JOURNAL OF BACTERIOLOGY, Jan. 1980, p. 67-73
0021-9193/80/01-0067/07$02.00/0

Vol. 141, No. 1

Growth Rate-Dependent Regulation of Transfer Ribonucleic Acid (5-Methyluridine)Methyltransferase in *Escherichia coli* B/r

TOR NY AND GLENN R. BJÖRK*

Department of Microbiology, University of Umeå, S-901 87 Umeå, Sweden

Enzymes catalyzing the transfer of methyl groups from *S*-adenosyl-L-methionine to a precursor transfer ribonucleic acid (tRNA) and forming 5-methyluridine (m^5U), 1-methylguanine (m^1G), or 5-methylaminomethyl-2-thio-uridine (mam^5s^2U) are denoted tRNA(m^5U)-(EC 2.1.1.35), tRNA(m^1G)-(EC 2.1.1.31), and tRNA(mam^5s^2U)methyltransferase. We have studied the regulation of these tRNA biosynthetic enzymes in *Escherichia coli* under various physiological conditions and in bacterial mutants known to affect the regulation of components of the translational apparatus. Such studies have revealed that tRNA(m^5U)-methyltransferase increases with the growth rate in the same fashion as stable RNA, whereas the activity of two other tRNA methyltransferases remains constant in relation to the growth rate. Thus, these tRNA biosynthetic enzymes were not coordinately regulated. Regulation of both tRNA(m^5U)methyltransferase and stable RNA was similar during shift-up and shift-down experiments. This enzyme showed a stringent regulation in *relA*$^+$ strain (T. Ny and G. R. Björk, J. Bacteriol. **130**:635–641, 1977) but also in two temperature-sensitive mutants, *fusA* and *fusB*, known to influence the accumulation of guanosine 5′-diphosphate 3′-diphosphate and RNA synthesis at nonpermissive temperatures. The tRNA(m^5U)methyltransferase showed a gene dose effect when its structural gene, *trmA*, was carried on a plasmid or on λ transducing phages. Although the regulation of tRNA-(m^5U)methyltransferase was surprisingly coupled to that of stable RNA, this enzyme was expressed at a much lower level.

tRNA plays a central role in the metabolism of the cell not only in its well-known function as an activator of amino acids for protein synthesis, but also as a regulatory element in many amino acid biosynthetic pathways (17). During the last few years, the mechanism of the biosynthesis of tRNA has been intensively studied (33, 34). It is known that the primary transcription product is larger than the mature tRNA. This primary transcription product is cleaved by several endo- and exonucleases. Polymeric as well as monomeric precursors have been identified. During this maturation process, several modification reactions such as tRNA methylation must occur. The methylated nucleoside m^5U (ribothymidine) is present in all tRNA chains in *Escherichia coli* and is always positioned in the TΨCG-loop (loop IV) of the tRNA (31). The enzyme which catalyzes this methylation, tRNA(m^5U)methyltransferase, is known to work at the precursor level, as well as on mature tRNA which lacks m^5U (3). Many other modification reactions are known to occur, but they usually only operate on a few of the tRNA species in *E. coli*. Thus, m^1G and mam^5s^2U are present only in 12 and 4% of the tRNA chains, respectively (5). At high growth rates, tRNA constitutes about 4% of cell mass, i.e., the biosynthesis of tRNA is a major biosynthetic pathway in the cell (19, 25).

The regulation of different components of the translational apparatus has been under intensive study for several years (19, 23, 25). Thus, it is known that the level of rRNA, ribosomal proteins, tRNA, and elongation factors G and T increases with increasing growth rate (19, 25). The aminoacyl-tRNA ligases were shown to have both an amino acid- and a growth rate-dependent regulation (23). Little is known about the interrelationship between the amount of enzyme involved in the biosynthesis of tRNA and the amount of the final product. Therefore, it is important to elucidate not only the stepwise maturation of the tRNA biosynthesis, but also how the different elements involved in its biosynthesis are regulated. Especially interesting is the behavior of the tRNA biosynthetic enzymes in relation to the behavior of RNA polymerase and different components in the translational apparatus. When mutants such as *trmA*, *trmC*,

and *trmD* were isolated, a specific assay procedure for tRNA biosynthetic enzymes became available, since tRNAs from such mutants were specific substrates for the tRNA(m^5U)-, tRNA(mam^5s^2U)-, and tRNA(m^1G)methyltransferase, respectively (4, 5). This made it possible for us to initiate a systematic study of the regulation of tRNA(m^5U)methyltransferase and two other tRNA methyltransferases under various physiological conditions and in bacterial mutants known to affect the regulation of the translational apparatus.

