Anaerobiosis-Induced Changes in an Isoleucyl Transfer Ribonucleic Acid and the 50S Ribosomes of *Escherichia coli**

Catherine Ning Kwan, David Apirion, and David Schlessinger

ABSTRACT: Extracts from aerobically and anaerobically grown *Escherichia coli* cells were compared with respect to their capacities to support *in vitro* polypeptide synthesis. For the miscoding of isoleucine (directed by polyuridylic acid (poly U)) in the presence of streptomycin, the optimal Mg^{2+} concentration was lower in extracts from the anaerobically grown cells. The optimal Mg^{2+} concentration for poly U directed binding of isoleucyl transfer ribonucleic acid (isoleucyl-tRNA) from anaerobically grown cells to ribosomes was also lower in comparison to that for binding isoleucyl-tRNA from aerobically grown cells. In addition, isoleucyl-tRNA isolated from aerobically and anaerobically grown cells chromatographed differently on a DEAE Sephadex column, indicating the existence of an additional or modified species of isoleucyl-tRNA in anaerobically grown cells. Ribosomes from aerobically and anaerobically grown cells responded differently to the addition of tRNA in polylysine formation directed by polyadenylic acid. The 50S subunit determined this difference.

Mutations that modify activating enzymes (Eidlic and Neidhardt, 1965), tRNA (Capecchi and Gussin, 1965), and ribosomes (Speyer *et al.*, 1962; Flaks *et al.*, 1962) have been detected. The existence of viable genetic changes in the protein synthetic machinery raises the question of whether similar alterations in the protein synthetic machinery can be induced physiologically.

We have, therefore, sought for and found such changes in cells grown anaerobically. Anaerobiosis was chosen for several reasons. First, it has long been known that some bacterial strains have altered growth requirements when grown anaerobically (Wilson and Miles, 1946); secondly, anaerobiosis markedly reduces the antibacterial effect of streptomycin (Bondi *et al.*, 1946; Stern *et al.*, 1966), an antibiotic known to affect the briosomes. Differences in growth response to other antibiotics under anaerobic conditions have also been noted (Schlessinger and Apirion, 1966). These observations suggested that protein synthesis might be somehow modified during anaerobic growth.

Evidence for an inducible change was obtained when extracts of aerobically and anaerobically grown *Escherichia coli* were found to differ in their optimal Mg^{2+} concentration for isoleucine incorporation (miscoding) directed by polyuridylic acid (poly U), in the presence of streptomycin. The component responsible for the shift in the Mg^{2+} optimum was found to be a supernatant factor (Schlessinger and Apirion, 1966). We report here that this altered component in the supernatant is isoleucyl-tRNA. Studies have been also extended to polylysine synthesis directed by polyadenylic acid (poly A), and we have thereby detected a difference between the 50S ribosomes of cells grown aerobically and anaerobically.

Methods

Growth of Bacteria. E. coli AB301 auxotrophic for methionine and carrying a λ prophage or *E. coli* AB301-1 (a derivative of AB301 cured of λ) were grown in trypticase medium (Baltimore Biological Laboratories) to which sodium thioglycolate (0.5 g/l.) was added for anaerobic growth. Cells were grown under vigorous aeration (aerobic cultures) or under 95% N_2 and 5% CO_2 (anaerobic cultures) in a Fermentor CF 130 (New Brunswick Co., New Brunswick, N. J.). Under aerobic condition, both strains were sensitive to 5 μg/ml of streptomycin but under the anaerobic conditions, 50 μg/ml of streptomycin did not inhibit growth.

Preparation of Cell Extracts. Cells were harvested in early log phase. Ribosomes, ribosomal subunits, and S-100[1] were isolated according to Tissières *et al.* (1959) and Nirenberg and Matthaei (1961). Purified ribosomes were prepared by precipitating the ribosomes with ammonium sulfate at 35–60% saturation (Williams, 1967). S-100 was separated into two fractions on Sephadex G-100 as described by Hung *et al.* (1966), with the following modification. Protein (60 mg) in 10 ml of S-100 was passed through a 1.5 × 60 cm column (void volume, 30 ml) at 4° in 0.01 M Tris-HCl (pH 7.8) and 0.01 M Mg^{2+}, at a flow rate of 0.25 ml/min. Two peaks

* From the Department of Microbiology, Washington University School of Medicine, St. Louis, Missouri 63110. *Received June 22, 1967.* Supported in part by research and training grants from the National Institutes of Health (HD 01956, GM 10447, and TI AI-257).

[1] Ribosomes, S-100, and tRNA from aerobically or anaerobically grown cells are referred to as aerobic (or anaerobic) ribosomes, aerobic (or anaerobic) S-100, and aerobic (or anaerobic) tRNA.

