Invariance of the Nucleoside Triphosphate Pools of *Escherichia coli* with Growth Rate*

(Received for publication, November 2, 1999, and in revised form, December 9, 1999)

Carsten Petersen‡ and Lisbeth Birk Møller§

*From the ‡Department of Biological Chemistry, Institute of Molecular Biology, University of Copenhagen, Solgade 83H, DK-1397 Copenhagen K, Denmark and §The John F. Kennedy Institute, Gl. Landevej 7, DK-2600 Glostrup, Denmark*

The ATP and GTP pools of *Escherichia coli* have recently been reported to increase approximately 10-fold with increasing growth rates in the range from 0.4 to 1.4 generations/hour (Gaal, T., Bartlett, M. S., Ross, W., Turnbough, C. L., and Gourse, R. L. (1997) *Science* 278, 2092–2097). Moreover, it was proposed that this variation of the nucleotide pools, particularly the ATP pool, might be responsible for the well known growth rate-dependent regulation of rRNA synthesis in *E. coli*. To test this hypothesis we have measured the nucleoside triphosphate pools as a function of growth rate for several *E. coli* strains. We found that the size of all four RNA precursor pools are essentially invariant with growth rate, in the range from 0.5 to 2.3 generations/hour. Nevertheless we observed the expected growth rate-dependent increase of RNA accumulation in these strains. In light of these results, it seems unlikely that nucleotide pool variations should be responsible for the growth rate-dependent regulation of rRNA synthesis.

It has been known for more than 30 years that the ribosome content of bacterial cells increases with increasing growth rates, governed by regulatory mechanisms which adjust the rate of ribosome biosynthesis to match the available resources present in the growth medium (for reviews, see Refs. 1 and 2). The seven rRNA operons of *Escherichia coli* are each preceded by two unusually strong promoters and are influenced by several elaborate control mechanisms. These include transcriptional activation by the FIS protein, repression by the regulatory nucleotide guanosine 5',3'-bispyrophosphate (ppGpp), as well as an antitermination mechanism that depends on the interaction of RNA polymerase with several protein factors (reviewed in Ref. 2).

While it is generally accepted that ppGpp is responsible for the abrupt decrease of rRNA synthesis that occurs in response to sudden restrictions of the amino acid or carbon source supply, the mechanisms responsible for the growth rate-dependent regulation of RNA synthesis during exponential growth have been a subject of controversy (reviewed in Ref. 3). Many observations indicate that growth rate-dependent control of rRNA synthesis may be achieved by a feedback-mechanism, which somehow senses the presence of excess functional ribosomes and regulates rRNA transcription accordingly (4). However, the molecular nature of the feedback signal generated by excess translation has remained obscure.

Recently Gaal et al. (5) showed that the activity of the ribosomal RNA P1 promoters *in vitro* correlates specifically with the concentration of the initiating nucleotide, which is GTP for the *rrnD* operon and ATP for the remaining six ribosomal RNA operons of *E. coli*. Moreover, it was reported that the ATP and GTP pools increase approximately 10-fold with increasing growth rates in the range from 0.4 to 1.4 generations/h. Based on these observations it was proposed that the ATP and GTP pool variations may be responsible for the growth rate-dependent regulation of rRNA synthesis *in vivo*. Furthermore, it was proposed that the size of the ATP and GTP pools might constitute the elusive feedback signal that is sensed by the ribosomal RNA operons as an indicator of the translational capacity in the cell. Excessive translation should drain these nucleotide pools resulting in a reduction of ribosome synthesis, whereas insufficient translational activity should cause an increase of the nucleoside triphosphate (NTP) pools, thereby increasing ribosome synthesis.

Here we have reinvestigated the relationship between growth rate and the size of the NTP pools. We found that the ATP, GTP, CTP, and UTP pools of several *E. coli* strains were essentially invariant with growth rate. Nevertheless, these strains showed the normal growth rate-dependent increase of RNA accumulation. In light of these results we find it unlikely that growth rate-dependent regulation of ribosome synthesis should be mediated by variation of the ATP or GTP pools. Moreover, we argue that these nucleotide pools do not function as sensitive feedback signals reflecting the total translational activity of the cell, since they are essentially unaffected by perturbations of the translation process.