MATERIALS AND METHODS

Abbreviations. The abbreviations used in this paper are those recommended by the Commission on Biochemical Nomenclature (CBN-1970): m^5U, 5-methyluridine (ribothymidine); m^1G, 1-methylguanosine; mam^5s^2U, 5-methylaminomethyl-2-thio-uridine; tRNA methyltransferases producing m^5U, m^1G and mam^5s^2U are called tRNA(m^5U)-(EC 2.1.1.35), tRNA(m^1G)-(EG 2.1.1.31), and tRNA(mam^5s^2U)-methyltransferase, respectively.

Bacterial strains. *Escherichia coli* strain NC3 was used in most experiments (21). This strain is *E. coli* B/r, substrain A, obtained from F. C. Neidhardt, Ann Arbor, Mich. Different mutants of strain NC3 were used: NC40 (*fusA*) has a temperature-sensitive elongation factor G; strain NC36 (*leuA*) is temperature sensitive for growth as a result of an altered first enzyme in the leucine biosynthetic pathway; and strain NC39 (*cya*) is pleiotrophic negative for growth on arabinose, galactose, and lactose. In earlier experiments a wild-type *E. coli* B/r, substrain F, obtained from N. Fiil, Copenhagen, Denmark, was used. The two *E. coli* B/r substrains A and F used have somewhat different DNA replication patterns (15). Several *E. coli* K-12 strains have also been used. Strain 72c (*fusB*) obtained from L. Isaksson, Uppsala, Sweden, is temperature sensitive for growth due to a failure to make stable RNA at high temperatures (35). Strains AB1932-5-39 (*trmA5*), IB1111 (*trmD1*), and a recombinant of IB1102 and IB1302 (*trmC2*) were used as sources for tRNA preparations (4, 5).

Plasmids and λ phages. ColE1 hybrid plasmid pTN035 carries a 10.1-kilobase chromosomal fragment which contains the *argCBH* and the *trmA* genes, whereas plasmid pTN102, which is a pBR322 derivative carries a 2.9-kilobase *Eco*RI fragment which contains the *trmA* gene. The two λd*arg* phages, λd*arg*13 and λd*arg*50, carry the chromosomal fragments which contain the *ppc*, *argECBH*, *trmA*, *bfe* genes, and *argECBH* and *trmA* genes, respectively. The chromosomal fragment is inserted in two different orientations, and at two different sites in the λ genome, in these two phages (10).

Chemicals and radiochemicals. *S*-[*methyl*-^{14}C]adenosyl-L-methionine (58 mCi/mmol, or 0.5 mCi/mmol) was from Radiochemical Centre, Amersham, Buckinghamshire, England.

Bacterial growth conditions. Bacterial cultures were grown aerobically on a rotary shaker at 37°C. All growth studies were carried out in a salt medium (morpholinepropanesulfonic acid [MOPS]), by the method of Neidhardt et al. (24). The carbon sources used were 0.4% potassium acetate, 0.4% succinic acid (disodium salt), and 0.4% D-glucose. For richer media, the salt medium containing glucose as carbon source (MOPS-glucose) was supplemented with either 1.5% Casamino Acids (MOPS-glucose-CA), 1.0% tryptone (Difco), and 0.5% yeast extract (MOPS-TGYE) or a supplement (defined rich) media containing 20 amino acids, 2 purines, 2 pyrimidines, and 5 vitamins (Rich-MOPS) as described by Neidhardt et al. (22). The MOPS salt medium was made up to 10 times the final concentration and filter sterilized. Water was autoclaved, and the media used were mixed under aseptic conditions. Shifts between different media were performed by collecting cells on a filter, washing, and quickly resuspending them in the new growth media.

Growth determination. The cells were pregrown for several (6 to 10) generations in the same medium, and kept below an optical density at 420 nm of 1.0 (150 μg total dry weight per ml) to assure that the cells were in balanced growth. The growth rate was determined by the rate of increase in cell mass between an optical density of 0.1 and 1.0. At least three measurements were made during each doubling. Growth rate was expressed as the specific growth rate constant, k, where $k = (\ln 2)/\text{mass doubling time in hours}$. Optical density was determined with a Zeiss PMQII or PMQIII spectrophotometer at 420 nm, with a path length of 1 cm.

Preparation of extract. Crude extracts were prepared as described by Ny and Björk (26). The method involves sonic disruption of the cells for 10 × 20 s at power setting 3.0 on a Branson sonifier B12 and centrifugation at 27,000 × *g* for 15 min. After dialysis overnight, the enzyme activity was measured. When enzyme extracts were stored at 4°C, the preparation retained 90% of the tRNA(m^5U)methyltransferase activity for 7 days.

Preparation of tRNA. The tRNA preparations used for assaying the tRNA-methyltransferases were prepared from strain GB1-5-39 [tRNA(m^5U)methyltransferase], from strain IB1111 [tRNA(m^1G)methyltransferase], and from strain IB1302 [tRNA(mam^5s^2U)methyltransferase] by the method of Avital and Elson (2). The cells were grown in a rich medium, harvested in late logarithmic phase, and washed with 0.9% NaCl. The method involves phenol extraction in the presence of chloroform, stripping of the amino acids, and fractionation of rRNA and tRNA with 2 M LiCl. The tRNA was dialyzed for 22 h against 6 liters of 10^{-6} M Mg^{2+}.

