulation after shift; o , accumulation without shift; Δ , accumulation in cells growing at 40°C (control). Radioactivities (cpm) per 50 µl culture are plotted on a logarithmic scale.

compare the postshift RNA accumulation with the normal rate for 40°C.

In contrast to RNA accumulation, protein accumulation (Fig. 2B) immediately attained the rate for 40°C when the cells were transferred from 23°C to 40°C. This differential effect of temperature shift on RNA and protein accumulation can also be illustrated by plotting the ratios of radioactivities of labeled RNA to labeled protein before and after the shift (Fig. 3). Upon shifting to 40°C, the RNA/protein ratio immediately began to fall, but by 20 min reached a

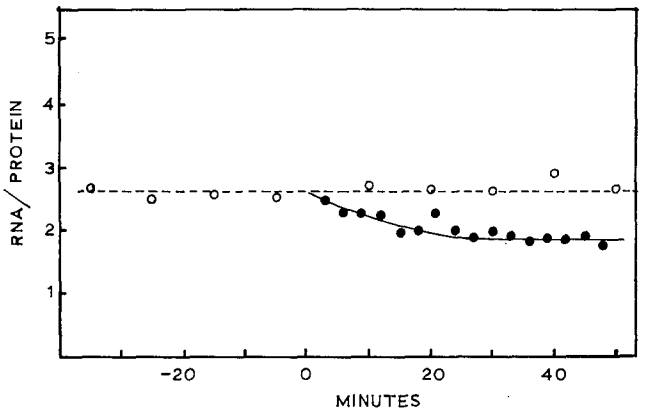

Fig. 3. Change in RNA/protein ratio during temperature shift. The radioactivities of labeled RNA (Fig. 2A) were divided by the radioactivities of labeled protein (Fig. 2B). Zero time corresponds to the time of shift from 23°C to 40°C. Symbols: ○, 23°C; ●, shift to 40°C.

steady ratio which corresponded to about 73% of the ratio at 23°C. Since this analysis indicates that the RNA/protein ratios are different at the two growing temperatures, colorimetic assays of RNA and protein were performed. It was confirmed that the RNA/protein ratio in the cells growing at 40°C was about 75% of the ratio in the cells growing at 23°C.

In summary, we can envisage the following course of events when an E. coli (relA$^+$ spoT$^+$) strain is transferred from 23°C to 40°C. There has to be a mechanism for adjusting the RNA/protein ratio to the lower value for the cells growing at 40°C. Upon shifting, protein synthesis is rapidly adjusted to the rate for 40°C. However, the supply of some amino acids will become a limiting factor, thus elevating the level of uncharged tRNA species. The uncharged tRNA species stimulate ribosome-dependent ppGpp synthesis. As ppGpp accumulates in the cells,stable RNA synthesis is partially inhibited and RNA accumulation proceeds at a rate slower than the normal rate for 40°C. As the supply of amino acids becomes sufficient, the ppGpp level falls and the cells are now able to accumulate RNA at the normal rate for 40°C.

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