**EXPERIMENTAL PROCEDURES**

**Bacterial Strains**

The bacterial strains used in this study are all derivatives of *E. coli* K12.

**CN1539**—ara Δ(gpt-pro-lac) thi zce 7cze/Tn10/F'(gpp' proAB' codAB' lacZΔlacZTn5) is the wild type member of an isogenic rne'/rne3071 strain pair, which was constructed by P1-mediated transduction of zce 7cze/Tn10 from CH1826 (6) into CSH26 (7). The F' factor, which complements the Δ(gpt-pro-lac) deletion, was subsequently introduced by conjugation with NF1829 (8).

**CN1709**—F' ara Δ(codB-lacZΔlacZ) thi, is a derivative of CSH26, in which the Δ(gpt-pro-lac) deletion has been replaced by the shorter Δ(codB-lac) deletion, also known as Δ(lacZΔlacZ), from NF1829.

**MG1655**—F' rph1(9) was obtained from Dr. K. F. Jensen.

**CF7968**—F' Δ(lacI) is an rph' derivative of MG1655, kindly provided by Dr. M. Cashel.

**Measurements of Nucleotide Pools**

Bacteria were grown with shaking in Tris-buffered medium (10) with the phosphate concentration lowered to 0.3 mM. The medium was supplemented with 1 µg/ml thiamine, and the different carbon sources were added to a final concentration of 0.2%. When appropriate cultures were supplemented with all 20 amino acids in the concentrations specified by Neidhardt et al. (11). Procedures for labeling of cultures with [32P]phosphate and for determination of nucleotide pools by thin layer
chromatography have been described previously (12). These experiments were performed two or three times for each strain with similar results. The data shown in Fig. 1 are from a single representative experiment.

Measurements of RNA Accumulation

The accumulation of total RNA per A_{260} was determined by measuring the incorporation of 32P-phosphate into acid-precipitable, KCl-soluble material by a modification of the procedure described by Vogel et al. (13). Briefly, [32P]phosphate-labeled samples, harvested as for nucleotide determinations, were mixed with 1 ml of 0.5 M perchloric acid containing unlabeled cells (A_{260} = 1). After 10 min on ice the acid precipitate was collected by centrifugation, and the resulting pellet was washed twice with 1 ml of 0.5 M perchloric acid to remove acid-soluble material and subsequently with 1 ml of 96% ethanol to remove phospholipids. The washed pellet was resuspended in 0.5 ml of 0.5 M KOH and incubated at 37 °C for 20 h. Alkali-stable DNA was then precipitated by addition of 100 μl of 6 M perchloric acid, and following a 2 min centrifugation, the radioactivity in the supernatant was determined by liquid scintillation counting. In control experiments we subjected supernatant samples to two-dimensional thin layer chromatography and found that 80–90% of the radioactivity was present in the eight spots corresponding to the 2'- or 3'-ribonucleoside monophosphates formed by alkaline hydrolysis of RNA. No other distinct products were detectable.

RESULTS

Effect of Growth Rate on the NTP Pools—In connection with studies of nucleotide metabolism in RNase-deficient strains we found that the nucleotide pools in a wild type control strain, CN1539, were not increased by supplementation of glycerol minimal medium with all 20 amino acids, even though the amino acid enrichment more than doubled the growth rate (Fig. 1a). This result was in direct conflict with the recent report by Gaal et al. (5), according to which a doubling of the growth rate should lead to a 4-fold increase of the ATP and GTP pools.

Consequently, we decided to investigate the growth rate dependence of the nucleotide pools in more detail using several different growth media giving a larger span of growth rates. For these measurements we used the strain CN1709, which like CN1539 is a derivative of strain CSH26 (see “Experimental Procedures”). As shown in Fig. 1b, the size of all four rNTP pools were essentially invariant in the investigated range of growth rates from 0.5 to 2.3 generations/h, in marked contrast to the 10-fold increase reported in Ref. 5.