Determination of tRNA(m^5U)methyltransferase activity. The activity of this tRNA-methyltransferase was measured as described (26) with tRNA from a *trmA5* mutant, which completely lacks m^5U in its tRNA, as specific substrate (4). One enzymatic unit transfers 1 μmol of methyl group to tRNA per min at 37°C.

Determination of tRNA(m^1G)methyltransferase activity. tRNA from strain IB1111 (*trmD1*), which lacks m^1G, was used as a specific substrate for the tRNA(m^1G)methyltransferase (5). The assays were performed in a reaction mixture of 100 μl containing: 10 μmol of Tris-hydrochloride (pH 8.0), 0.1

μmol of dithiothreitol, 0.01 μmol of EDTA, 0.6 μmol of $MgCl_2$, 2.4 μmol of NH_4Cl, 5.2 nmol of *S*-[*methyl*-^{14}C]adenosyl-L-methionine (specific activity, about 20 mCi/mmol), and 260 μg of tRNA from strain IB1111 and varying amounts of enzyme. The reaction was carried out at 37°C for 15 min and stopped by adding 3 ml of ice-cold 0.01 M $La(NO_3)_3$ in 0.5 M $HClO_4$. One enzymatic unit is defined above.

Determination of tRNA(mam^{5}s^{2}U)methyltransferase activity. tRNA from strain IB1302 (*trmC2*), which lacks mam^{5}s^{2}U, was used as specific substrate (5). The reaction mixture (100 μl) contained: 10 μmol of Tris-hydrochloride (pH 8.5), 0.1 μmol of dithiothreitol, 0.01 μmol of EDTA, 6 μmol of NH_4Cl, 13.4 nmol of *S*-adenosyl-L-methionine (specific activity of about 30 or 500 mCi/mmol of *methyl*-^{14}C or *methyl*-^{3}H label, respectively), and 100 μg of tRNA from strain IB1302 and varying amounts of enzyme. The reaction mixture was incubated at 37°C for 10 min. Mg^{2+} ions were omitted since they are inhibitory for this tRNA methyltransferase. One enzymatic unit is defined as above.

Determination of plasmid copy number. Plasmid copy number was determined by ethidium bromide-cesium chloride gradient centrifugation of [^{3}H]-thymidine-labeled cell lysates (13).

Antibody formation. Fraction VII (300 μg) of purified tRNA(m^{5}U)methyltransferase (Ny and Björk, manuscript in preparation) was injected into an inguinal lymph node of a rabbit, together with complete Freund adjuvant. After 1 month, an additional 300 μg of fraction VII was injected. Six days thereafter, the rabbit was bled. Whole serum was stored frozen. Such serum inhibits the activity of tRNA(m^{5}U)methyltransferase.

Other methods. Protein was determined by the method of Lowry et al. with bovine serum albumin as the standard (18). RNA was measured by the orcinol method with yeast RNA type III as standard (32).

RESULTS

tRNA methyltransferase level at different growth rates. *E. coli* strain NC3 was grown in different media to elicit a wide range of growth rates. Cells were harvested during steady-state growth at an optical density of 1.0, and the specific activity of the tRNA(m^{5}U)-, tRNA(m^{1}G)-, and tRNA(mam^{5}s^{2}U)methyltransferases was determined. Figure 1 shows that the tRNA(m^{5}U)methyltransferase level increased about 3.7-fold when the growth rate was changed approximately 5.5-fold. This was due to the growth rate and not to the media composition, since the level of this enzyme decreased with growth at semipermissive temperatures in strain NC36 [*leuA*(Ts)] (Fig. 5). However, the level of tRNA(m^{1}G)- and tRNA(mam^{5}s^{2}U)methyltransferases did not vary in the same range of growth rate (Fig. 1). In addition we have used another substrain (F) of *E. coli* B/r which shows a somewhat different DNA replication pattern (15). No significant difference was observed when this strain was compared with strain NC3.

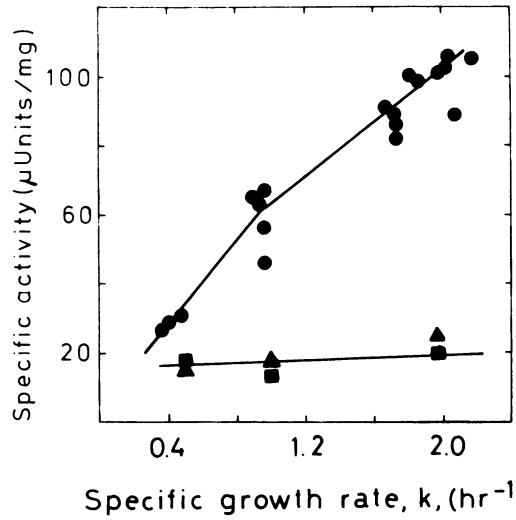

FIG. 1. *Levels of different tRNA methyltransferases in different media during the steady state of growth. Symbols represent the specific activity of tRNA(m^{5}U)-* (●), *tRNA(m^{1}G)-* (■), *and tRNA(mam^{5}s^{2}U)-* (▲) *methyltransferase.*