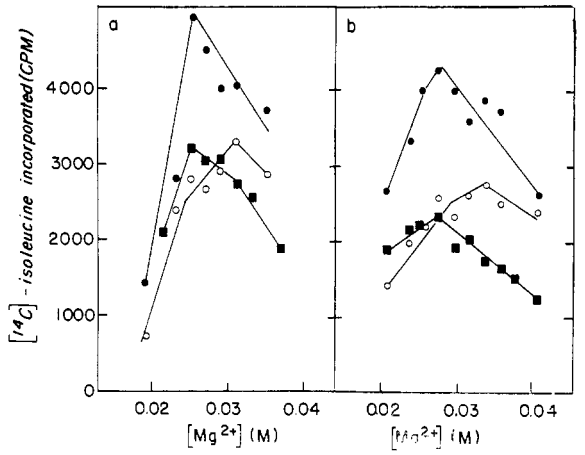

FIGURE 1: Incorporation of isoleucine in polypeptide, directed by poly U, as a function of Mg^{2+} concentration. (a) To each reaction mixture of 50 μl (see Methods), 50 μg of anaerobic ribosomes, 2 μg of streptomycin, and Mg^{2+} as indicated were added; 5 μl of unheated S-100 and 10 μl of heated S-100 were used: unheated aerobic S-100 (○—○); unheated anaerobic S-100 (●—●); unheated aerobic S-100 and heated anaerobic S-100 (■—■). (b) Same as a except that aerobic ribosomes were used: unheated aerobic S-100 (○—○); unheated anerobic S-100 (●—●); unheated anaerobic S-100 and heated aerobic S-100 (■—■).

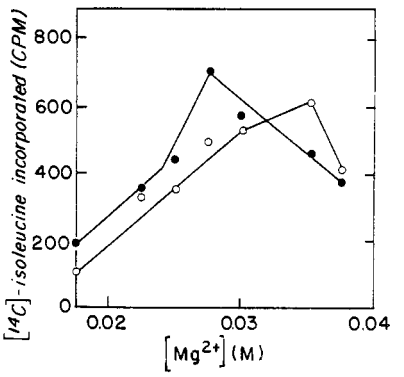

FIGURE 2: Isoleucine miscoding directed by poly U with S-100 depleted of tRNA. S-100 was fractionated (see Methods) and 10 μl of a fraction of the first peak was used with 22.5 μg of aerobic tRNA (○—○) or 18.0 μg of anaerobic tRNA (●—●). A background of incorporation of about 200 cpm in which no tRNA was added had been subtracted from each sample.

were detected by determining the absorbancy of each fraction at 260 and 280 mμ. The first excluded peak had an A_{280}:A_{260} ratio of 0.75–0.86 and did not contain certain t-RNAs, as incorporation of [^{14}C]phenylalanine, -isoleucine, or -lysine using material from this peak required the addition of tRNA.

Incorporation of Amino Acids in Vitro. Conditions for incorporation of amino acids into acid-insoluble polypeptide were those described by Apirion (1966, 1967). Poly A directed reactions were stopped as described by Davies *et al.* (1965).

Preparation of Total tRNA and Labeled Isoleucyl-tRNA. Total tRNA was prepared by the method of Tissières (1959). Labeled isoleucyl-tRNA was prepared by the method of Weisblum *et al.* (1962) with the following minor modifications; 1 ml of reaction mixture contained 100 μmoles of Tris-HCl (pH 7.0), 1 μmole of ATP,[2] 10 μmoles of $MgCl_2$, 10 μmoles of KCl, 0.2 mg of bovine serum albumin, 0.24 mg of S-100, 1.2 mg of tRNA (bulk tRNA), 0.5 μg of L-[^{14}C] isoleucine (2 μc/μg), or 10 μg of L-[^{3}H]isoleucine (3 μc/μg). The charged tRNA was not further purified.

Binding of Isoleucyl-tRNA to Ribosomes. Binding experiments were performed according to the procedures of Nirenberg and Leder (1964); 10 μg of streptomycin was added/50 μl of reaction mixture.

Fractionation of Isoleucyl-tRNA. [^{14}C]- and [^{3}H]isoleucyl-tRNA were fractionated on a DEAE Sephadex column, as described by Cherayil and Bock (1965). The column (1 × 27 cm) was eluted with a linear gradient of 150 ml in which the first reservoir contained 0.5 M NaCl and 0.01 M $MgCl_2$ in 0.02 M potassium acetate buffer (pH 5.1) and the second contained 0.75 M NaCl and 0.02 M $MgCl_2$ in 0.02 M potassium acetate buffer (pH 5.1). Fractions (1 ml) were collected at a flow rate of 1 ml/15 min. For each fraction absorbancy at 260 mμ was determined and 3 ml of ice-cold 5% trichloracetic acid was added. The precipitates were collected on a glass fiber filter (Reeve Angel, 934AH), washed with two 3-ml portions of 5% cold trichloroacetic acid, and then with 2 ml of 0.1 N HCl. Filters were dried, placed in vials containing 10 ml of scintillator solution (4 g of PPO and 0.1 g of POPOP per l. of toluene), and counted in a Packard liquid scintillation spectrophotometer. The efficiency for ^{14}C was 48% with 20% overlap in the ^{3}H channel; ^{3}H efficiency was 14% with an overlap of 0.1% in the ^{14}C channel. The recovery of radioactivity from the column was greater than 90%.