We considered the possibility that the conflicting results might be caused by strain differences. Noting that the strains used by Gaal et al. (5) were derivatives of MG1655, we performed some preliminary experiments to measure the nucleotide pools in MG1655 itself. We found that the ATP and GTP pools were essentially constant for growth rates between 0.4 and 1.9 generations/h, if the growth medium was supplemented with uracil (data not shown). MG1655 is not a pyrimidine requiring strain, but it suffers from pyrimidine limitation at fast growth rates due to the rph1 mutation, which interferes with expression of the downstream pyrE gene (14). Pyrimidine limitation is known to cause a swelling of the purine nucleotide pools (13), and the ATP and GTP pools of MG1655 were indeed 2.5-fold higher during rapid growth in the absence of uracil, whereas uracil supplementation had no significant effect on the purine nucleotide pools during slow growth (data not shown).

To avoid the complications with uracil supplementation, we chose to measure the nucleotide pools in CF7968, a derivative of MG1655, in which the rph1 allele have been replaced by the wild type gene (kindly provided by Dr. M. Cashel). As shown in Fig. 1c, the nucleotide pools of CF7968 showed very little variation with growth rate in agreement with the findings for CN1539, CN1709, and MG1655 (in the presence of uracil). In the experiment shown in Fig. 1, the nucleotide pools of CF7968 appeared slightly larger than those of CN1709. However, this difference was not observed in other experiments and, thus, was probably not significant.

In general determinations of the absolute size of the nucleotide pools may show some day to day variation, due to slight differences of the phosphate concentration in different batches of growth medium or due to minor pipetting errors occurring during sample preparation and loading of the thin layer chromatograms. The relative size of the nucleotide pools determined from a single chromatogram, however, is not affected by these uncertainties and thus can generally be determined with greater accuracy. As shown in Fig. 1f, the relative size of the four rNTP pools was also largely invariant with growth rate, except perhaps for a tendency of the GTP pool to increase slightly with growth rate relatively to the three other pools.

Growth Rate-dependent RNA Accumulation—To see if the strains used here would show the normal growth rate-dependent increase of stable RNA accumulation despite their essentially constant nucleotide pools, we measured the accumulation
The slight difference between growth rate, due to partial pyrimidine limitation at the higher with a slightly compromised pyrimidine biosynthesis may show discrepancies, but our results with MG1655 show that strains (1.5- and 2-fold, respectively). We cannot account for these ATP pool was reported to increase moderately with growth rate and the rate of rRNA synthesis in opposite directions (21, 22).

The present work adds to the large body of evidence showing that the ATP pool is strongly buffered around a fairly constant value, varying less than 2-fold over a wide range of growth rates. We cannot explain the discrepancy with the 10-fold variation reported for ATP and GTP by Gaal et al. (5), which primarily reflects their finding of approximately 4-fold greater pools at the highest growth rates compared with the present work. It should be emphasized, however, that this difference was not caused by a technical limitation in our extraction procedure, which has been used to measure nucleotide concentrations that are even 10-fold higher than the normal ATP concentration (12). The only difference between the strain CF7968 used here, and RLG3492 used in Ref. 5, is the presence in the latter strain of a lambda prophage with an rrrP1-lacZ fusion. Considering the low growth rate of RLG3492 in amino acid-supplemented glucose medium (μ = 1.35 compared with μ = 2.3 for CF7968, Fig. 1c), it is conceivable that the high rate of β-galactosidase synthesis from this fusion at the higher growth rates may have perturbed cellular physiology to cause an abnormal swelling of the ATP and GTP pools, for instance by imposing some kind of pyrimidine restriction. It is known that gratuitous protein overproduction can severely distort cellular metabolism (29).