Activators or inhibitors produced during growth in different media may influence the activity in the different cell extracts. To examine this possibility, we prepared enzyme extracts from slow-growing cells (MOPS-acetate) and fast-growing cells (MOPS-TGYE) and mixed them in different proportions. The activities of the tRNA(m^{5}U)methyltransferase were always strictly additive, and no indication of an activator or inhibitor was observed (data not shown). To directly correlate the specific activity with enzyme molecules, we made an antibody neutralization curve. Figure 2 shows the results with enzyme extract from slow-growing cells (MOPS-acetate) and fast-growing cells (Rich-MOPS). Since the initial slopes are very similar, the enzyme extract must contain the same amount of enzyme molecules per unit of activity. Thus, the results in Fig. 1 reflect changes in the level of tRNA(m^{5}U)methyltransferase molecules. Since this enzyme shows the interesting growth rate-dependent regulation, we have studied it in more detail.

Regulation of the tRNA(m^{5}U)methyltransferase during shifts in growth rate. Figure 3 shows the results of a nutritional shift-up experiment from MOPS-acetate (k = 0.42) to MOPS-Casamino Acids (k = 1.5). The differential rate of the tRNA(m^{5}U)methyltransferase synthesis changed immediately to the differential rate characteristic for the faster growth rate. These kinetics are identical to the kinetics for accumulation of stable RNA during a similar shift (20). Thus, the reg-

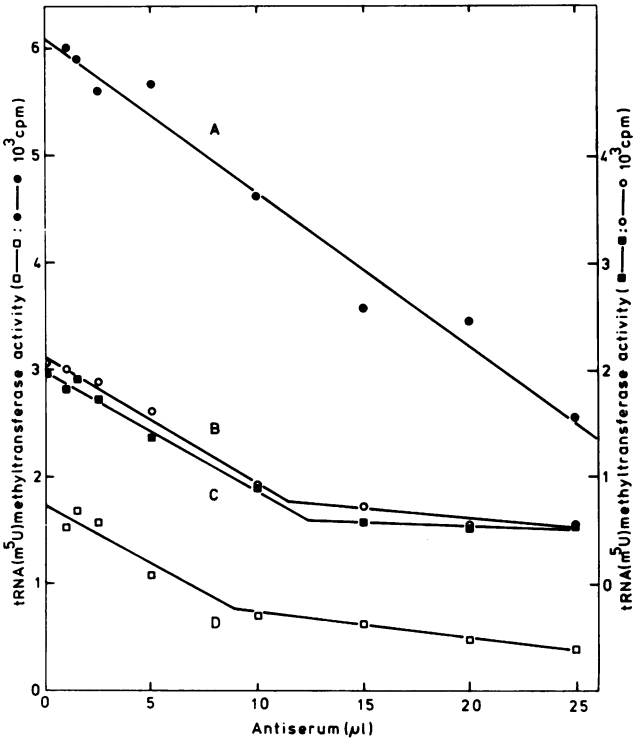

FIG. 2. *Antibody neutralization of tRNA(m⁵U)methyltransferase activity from E. coli NC3 grown at different growth rates. Cultures of strain NC3 were grown in MOPS-acetate (k = 0.42) and in Rich-MOPS (k = 2.06). The specific activity of tRNA(m⁵U)methyltransferase was 3.2 × 10⁻⁵ in the MOPS-acetate and 11.6 × 10⁻⁵ in the Rich-MOPS media. These extracts were mixed with varying amounts of diluted (1:40) antiserum directed against the tRNA(m⁵U)methyltransferase. Various amounts of the diluted antiserum were incubated in a 50-μl volume at 0°C for 1 h in the presence of crude extracts. Thereafter, duplicate samples were assayed for tRNA(m⁵U)methyltransferase activity. The circles are values from the Rich-MOPS culture; squares are from MOPS-acetate culture. The solid symbols in each case represent higher amounts of proteins used than the open symbols. Curves A and D were generated by using equal amounts of protein (42.4 μg), and curves B and C were generated by using equal amounts of activity (1.8 × 10⁻⁶ U).*

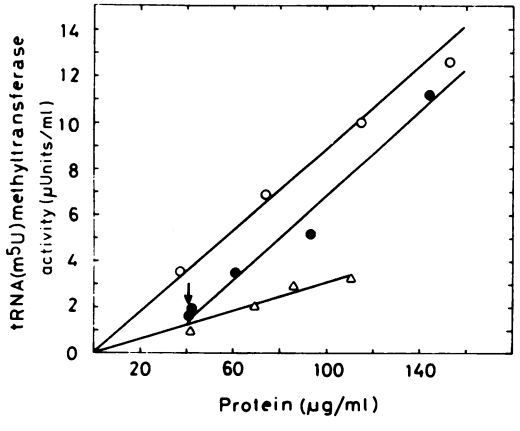

FIG. 3. *Differential rate of accumulation of tRNA(m⁵U)methyltransferase in MOPS-acetate (k = 0.42, △) and MOPS-Casamino Acids (k = 1.5, ○) and after a shift up (●).*

ulatory pattern of tRNA(m⁵U)methyltransferase during a shift up was found to be identical to that of stable RNA and did not follow that of bulk protein.