Materials

L-[^{14}C]Phenylalanine (2 μc/μg), L-[^{14}C]isoleucine (2 μc/μg), L-[^{14}C]lysine (2 μc/μg), and L-[^{3}H]isoleucine (3 μc/μg) were obtained from Schwarz BioResearch, Orangeburg, N. Y. Poly U and poly A were from Miles Laboratory, Elkhart, Ind.; *E. coli* B tRNA from General Biochemical, Chagrin Falls, Ohio; and DEAE Sephadex (3.5 ± 0.5 mequiv/g, 100–200 mesh) and Sephadex G-100 from Pharmacia, Uppsala, Sweden.

Results

Modified Isoleucyl-tRNA in Anaerobically Grown Cells. Poly U directed amino acid incorporations were

[2] Abbreviations used: ATP, adenosine triphosphate; PPO, 2,5-diphenyloxazole; POPOP 1,4-bis[2-(5-phenyloxazolyl)]-benzene; PEP, phosphoenolpyruvate.

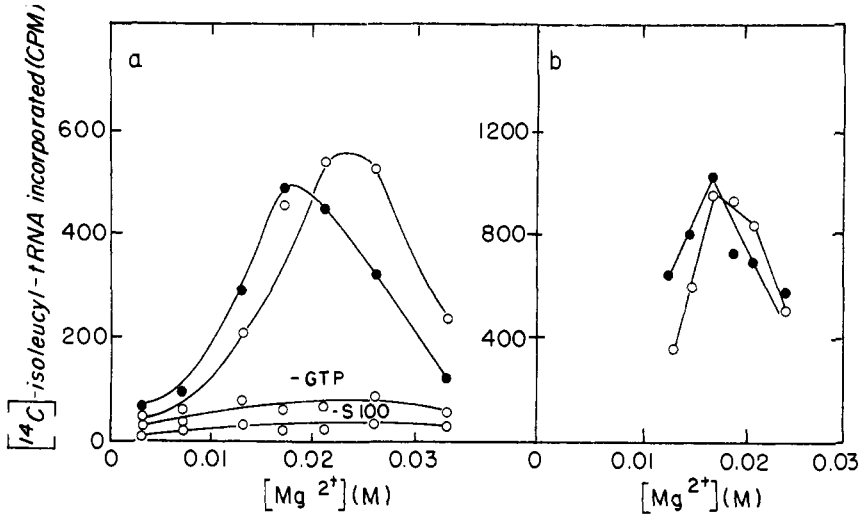

FIGURE 3: Poly U directed miscoding of [^{14}C]isoleucyl-tRNA into polypeptide as a function of Mg^{2+}. (a) Reaction mixture as in Methods, except that ATP, PEP, and pyruvate kinase were left out, aerobic ribosomes (50 μg) and aerobic S-100 were used, and [^{14}C]isoleucine was replaced by aerobic [^{14}C]isoleucyl-tRNA (○——○) (A_{260} 2.30, 1232 cpm) or anaerobic [^{14}C]isoleucyl-tRNA (●——●) (A_{260} 1.20, 1160 cpm). (b) Reaction mixture as in a; anaerobic ribosomes and anaerobic [^{14}C]isoleucyl-tRNA were used with either aerobic S-100 (○——○) or anaerobic S-100 (●——●).

compared in aerobic and anaerobic subcellular fractions. Phenylalanine, leucine, and isoleucine incorporations were studied in the presence or absence of streptomycin. Only the miscoded incorporation of isoleucine in the presence of streptomycin differed in the anaerobic fractions compared to the aerobic. The difference was a shift in the Mg^{2+} optimum concentration required for isoleucine incorporation and was found to be due to the S-100 (Schlessinger and Apirion, 1966).

As also can be seen in Figure 1a,b, the Mg^{2+} optimum for incorporation of isoleucine was about 0.033 M for the aerobic fractions while for the anaerobic fractions it was about 0.027 M, regardless of the source of the ribosomes used. The critical component in the S-100 was heat stable (2 min at 100°) and nondialyzable. As is shown in Figure 1a, when heated anaerobic S-100 was added to nonheated aerobic S-100, the optimal Mg^{2+} concentration was shifted from 0.033 to 0.027 M. However, when heated aerobic S-100 was added to anaerobic S-100 (Figure 1b) the Mg^{2+} optimum remained at about 0.027 M. This showed that the S-100 factor of anaerobic cells was dominant in determining the Mg^{2+} optimum for isoleucine incorporation.