Mechanisms Controlling the Size of the ATP Pool—The size of the ATP pool is determined by the interplay of many metabolic fluxes as illustrated in Fig. 3. All the biosynthetic reactions, that are driven by the conversion of ATP to ADP or AMP, together with the reactions that regenerate ATP constitute a large cyclic flux, which is 2 orders of magnitude greater than the fluxes that feed and drain the total adenylate pool, i.e. the de novo synthesis flux and the incorporation of adenine nucleotides into nucleic acids and metabolites. Most importantly the recycling fluxes between the ATP, ADP, and AMP pools are also very large compared with the size of these pools, which turn over in a fraction of a second (Fig. 3). Thus, the recycling fluxes must be strictly balanced, to avoid depletion of any of the pools, and to maintain the "energy charge," defined as ([ATP] + 0.5[ADP])/([ATP] + [ADP] + [AMP]) (30, 31). If for instance the ATP to ADP conversion should be blocked, the rate of ADP phosphorylation would have to be reduced accordingly within seconds, if not for anything else then for the lack of substrate.

Apart from substrate limitation, two major factors contribute to maintain the balance between the adenine nucleotide pools and the fluxes between them. One is the high capacity adenylate kinase enzyme, which catalyzes the reversible reaction, AMP + ADP = 2 ADP (32, 33). The other is the general tendency of enzymes that utilize or regenerate ATP, respectively, to respond reciprocally to changes in energy charge (30, 31). Together these mechanisms balance the phosphorylation-dephosphorylation fluxes on a time scale of seconds to maintain a constant energy charge over a wide range of growth conditions (17, 34–36).

Even though the translation process accounts for a substantial fraction of the ATP recycling (Fig. 3), it does not necessarily have a strong influence on the size of the ATP pool, because a reduction of translation and the associated ATP turnover may be balanced within a second by a corresponding decrease of ATP regeneration. Indeed the ATP and GTP pools remain unaffected for several minutes following a complete block of translation with chloramphenicol (28), as does the energy charge (34). Thus, the ATP pools do not function as a sensitive feedback signal informing the rRNA operons about the translational activity in the cell.

The total adenine nucleotide pool turns over in less than a minute determined by the balance between the de novo syn-

---

Invariance of NTP Pools with Growth Rate

**DISCUSSION**

**Invariance of NTP Pools with Growth Rate**—The present work shows that the NTP substrate pools for RNA polymerase are essentially invariant with growth rate, which is not compatible with the idea that nucleotide pool variations should be responsible for the growth rate-dependent regulation of rRNA synthesis. The NTP pools have been implicated in the regulation of rRNA synthesis before (e.g. Refs. 15 and 16). However, the idea was generally abandoned, because evidence accumulated, showing that shifts of growth conditions, which greatly affect the growth rate and rRNA synthesis, have little or no effect on the NTP pools (17–20) or even affect the NTP pools and the rate of rRNA synthesis in opposite directions (21, 22).

Moreover, numerous studies performed with cells in steady-state exponential growth have shown the ATP pool to be essentially invariant with growth rate (23–26), in agreement with the present results. In two studies (27, 28), however, the ATP pool was reported to increase moderately with growth rate (1.5- and 2-fold, respectively). We cannot account for these discrepancies, but our results with MG1655 show that strains with a slightly compromised pyrimidine biosynthesis may show such a 2-fold increase of the purine nucleotide pools with growth rate, due to partial pyrimidine limitation at the higher growth rates. This may not be an uncommon phenomenon.

In summary, the present work adds to the large body of

---

2 C. Petersen, unpublished data.
Invariance of NTP Pools with Growth Rate

thickness flux and the fluxes into nucleic acids and metabolites (Fig. 3). Like the adenine nucleotide distribution the size of the total pool seems to be strongly buffered against perturbations. Even when RNA synthesis is inhibited with rifampicin, the ATP pool increases less than 2-fold and the GTP pool even less² (37), while the energy charge remains unaffected (34). Apparently this homeostasis is predominantly mediated by regulation of the purine de novo synthesis via feedback signals from purine nucleotides to phosphoribosylpyrophosphate synthetase and to the PurF enzyme (see Ref. 12 for a discussion).