Several shift-down experiments were performed, and tRNA(m⁵U)methyltransferase activity, stable RNA, protein and optical density were monitored. Figure 4 shows a shift from a fully supplemented medium (MOPS-TGYE, k = 1.98) to a medium with the same carbon source but with no supplementation (MOPS-glucose, k = 1.02). The accumulation of both stable RNA and tRNA(m⁵U)methyltransferase stopped immediately, but the resumption of accumulation of the enzyme preceded significantly that of stable RNA. Two other shift-down experiments have been performed. One shift was from cells growing in MOPS-glucose (k = 1.02) to MOPS-acetate (k = 0.48). Another experiment utilized the fact that addition of α-meth-

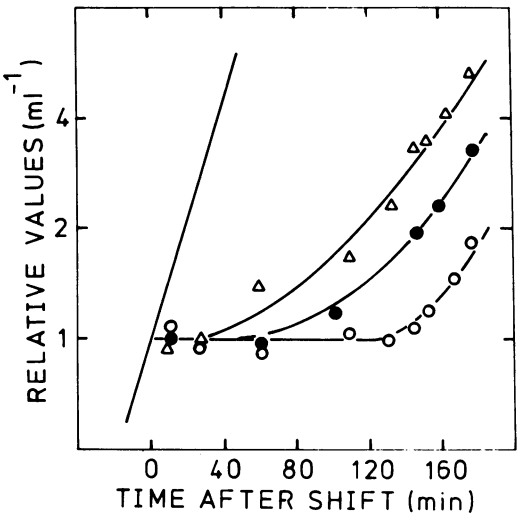

FIG. 4. *Accumulation of protein (△), tRNA-(m⁵U)methyltransferase (●), and stable RNA (○) after a shift down from MOPS-TGYE to MOPS-glucose. All values have been normalized to the value in MOPS-TGYE at the time of the shift. The accumulation in MOPS-TGYE control culture of all macromolecules is indicated by* (——).

ylglucoside to cells growing in glucose elicits a shift down due to the competition by the nonmetabolizable α-methylglucoside for glucose (14). Both experiments showed that the accumulation of tRNA(m⁵U)methyltransferase and stable RNA stopped immediately and resumed at the same time (data not shown).

Gene dose response. We have cloned the *trmA* gene on different plasmids, pTN035 and pTN102. Cells carrying the pTN035 or pTN102 plasmid overproduce the tRNA(m⁵U)methyltransferases 3.5-fold and 20-fold, respectively. In the latter case the plasmid content was determined by density centrifugation in cesium chloride, and it was found that such cells had a plasmid copy number that correlated with the enzyme level. The two λd*arg* transducing phages λd*arg*13 and λd*arg*50 carry the *trmA* gene (10; Ny and Björk, manuscript in preparation). Upon induction of the λd*arg*13 and λd*arg*50, a four- and sixfold increase, respectively, in specific activity was observed compared with induction of λd*arg* derivatives which do not carry the entire *trmA* gene. Thus, when the structural gene is present in extrachromosomal elements, the enzyme is overproduced. In one case, the enzyme was shown to be overproduced in a one-to-one ratio even when high gene copy numbers were present.

Regulation of tRNA(m⁵U)methyltransferase in mutants or under special physiological conditions with known effects on the accumulation of components of the translational apparatus. We have shown earlier that tRNA(m⁵U)methyltransferase is stringently regulated; i.e., in *relA*⁺ or *relA1* strains, under limiting levels of charged valyl-tRNA, the relative rate of synthesis is repressed or derepressed, respectively (26). Mutation in the elongation factor G is known to elicit a relaxed response, i.e., at semipermissive temperatures, a stable RNA is overproduced (1; Fig. 5). When the level of tRNA(m⁵U)methyltransferase was measured under such conditions, the level of the enzyme was raised in the same manner as stable RNA (Fig. 5). Strain 72c harbors a temperature-sensitive lesion in a gene, *fusB*. This mutation has a pleiotrophic effect, but shows a dramatic inhibition of stable RNA accumulation at high temperature, whereas protein synthesis continues (35). We followed the differential rate of tRNA (m⁵U)methyltransferase synthesis, and as a control, glucose 6-phosphate dehydrogenase. The rate of synthesis of the tRNA(m⁵U)-methyltransferase stopped in the same manner as stable RNA, whereas the synthesis of the glucose 6-phosphate dehydrogenase continued at the expected rate (data not shown).