S-100 can be fractionated into two fractions on a Sephadex column (see Methods); the first was relatively depleted of tRNA and depended on addition of tRNA for incorporation activities. When tRNA isolated from either aerobically or anaerobically grown cells was used with the fraction depleted of tRNA, the Mg^{2+} optimum for incorporation was determined by the source of the tRNA. The Mg^{2+} optimum was 0.034 M when aerobic tRNA was used and 0.028 M when anaerobic tRNA was used (Figure 2). In this experiment aerobic ribosomes and S-100 were used; however, similar results were obtained using anaerobic fractions.

To determine whether the factor involved in the shift of the Mg^{2+} optimum is isoleucyl-tRNA, [^{14}C]isoleucyl-tRNA prepared from aerobic S-100 or anaerobic S-100 was used in incorporation experiments. In these reactions less Mg^{2+} was required than in those in which the free amino acid was used. Nevertheless, the relative difference in the optimal Mg^{2+} concentration

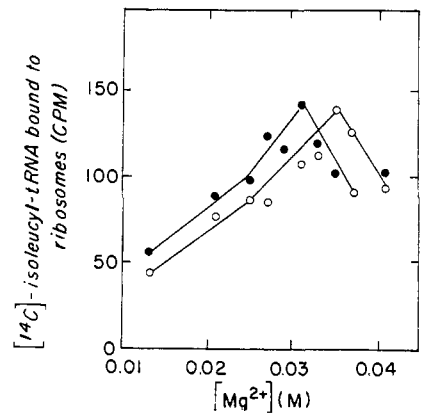

FIGURE 4: Binding of [^{14}C]isoleucyl-tRNA to ribosomes as a function of Mg^{2+} concentration. Reaction mixtures are as described in Methods. Aerobic [^{14}C]isoleucyl-tRNA (OD_{260} 1.10, 1300 cpm) or anaerobic [^{14}C]-isoleucyl-tRNA (OD_{260} 1.00, 1180 cpm) were used for binding to anaerobic ribosomes. Aerobic [^{14}C]isoleucyl-tRNA (○—○); anaerobic [^{14}C]isoleucyl-tRNA (●—●).