The Mechanism of Growth Rate-dependent Control of rRNA Synthesis—We emphasize that the present finding of growth rate-independent NTP pools does not preclude that variations of the ATP and GTP concentrations, if they should occur, might affect the activity of the rRNA promoters. However, it should be noted that the rate of rRNA synthesis shows an inverse relationship with the ATP pool during pyrimidine limited growth (13) or during an amino acid-induced upshift (22). Thus, the effect of the initiating nucleotide concentration on the rrl P1 promoter activity can be completely overruled by other control mechanisms acting on rRNA synthesis.

Unfortunately the present results do not provide an alternative explanation for the growth rate-dependent control of rRNA synthesis. Several mechanisms may contribute to the phenomenon, e.g. growth rate-dependent inhibition of RNA synthesis by ppGpp (38), passive redistribution of RNA polymerase from biosynthetic genes to rRNA operons in richer media (39), degradation of nascent RNA during slow growth (40), growth rate-dependent variations in the concentration of free RNA polymerase (37), or modulation of antitermination or FIS-mediated activation (2). Most discussions of growth rate-dependent regulation have tacitly assumed that one mechanism should be solely responsible for the effect; however, the answer to the problem may well be that several mechanisms are involved (3, 13). It should be noted that the amount of RNA per cell mass barely increases 2-fold over the entire range of growth rates³ (Fig. 2b). If four or five different mechanisms each contributed say 15–20% to this increase, then any one of them could be eliminated without severely affecting the growth rate-dependent regulation.

Acknowledgments—We acknowledge the expert technical assistance of Jeanette Lundin, and we thank Drs. M. Cashel and K. F. Jensen for the generous donation of bacterial strains. Furthermore, we are grateful to K. F. Jensen for helpful comments on the manuscript.

REFERENCES
1. Bremer, H., and Dennis, P. P. (1996) in Escherichia coli and Salmonella: Cellular and Molecular Biology (Neidhardt, F. C., Curtiss, R., III, Ingraham, J. L., Lin, E. C. C., Low, K. B., Magasanik, B., Reznikoff, W. S., Riley, M., Schaechter, M., and Umbarger, H. E., ed) 2nd Ed., Vol. 2, pp. 1553–1569, American Society for Microbiology, Washington, D. C.
2. Gourse, R. L., Gaal, T., Bartlett, M. S., Appleman, J. A., and Ross, W. (1996) Annu. Rev. Microbiol. 50, 645–677
3. Cashel, M., Gentry, D. R., Hernandez, V. J., and Vinel, D. (1996) in Escherichia coli and Salmonella: Cellular and Molecular Biology (Neidhardt, F. C., Curtiss, R., III, Ingraham, J. L., Lin, E. C. C., Low, K. B., Magasanik, B., Reznikoff, W. S., Riley, M., Schaechter, M., and Umbarger, H. E., ed) 2nd Ed., Vol. 1, pp. 1458–1496, American Society for Microbiology, Washington, D. C.
4. Jinke-Robertson, S., and Nomura, M. (1987) in Escherichia coli and Salmonella typhimurium: Cellular and Molecular Biology (Ingraham, J. L., Low, K. B., Magasanik, B., Neidhardt, F. C., Schaechter, M., and Umbarger, H. E., ed) Vol. 2, pp. 1388–1385, American Society for Microbiology, Washington, D. C.
5. Gaal, T., Bartlett, M. S., Ross, W., Turnbaugh, C. L., and Gourse, R. L. (1997) Science 278, 2092–2097
6. Mudd, E. A., Krisch, H. M., Higgins, C. F. (1990) Mol. Microbiol. 4, 2127–2135
7. Miller, J. H. (1972) Experiments in Molecular Genetics, p. 17, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
8. Shultz, J., Stihary, T. J., Berman, M. L., Fiil, N., and Emr, S. D. (1982) Cell 31, 257–265
9. Guyer, M. S., Reed, R. R., Steitz, J. A., and Low, K. B. (1981) Cold Spring...
Harbor Symp. Quant. Biol. 45, 135–140