McKeever and Neidhardt (21) have found several physiological conditions where the level of many aminoacyl-tRNA ligases failed to adjust to that expected from the growth rate. These conditions included growth of strain NC39 (*leuA*) at semipermissive temperature, growth of strain NC3 in Rich-MOPS lacking only arginine, and growth of strain NC39 (*cya*) in Rich-MOPS. Figure 5 shows the level of tRNA-(m⁵U)methyltransferase and the RNA/protein ratio under the same conditions. It is clear that in all these experiments the tRNA(m⁵U)meth-

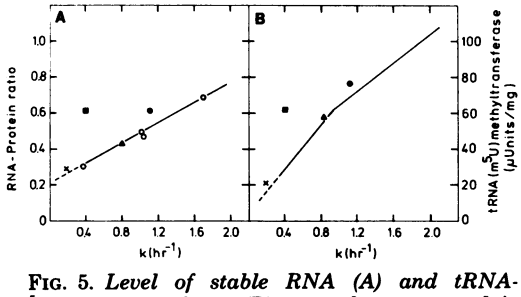

FIG. 5. *Level of stable RNA (A) and tRNA-(m⁵U)methyltransferase (B) at steady-state growth in different mutants or under different physiological conditions. Symbols indicate the levels in NC39 (cya) at 37°C (×), in NC40 (fusA) at the semipermissive temperature 37°C (■), in NC36 (leuA) at semipermissive temperature 37°C (▲), and NC3 in Rich-MOPS lacking only arginine 37°C (●). Symbols (○) and* (——) *denote the steady-state level of RNA and tRNA(m⁵U)methyltransferase in NC3, respectively.*

yltransferase level, unlike the level of many aminoacyl-tRNA ligases, was adjusted in the same manner as that of stable RNA.

DISCUSSION

We have shown that the regulation of a tRNA biosynthetic enzyme, tRNA(m^5U)methyltransferase, is surprisingly similar to that of stable RNA. Since all experiments were performed by activity measurements, some results may be questionable. However, no indication of an activator/inhibitor was detected. Furthermore, the lower activity at slow growth rates cannot be due to poor sonic disruption, since a different sonication treatment did not influence the specific activity (data not shown). A direct measurement of enzyme molecules by antibodies showed that the specific activity is a true measurement of the level of enzyme molecules (Fig. 2). However, the changed level of the tRNA(m^5U)methyltransferase might be due to a changed turnover rate of the enzyme. We found this unlikely, since such mechanism has not been found valid for the regulation of ribosomal proteins and aminoacyl-tRNA ligases (11, 28).

Since cells dividing more than once per hour have multiple growing forks of DNA, genes located near the replication origin will be amplified compared with genes closer to the replication terminus. To investigate whether or not the increasing specific activity of the tRNA(m^5U)-methyltransferase might be due to differences in gene dose, we calculated the *trmA* gene density, i.e., gene copies per genome (7, 9). We found for the *trmA* gene, which is the structural gene for the tRNA(m^5U)methyltransferase, has a gene density of 1.13 at $k = 0.4$ and 1.72 at $k = 2.0$. The ratio between the gene densities at the two growth rates is only 1.52, which is why only a minor part of the 3.7-fold increase in the level of tRNA(m^5U)methyltransferase can be explained by such a mechanism. Furthermore, it is known that the kinetics of accumulation of DNA after shift up or shift down continue for one round of replication at the preshift rate (8, 20). Thus, the kinetics of the accumulation or tRNA(m^5U)-methyltransferase upon an increase in growth rate cannot be explained by gene dose alone. However, upon increasing the number of *trmA* genes by introducing plasmids containing this gene, a gene dose response was observed. This is contrary to the lack of gene dose response by most ribosomal proteins, but similar to the behavior of rRNA and aminoacyl-tRNA ligase operons (6, 13, 16, 27, 30). The gene dose response for the *trmA* gene shows that no specific positive effector can be operating in the regulation of the tRNA(m^5U)methyltransferase. If the plasmid contains the native promoter of the *trmA* gene, our result shows that the cell has the capability to transcribe a surplus of *trmA* genes, indicating that no hypothetical effector is limiting in the cell, but rather that the limiting component may be the capacity of the promoter.