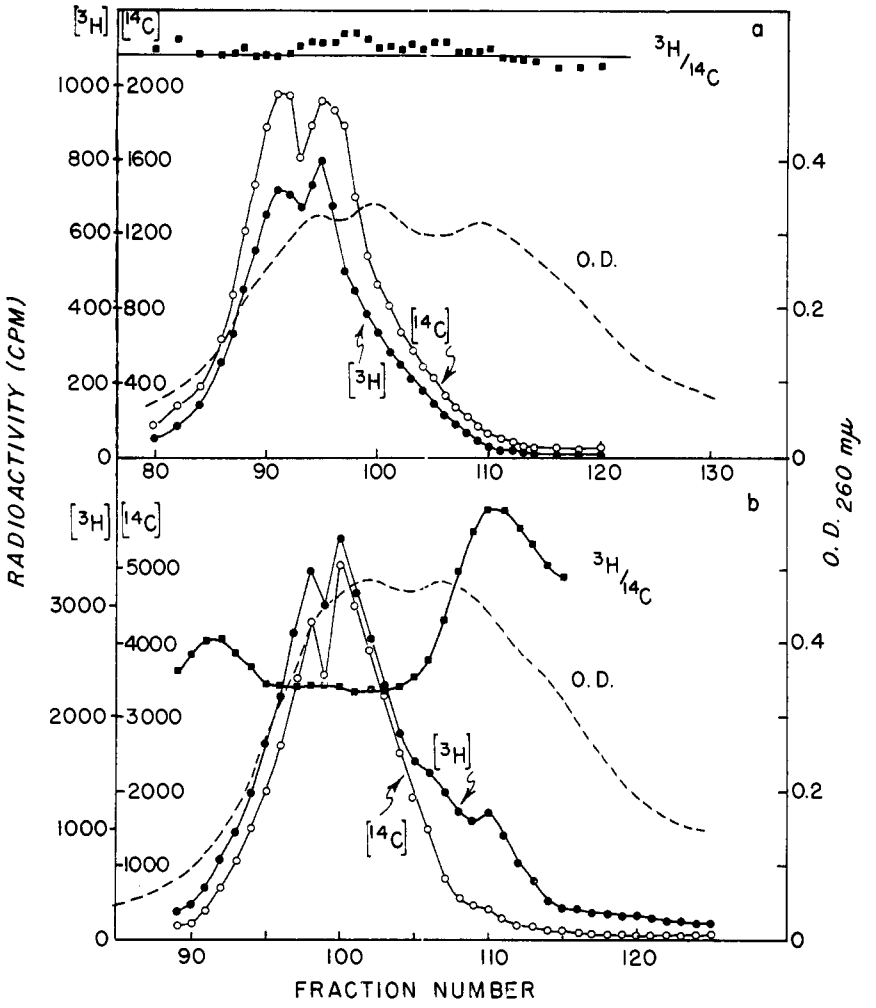

FIGURE 5: Elution pattern of isoleucyl-tRNA on DEAE Sephadex columns. (a) Aerobic [^{14}C]isoleucyl-tRNA (OD_{260} 15.6, 32,000 cpm) and aerobic [^{3}H]isoleucyl-tRNA (OD_{260} 16.0, 12,400 cpm) were mixed and placed on a column. ^{14}C counts per minute (○——○); ^{3}H counts per minute (●——●); ^{3}H:^{14}C (■——■). (b) Aerobic [^{14}C]isoleucyl-tRNA (A_{260} 23.0, 54,900 cpm) and anaerobic [^{3}H]isoleucyl-tRNA (A_{260} 50.0, 33,500 cpm) were mixed and placed on a column. ^{14}C counts per minute (○——○); ^{3}H counts per minute (●——●); ^{3}H:^{14}C (■——■).

remained the same; with aerobic [^{14}C]isoleucyl-tRNA it was 0.024 M, while it shifted to 0.018 M with anaerobic [^{14}C]isoleucyl-tRNA (Figure 3a). These Mg^{2+} optima were dependent only on the source of the isoleucyl-tRNA, while the ribosomes and the S-100 were interchangeable (Figure 3a,b). The difference in the Mg^{2+} requirements could be due either to differences in the binding of tRNA to ribosomes or to the polymerization of isoleucine. Binding experiments were therefore carried out to distinguish between these two alternatives.

Anaerobic [^{14}C]isoleucyl-tRNA bound better to anaerobic ribosomes at lower Mg^{2+} concentration than aerobic [^{14}C]isoleucyl-tRNA, with an optimum of 0.031 M for the former, and 0.037 for the latter (Figure 4); similar results were obtained with aerobic ribosomes. The relatively high Mg^{2+} requirements agree with the findings of Nirenberg and Leder (1964), that binding requires more Mg^{2+} than does incorporation.

The above functional tests implied a structural difference between aerobic and anaerobic isoleucyl-tRNA. Since column fractionation might detect differences in tRNA, we labeled the two types of tRNAs by charging one with [^{3}H]- and the other with [^{14}C]isoleucine and fractionated them on a DEAE Sephadex column (Figure 5). In the control experiment (Figure 5a), aerobic tRNA charged with both [^{3}H]- and [^{14}C]isoleucine was mixed and fractionated on a column. The isoleucyl-tRNA eluted just before the bulk of the tRNA in a broad bimodal pattern. The ratio of ^{3}H to ^{14}C remained constant across the peaks. In contrast, when aerobic tRNA, charged with [^{14}C]isoleucine, was mixed with anaerobic tRNA, charged with [^{3}H]isoleucine, and the mixture was fractionated on an identical column, a third peak of anaerobic tRNA eluted at higher salt concentrations (Figure 5b). This peak accounted for about 5 to 10% of the total [^{3}H]isoleucyl-tRNA. The plot of ^{3}H:^{14}C revealed a relative deficit in the [^{3}H]iso-