10. Edlin, G., and Maaløe, O. (1966) J. Mol. Biol. 15, 428–434
11. Neidhardt, F. C., Bloch, P. L., and Smith, D. F. (1974) J. Bacteriol. 119, 736–747
12. Petersen, C. (1999) J. Biol. Chem. 274, 5348–5356
13. Vogel, U., Pedersen, S., and Jensen, K. F. (1991) J. Bacteriol. 173, 1168–1174
14. Jensen, K. F. (1993) J. Bacteriol. 175, 3401–3407
15. Gallant, J., and Harada, B. (1969) J. Biol. Chem. 244, 3125–3132
16. Erlich, H., Gallant, J., and Lazzarini, R. A. (1975) J. Biol. Chem. 250, 3057–3061
17. Chapman, A. G., Fall, L., and Atkinson, D. E. (1971) J. Bacteriol. 108, 1072–1086
18. Friesen, J. D., Fiil, N. P., and von Meyenburg, K. (1975) J. Biol. Chem. 250, 304–309
19. Nazar, R. N., and Wong, J. T. F. (1972) J. Biol. Chem. 247, 790–797
20. Winslow, R. M. (1971) J. Biol. Chem. 246, 4872–4877
21. Bagnara, A. S., and Finch, L. R. (1968) Biochim. Biophys. Res. Commun. 33, 15–21
22. Beck, C. H., Ingraham, J., Maaløe, O., and Neuhard, J. (1973) J. Mol. Biol. 78, 117–121
23. Franzen, J. S., and Binkley, S. B. (1961) J. Biol. Chem. 236, 515–519
24. Holms, W. H., Hamilton, I. D., and Robertson, A. G. (1972) Arch. Microbiol. 83, 95–109
25. Lowry, O. H., Carter, J., Ward, J. B., and Glaser, L. (1971) J. Biol. Chem. 246, 6511–6521
26. Smith, R. C., and Maaløe, O. (1964) Biochim. Biophys. Acta 66, 229–234
27. Moses, V., and Sharp, P. B. (1972) J. Gen. Microbiol. 71, 181–190
28. Bagnara, A. S., and Finch, L. R. (1973) Eur. J. Biochem. 36, 422–427
29. Kurland, C. G., and Dong, H. (1996) Mol. Microbiol. 21, 1–4
30. Atkinson, D. E. (1968) Biochemistry 7, 4030–4034
31. Chapman, A. G., and Atkinson, D. E. (1977) Adv. Microbiol. Physiol. 15, 253–266
32. Glaser, M., Nulty, W., and Vageos, P. R. (1975) J. Bacteriol. 123, 128–136
33. Glombotski, C. C., Chapman, A. G., and Atkinson, D. E. (1981) J. Bacteriol. 145, 1374–1385
34. Andersen, K. B., and von Meyenburg, K. (1977) J. Biol. Chem. 252, 4151–4156
35. Sadler, W. C., and Switzer, R. L. (1977) J. Biol. Chem. 252, 8504–8511
36. Swedes, J. S., Sedo, R. J., and Atkinson, D. E. (1975) J. Biol. Chem. 250, 6930–6938
37. Jensen, K. F., and Pedersen, S. (1999) Microbiol. Rev. 54, 89–100
38. Ryals, J., Little, R., and Bremer, H. (1969) J. Bacteriol. 151, 1261–1268
39. Maaløe, O. (1979) in Biological Regulation and Development (Goldberger, R., ed) Vol. 1, pp. 487–542, Plenum Publishing Corp., New York
40. Gauze (1977) J. Mol. Biol. 115, 335–354
41. Neidhardt, F. C., Ingraham, J. L., and Schaechter, M. (1990) Physiology of the Bacterial Cell, Sinauer Associates Inc., Sunderland, MA
42. Neuhard, J., and Nygaard, P. (1987) in Escherichia coli and Salmonella typhi-
murium: Cellular and Molecular Biology (Ingraham, J. L., Low, K. B., Magasanik, B., Neidhardt, F. C., Schaechter, M., and Umbarger, H. E., eds) Vol. 1, pp. 445–473, American Society for Microbiology, Washington, D. C.
43. Varma, A., and Palsson, B. O. (1994) Appl. Environ. Microbiol. 60, 3724–3731