The mechanism which governs the regulation of cell growth has not been elucidated. A passive model has been suggested, while other investigators have put forward a model including a general signal measuring metabolic rate (19, 23). Our result does not distinguish between these models, but the growth rate-dependent regulation might be composed of several regulatory components, such as gene dose, and the distribution of RNA polymerase molecules and specific metabolic effectors. Whatever the mechanism is, the regulation of tRNA(m^5U)methyltransferase shares most of the hypothetical regulatory elements with that of the regulation of stable RNA and proteins involved in the translational machinery. However, there is a major difference between the amount of product made from the rRNA, ribosomal protein genes, and the *trmA* gene. The weight fraction in glucose minimal medium media of, for instance, ribosomal proteins S1, L7/L12, and S6 is 1.51, 0.74, and 0.1%, respectively, (corresponding to 10,220, 25,390, and 2,820 molecules per genome) (29). Our purification of tRNA(m^5U)methyltransferase has revealed that the weight fraction of this enzyme in cells grown in the same media is, at the most, 0.002% (23 molecules per genome) in glucose minimal medium (Ny and Björk, manuscript in preparation). Surveying the growth rate-dependent regulation of several proteins by the O'Farrel technique, Pedersen et al. (29) were able to identify groups of proteins with a similar regulatory behavior. Of the 105 proteins in group 1c which are regulated as ribosomal proteins, most also had a high weight fraction. This is clearly not the case for the tRNA(m^5U)-methyltransferase, and this enzyme might be a unique member of such a group. The other two tRNA methyltransferases investigated, tRNA(m^1G)- and tRNA(mam^5s^2U)methyltransferase, are also found in low amount in the cell (unpublished data). Although the corresponding genes have a similar low expression as the *trmA* gene, these three tRNA biosynthetic enzymes are not coordinately regulated (Fig. 1).

The strong growth rate-dependent regulation of tRNA(m^5U)methyltransferase, but the low expression of the *trmA* gene, makes it an interesting gene to study in more detail. A comparison of the *trmA* promoter with genes with similar regulation, but with vastly different expres-

sion, will shed light on the elements determining that expression, but may also allow some insight into the promoter structure leading to growth rate-dependent regulation.

ACKNOWLEDGMENTS

This work was supported by the Swedish Cancer Society (project no 680).

We are indebted to S. Holm for guidance and practical advise in preparation of antibodies. Critical reading and linguistic improvement of the manuscript by Staffan Normark and John Davis are appreciated. The expert technical support by Arne Olsén is greatfully acknowledged.