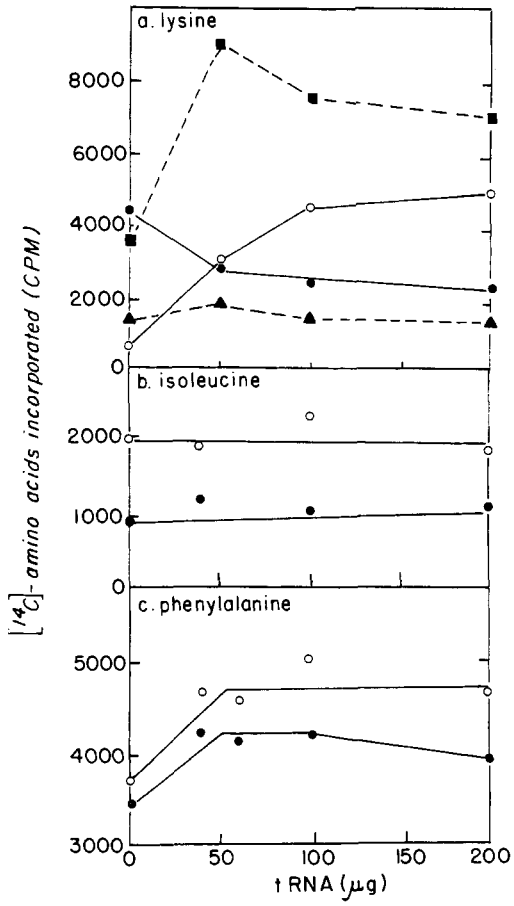

FIGURE 6: The effect of tRNA concentration on amino acid incorporation in aerobic and anaerobic extracts. (a) Lysine incorporation directed by poly A. Ribosomes (50 μg) were used per incubation mixture (see Methods). Reactions were carried to completion. (1) Aerobic ribosomes and aerobic S-100 (○—○); (2) aerobic ribosomes and anaerobic S-100 (■—■); (3) anaerobic ribosomes and anaerobic S-100 (▲—▲); and (4) anaerobic ribosomes and aerobic S-100 (●—●). (b) Isoleucine incorporation directed by poly U. Reaction mixture similar to those in a; 2 μg of streptomycin was added/reaction mixture; anaerobic ribosomes and anaerobic S-100 (●—●); aerobic ribosomes and aerobic S-100 (○—○). (c) Phenylalanine incorporation directed by poly U. Reaction mixture similar to those in a. Anaerobic ribosomes and anaerobic S-100 (●—●); aerobic ribosomes and aerobic S-100 (○—○).

leucyl-tRNA that eluted at low salt concentration and a compensatory excess of it in fractions eluted at high salt concentration, suggesting that a modified rather than a completely new isoleucyl-tRNA is formed during anaerobic growth.

Modification of 50S Ribosomes in Anaerobically Grown Cells. Addition of tRNA to crude extracts stimulates greatly the incorporation of lysine directed by poly A but has little or no effect on poly U directed L-phenylalanine and L-isoleucine incorporation (Davies

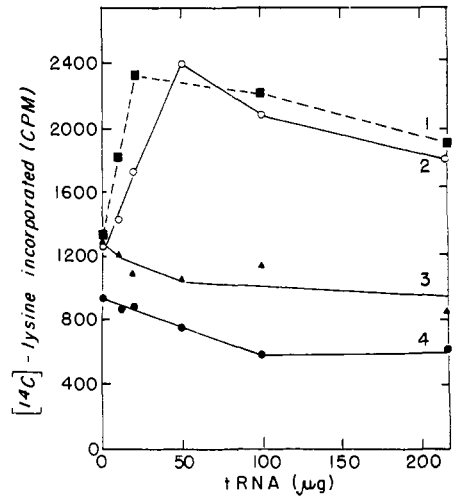

FIGURE 7: The effect of tRNA on poly A directed lysine incorporation with crude and purified ribosomes. Ribosomes (50 μg) were tested for incorporation with aerobic S-100 in each sample (see Methods). (1) Aerobic ribosomes washed with ammonium sulfate (■- - -■); (2) crude aerobic ribosomes (○—○); (3) anaerobic ribosomes washed with ammonium sulfate (▲—▲); and (4) crude anaerobic ribosomes (●—●).

et al., 1965). We confirmed (Figure 6b,c) these results for L-phenylalanine and L-isoleucine using aerobic or anaerobic fractions. The stimulation of lysine incorporation by tRNA was observed when aerobic fractions were used, but no such stimulation was found when anaerobic fractions were used (Figure 6a). The ribosomes and not the S-100 caused this effect (Figure 6a). Commercial aerobic tRNA was used in most experiments, but tRNA extracted from anaerobically grown cells gave the same results. The incorporation varied somewhat with the S-100 used (some S-100 preparations were more active in lysine incorporation than others) probably due to the instability of the lysyl-tRNA synthetase (Stern and Mehler, 1965). More lysine was incorporated with the anaerobic than the aerobic S-100 (Figure 6), though the reverse was true for incorporations of phenylalanine and isoleucine. Although the degree of stimulation or inhibition varied with different preparation of ribosomes, the incorporations with aerobic ribosomes were always stimulated by tRNA while those with anaerobic ribosomes were not stimulated and were even inhibited in some cases.

In order to see whether these results could be accounted for by factors bound to ribosomes, ribosomes were washed with ammonium sulfate (see Methods). These ribosomes retained their initial incorporaton activity but lost contaminating tRNA, fragments of mRNA, and supernatant factors (F. R. Williams, personal communication). Figure 7 shows that the washed ribosomes responded to tRNA in the same way as did crude ribosomes; *i.e.*, lysine incorporation was stimulated by tRNA on aerobic ribosomes and in-