LITERATURE CITED

1. **Atherly, A. G.** 1973. Temperature-sensitive relaxed phenotype in a stringent strain of *Escherichia coli*. J. Bacteriol. **113**:178–182.
2. **Avital, S., and D. Elson.** 1969. A convenient procedure for preparing transfer ribonucleic acid from *Escherichia coli*. Biochim. Biophys. Acta **179**:297–307.
3. **Björk, G. R.** 1975. Bacterial RNA methyltransferase mutants: tools in studying the biosynthesis of phage T4 tRNA, p. 53–66. *In* F. Antoni, and A. Faragó (ed.), FEBS Proceedings of the Ninth Meeting, Budapest 1974, vol. 34. North Holland Publishing Co., Amsterdam.
4. **Björk, G. R., and L. A. Isaksson.** 1970. Isolation of mutants of *Escherichia coli* lacking 5-methyluracil in transfer ribonucleic acid or 1-methylguanine in ribosomal RNA. J. Mol. Biol. **51**:83–100.
5. **Björk, G. R., and K. Kjellin-Stråby.** 1978. *Escherichia coli* mutants with defects in the biosynthesis of 5-methylaminomethyl-2-thio-uridine or 1-methylguanosine in their tRNA. J. Bacteriol. **133**:508–517.
6. **Cassio, D., Y. Mathien, and J. P. Waller.** 1975. Enhanced level and metabolic regulation of methionyl-transfer ribonucleic acid synthetase in different strains of *Escherichia coli* K-12. J. Bacteriol. **123**:580–588.
7. **Chandler, M. G., and R. M. Pritchard.** 1975. The effect of gene concentration and relative gene dosage on gene output in *Escherichia coli*. Mol. Gen. Genet. **138**:127–141.
8. **Cooper, S.** 1969. Cell division and DNA replication following a shift to a richer medium. J. Mol. Biol. **43**:1–11.
9. **Cooper, S., and C. E. Helmstetter.** 1968. Chromosome replication and the division cycle of *Escherichia coli* B/r. J. Mol. Biol. **31**:519–540.
10. **Crabeel, M., D. Charlier, N. Glansdorff, S. Palchaudhuri, and W. K. Mass.** 1977. Studies on the biopolar *argECBH* operon of *E. coli*: characterization of restriction endonuclease fragments obtained from *dargECBH* transducing phages and ColE1 *argECBH* plasmid. Mol. Gen. Genet. **151**:161–168.
11. **Dennis, P. P.** 1974. *In vivo* stability, maturation and relative differential synthesis rates of individual ribosomal proteins in *Escherichia* coli B/r. J. Mol. Biol. **88**: 25–41.
12. **Geyl, D., and A. Böck.** 1977. Synthesis of ribosomal proteins in merodiploid strains and in minicells of *Escherichia coli*. Mol. Gen. Genet. **154**:327–334.
13. **Grinsted J., Jr., L. Saunders, C. Ingram, R. B. Sykes, and M. H. Richmond.** 1972. Properties of an R-factor which originated in *Pseudomonas aeruginosa* 1822. J. Bacteriol. **110**:529–537.
14. **Hansen, M. T., M. L. Pato, S. Molin, N. P. Fiil, and K. von Meyenburg.** 1975. Simple downshift and resulting lack of correlation between ppGpp pool size and ribonucleic acid accumulation. J. Bacteriol. **122**:585–591.
15. **Helmstetter, C. E., and O. Pierucci.** 1976. DNA synthesis during the division cycle of three substrains of *Escherichia coli* B/r. J. Mol. Biol. **102**:477–486.
16. **Ikemura, T., and M. Nomura.** 1977. Expression of spacer tRNA genes in ribosomal RNA transcription units carried by hybrid ColE1 plasmids in *E. coli*. Cell **11**:779–793.
17. **Littauer, U. Z., and H. Inouye.** 1973. Regulation of tRNA. Annu. Rev. Biochem. **42**:439–470.
18. **Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall.** 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. **193**:265–275.
19. **Maaløe, O.** 1979. Regulation of the protein-synthesizing machinery—ribosomes, tRNA, factors, and so on. *In* R. F. Goldberger (ed.), Biological regulation and development, vol. 1. Plenum Publishing Corp., New York.
20. **Maaløe, O., and N. O. Kjeldgaard.** 1966. Control of macromolecular synthesis. W. A. Benjamin, Inc. New York.
21. **McKeever, W. G., and F. C. Neidhardt.** 1976. Growth rate modulation of four aminoacyl-transfer ribonucleic acid synthetases in enteric bacteria. J. Bacteriol. **126**: 634–645.
22. **Neidhardt, F. C., P. L. Bloch, S. Pedersen, and S. Reeh.** 1977. Chemical measurement of steady-state levels of ten aminoacyl-transfer ribonucleic acid synthetases in *Escherichia coli*. J. Bacteriol. **129**:378–387.
23. **Neidhardt, F. C., J. Parker, and W. G. McKeever.** 1975. Function and regulation of aminoacyl-tRNA synthetases in prokaryotic and eukaryotic cells. Annu. Rev. Microbiol. **29**:215–250.
24. **Neidhardt, F. C., P. L. Bloch, and D. F. Smith.** 1974. Culture medium for enterobacteria. J. Bacteriol. **119**: 736–747.
25. **Nierlich, D. P.** 1978. Regulation of bacterial growth, RNA, and protein synthesis. Annu. Rev. Microbiol. **32**: 393–432.
26. **Ny, T., and G. R. Björk.** 1977. Stringent regulation of the synthesis of a transfer ribonucleic acid biosynthetic enzyme: transfer ribonucleic acid(m^{5}U)methyltransferase from *Escherichia coli*. J. Bacteriol. **130**:635–641.
27. **Olsson, M. O., and L. A. Isaksson.** 1979. Analysis of *rpsD* mutations in *Escherichia coli*. III. Effects of *rpsD* mutations on expression of some ribosomal protein genes. Mol. Gen. Genet. **169**:271–278.
28. **Parker, J., M. Flashner, W. G. McKeever, and F. C. Neidhardt.** 1974. Metabolic regulation of the arginyl and valyl transfer ribonucleic acid synthetases in bacteria. J. Biol. Chem. **249**:1044–1053.
29. **Pedersen, S., P. L. Bloch, S. Reeh, and F. C. Neidhardt.** 1978. Patterns of protein synthesis in *E. coli*: a catalog of the amount of 140 individual proteins at different growth rates. Cell **14**:179–190.
30. **Piepersberg, A., H. Hennecke, M. Engelhard, G. Nass, and A. Böck.** 1975. Cross-reactivity of phenylalanyl-transfer ribonucleic acid ligases from different microorganisms. J. Bacteriol. **124**:1482–1488.
31. **Rich, A., and U. L. RajBhandary.** 1976. Transfer RNA: molecular structure, sequence, and properties. Annu. Rev. Biochem. **45**:805–860.
32. **Schneider, W. C.** 1945. Phosphorus compounds in animal tissues. I. Extraction and estimation of desoxypentose nucleic acid and pentose nucleic acid. J. Biol. Chem. **161**:293–303.
33. **Shimura, Y., and H. Sakano.** 1977. Processing of tRNA precursors in *Escherichia coli*, p. 293–319. *In* H. J. Vogel (ed.), Nucleic acid-protein recognition. Academic Press, New York.
34. **Smith, J. D.** 1976. Transcription and processing of transfer RNA precursors. Prog. Nucleic Acid Res. Mol. Biol. **16**:25–73.
35. **Takata, R., and L. A. Isaksson.** 1978. The temperature sensitive mutant 72c. II. Accumulation at high temperature of ppGpp and pppGpp in the presence of protein synthesis. Mol. Gen. Genet. **161**:15–21.