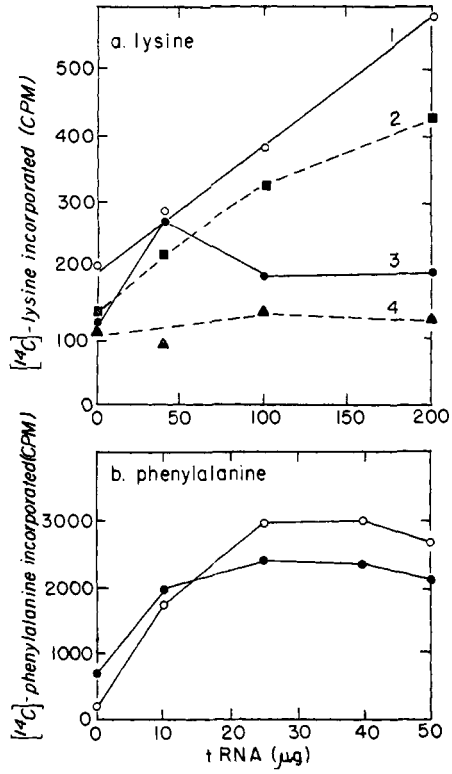

FIGURE 8: Effect of tRNA on amino acid incorporation with washed ribosomes and S-100 depleted of tRNA. Reaction mixtures were as described in Methods; 50 μg of ammonium sulfate washed ribosomes and 10 μl of depleted anaerobic S-100 (OD_{280} 0.053, OD_{260} 0.041) or 20 μl of depleted aerobic S-100 (OD_{280} 0.050, OD_{260} 0.040) were used in each case. (a) Poly A directed lysine incorporation. (1) Anaerobic S-100 and aerobic ribosomes (○—○); (2) aerobic S-100 and aerobic ribosomes (■- - -■); (3) anaerobic S-100 and anaerobic ribosomes (●—●); and (4) aerobic S-100 and anaerobic ribosomes (▲- - -▲). (b) Poly U directed phenylalanine incorporation with depleted anaerobic S-100 and aerobic ribosomes (○—○), or depleted anaerobic S-100 and anaerobic ribosomes (●—●).

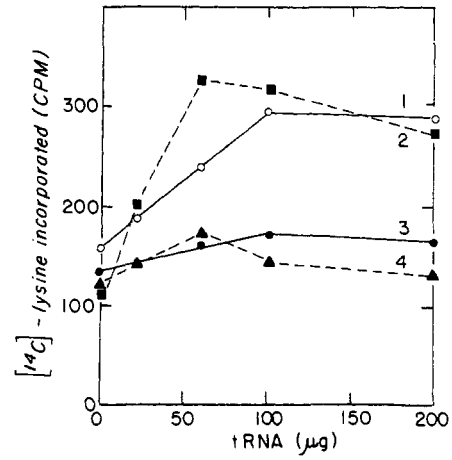

FIGURE 9: Effect of tRNA concentration on poly A directed lysine incorporation with reconstructed mixtures of 30S and 50S ribosomal particles. Reaction mixtures were as described under Methods. Reconstructed ribosomes (9 μg) (5.4 μg of 50 S and 3.6 μg of 30 S) were used with aerobic S-100 in each case. (1) Aerobic 30S and aerobic 50S (○—○); (2) anaerobic 30S and aerobic 50S (■- - -■); (3) anaerobic 30S and anaerobic 50S (●—●); and (4) aerobic 30S and anaerobic 50S (▲- - -▲).

hibited on anaerobic ribosomes (aerobic S-100 was used in all cases).

To eliminate possible interactions of endogenous and added tRNA, the S-100 was depleted of tRNA by gel filtration on Sephadex G-100 (see Methods). The capacity of the depleted S-100 to support polylysine synthesis was variable and generally poor, probably due to instability of lysine tRNA synthetase. Figure 8a shows an experiment where the effect of added tRNA on lysine incorporation was tested with aerobic or anaerobic ribosomes and aerobic or anaerobic S-100 depleted of tRNA. At low levels of tRNA the incorporation of lysine with both kinds of ribosomes was stimulated; as the concentration of tRNA increased, it continued to stimulate the incorporation on aerobic ribosomes, but inhibited that on anaerobic ribosomes. This indicates that tRNA can also stimulate lysine incorporation with anaerobic ribosomes, but these ribosomes become saturated with tRNA at a much lower level than aerobic ribosomes. That the anaerobic ribosomes do not contain more tRNA than aerobic ribosomes is indicated by the same response to added tRNA of crude or purified ribosomes (Figure 7), and by the fact that even though they respond to added tRNA, their maximum response is almost always less than that achieved with aerobic ribosomes. In contrast to lysine incorporation, phenylalanine incorporation directed by poly U in the presence of depleted S-100 was identical when aerobic or anaerobic ribosomes were used (Figure 8b).

Ribosomes isolated from aerobic or anaerobic grown cells were separated into 30S and 50S subunits to determine which of the subunits was responsible for the tRNA effects on lysine incorporation. When the four possible mixtures of 30S and 50S particles were tested, the results revealed that the 50S subunit of anaerobic ribosomes was the component responsible for the inability of tRNA to stimulate polylysine formation (Figure 9). Preliminary experiments comparing the ribosomal proteins from aerobic and anaerobic 50S ribosomes show no difference in the electrophoretic patterns in acrylamide gels.

Discussion

The results presented here show that inducible modifications of isoleucyl-tRNA and 50S ribosomes occur in anaerobically grown cells, though the precise chemical

changes involved are unknown. It is likely that the modification in the anaerobic isoleucyl-tRNA is in the primary structure, since anaerobic S-100 can be heated at 100° for 2 min and maintains its dominant effect in determining the Mg^{2+} optimum for miscoding (see Results). The modification might be, for instance, a methylation or demethylation.

As yet, there is no direct evidence that the changes we report here have any adaptive value. However, we have examined only a few amino acid incorporating systems (poly U–phenylalanine, –leucine, and –isoleucine; poly A–lysine; and poly C–proline (unpublished results)) and have found alterations in a tRNA as well as in the ribosomes. Therefore, the modifications in the protein synthetic machinery are likely to be extensive, and are unlikely to be gratuitous.

An obvious adaptive advantage of anaerobiosis is that it markedly reduces the bactericidal effect of streptomycin. Most likely the resistance is caused by modification in the protein-synthesizing machinery, since streptomycin definitely enters anaerobically growing cells (Schlessinger and Apirion, 1966). However, the relationship between inducible alterations like those described here, increased resistance to streptomycin during anaerobic growth and mutations to streptomycin resistance is unclear, especially since the mutations to streptomycin resistance affect the 30S ribosome, while the modifications described here involve the 50S ribosome and isoleucyl-tRNA. Nevertheless, since we do not know the exact molecular events that lead to streptomycin killing, it is possible that mutations leading to streptomycin resistance interfere with the same event as a modification induced in the protein-synthesizing machinery by anaerobiosis.

Several other changes in tRNA and aminoacyl-tRNA synthetases of cells subjected to extreme environments have recently been reported. In the first such study, a modified leucyl-tRNA was isolated from *E coli* infected with phage (Sueoka and Kano-Sueoka, 1964; Kano-Sueoka and Sueoka, 1966). Subsequently, a valyl-tRNA synthetase modified after phage infection was reported (Niedhardt and Earhart, 1966), and a modified seryl-tRNA was found in sporulating *Bacillus subtilis* (Doi *et al.*, 1966). While the last instance might have adaptive value, the adaptive value is unclear in the first two cases, in which the bacteria die. Nevertheless, all these examples could be manifestations of an extensive repertoire of possible changes in the protein synthetic machinery, induced by changes in the cellular environment.

References

Apirion, D. (1966), *J. Mol. Biol. 16*, 285.

Apirion, D. (1967), *J. Mol. Biol. 30* (in press).

Bondi, A., Dietz, D. D., and Spaulding, E. H. (1946), *Science 103*, 399.

Capecchi, M. R., and Gussin, G. N. (1965), *Science 149*, 417.

Cherayil, J. D., and Bock, R. M. (1965), *Biochemistry 4*, 1174.

Davies, J., Gorini, L., and Davis, B. D. (1965), *Mol. Pharm. 1*, 93.

Doi, R. H., Kanako, I., and Goehler, B. (1966), *Proc. Natl. Acad. Sci. U. S. 56*, 1548.

Eidlic, L., and Neidhardt, F. C. (1965), *J. Bacteriol. 89*, 706.

Flaks, J., Cox, E. C., Whitting, M. L., and White, J. R. (1962), *Biochem. Biophys. Res. Commun. 7*, 390.

Hung, P., Straube, L., and Overby, L. (1966), *Biochem. Biophys. Res. Commun. 24*, 656.

Kano-Sueoka, T., and Sueoka, N. (1966), *J. Mol. Biol. 20*, 183.

Neidhardt, F. C., and Earhart, C. (1966), *Cold Spring Harbor Symp. Quant. Biol. 31*, 557.

Nirenberg, M. W., and Leder, P. (1964), *Science 145*, 1399.

Nirenberg, M. W., and Matthaei, J. H. (1961), *Proc. Natl. Acad. Sci. U. S. 47*, 1588.

Schlessinger, D., and Apirion, D. (1966), *Cold Spring Harbor Symp. Quant. Biol. 31*, 680.

Speyer, J. F., Lengyel, P., and Basilio, C. (1962), *Proc. Natl. Acad. Sci. U. S. 48*, 684.

Stern, J. L., Barner, H. D., and Cohen, S. S. (1966), *J. Mol. Biol. 17*, 188.

Stern, R., and Mehler, A. H. (1965), *Biochem. Z. 342*, 400.

Sueoka, N., and Kano-Sueoka, T. (1964), *Proc. Natl. Acad. Sci. U. S. 52*, 1535.

Tissières, A. (1959), *J. Mol. Biol. 1*, 365.

Tissières, A., Watson, J. D., Schlessinger, D., and Hollingworth, B. R. (1959), *J. Mol. Biol. 1*, 221.

Weisblum, B., Benzer, S., and Holley, R. W. (1962), *Proc. Natl. Acad. Sci. U. S. 48*, 1449.

Williams, F. R. (1967), *Biochim. Biophys. Acta* (in press).

Wilson, G. S., and Miles, A. A. (1946), *in* Principles of Bacteriology and Immunity, Vol. 1, Topley, and Wilson, 3rd ed, Baltimore, Md., Williams & Wilkins, p 70.