Decoding at the Ribosomal A Site

THE EFFECT OF A DEFINED CODON-ANTICODON MISMATCH UPON THE BEHAVIOR OF BOUND AMINOACYL TRANSFER RNA∗

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Ribosomes from Escherichia coli were programmed by being allowed to bind a molecule of tRNA^Phe or fMet-tRNA^Met and the hexanucleotide messenger AUGN1N2N3. The interaction of the ternary complex [EF-Tu·GTP·Phe-tRNA^Phe] with the A site (containing the codon N1N2N3) was then studied by measuring the extent of (i) the binding of Phe-tRNA^Phe to the ribosome, (ii) the hydrolysis of GTP, and (iii) the formation of the dipeptide fMet-Phe. By variation of N1, N2, and N3, a defined degree and position of mismatch could be obtained; the correct A-site codon UUU was compared with the incorrect codons CUU, UCU, GUU, and UUG. Each single-point alteration led to a catalytic hydrolysis of GTP and to a strong reduction in the amounts of Phe-tRNA^Phe binding and of dipeptide formation.

The observations were explicable qualitatively by a hypothesis according to which the behavior of the bound aa-tRNA, after hydrolysis of GTP and before peptidyl transfer, is determined principally by the energy of binding of the aminoacyl-tRNA to the A site. This binding in turn was found to depend upon both the nature and the position of the mismatch. The results further suggest a steric interplay between the 3′ (acceptor) end of the A-site tRNA and the second and third positions of the anticodon, so that a mismatch at one of these positions can impaire directly the interaction between the aminoacylated 3′ end and the ribosome and can thus reduce the rate of peptide bond formation and contribute to the overall fidelity of the elongation cycle.

Present understanding of the process of codon recognition at the A site of the proaryotic ribosome requires distinction between at least three steps: A site/ternary complex recognition (a reversible association equilibrium), GTP hydrolysis (an irreversible reaction), and peptide bond formation (generally presumed to be irreversible). Non-cognate aa-tRNA is rejected from the ribosome either by reversal of the first step or by virtually irreversible dissociation of the aa-tRNA from the ribosome between the events of GTP hydrolysis and peptide bond formation. The rates of both rejection processes depend, among other things, upon the “correctness” of the codon-anticodon matching, and thus both should contribute to the final accuracy of protein biosynthesis (cf. Refs. 1 and 2). This has been amply demonstrated in the case of dissociation after GTP hydrolysis, since the amount of hydrolysis can be measured (3–7); however, the mechanism of this reaction and the importance of the various kinds of mismatch remain unknown.

In earlier work addressed to the question of aa-tRNA binding (8) and in studies (7) preliminary to this work, we created an artificial codon-anticodon mismatch by shortening the messenger. It is true that excising a part of the codon leads to an exactly definable change in the interaction energy, whereas altering the messenger makes the interaction energy less well defined, principally because of the unknown importance of steric repulsion. However, altering the messenger has three advantages. (i) The interaction involves two mismatching triplets, which better reproduces misreading in vivo. (ii) The possible objection that a shortened codon can distort the A site and thus lead to artifacts does not apply. (iii) The degree and the position of mismatch can be controlled. An alternative approach, that of changing the aa-tRNA of the messenger, has been investigated by other authors but is open to uncertainty in interpretation, as we have discussed elsewhere (7).

In this paper we describe for the first time the effect of modifying the interaction of the Phe-tRNA^Phe with the cognate, programmed ribosomal complex [70 S·(fMet-)tRNA^Met·AUGUUU] by changing one of the A-site codon bases. In each case, the Phe-tRNA^Phe is introduced as the ternary complex [Phe-tRNA^Phe·EF-Tu·GTP] and the amounts of RNA binding, of GTP hydrolysis, and of dipeptide formation are documented. We are thus able to detail (i) the effects of gradual changes in the codon-anticodon interaction energy and their consequences for tRNA binding, for GTPase activity, and for the success of peptidyl transfer and (ii) the effects of mismatch directed at different positions of the codon/anticodon base pair triplet.

In addition, the effects of the antibiotics sparsomycin and viomycin upon the codon-anticodon mismatch were observed; these antibiotics were chosen since the former is believed to weaken the A-site interaction without direct involvement in the anticodon region (9) and the latter is believed to strengthen the A-site interaction with aa-tRNA (10).

MATERIALS AND METHODS

RESULTS

aa-tRNA Binding—A frequently used criterion of tRNA-ribosome affinity is the dependence of the degree of binding

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1 Portions of this paper (including "Materials and Methods" and Tables I and II) are presented in miniprint at the end of this paper. The abbreviations used are: DTT, dithiothreitol; Hepes, 4-(2-hydroxyethyl)-1-piperazineethane sulfonic acid; BSA, bovine serum albumin; GDPNP, guanyl-5′-y1 imidotriphosphate. Miniprint is easily
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FIG. 1. Mg++. dependence of enzymic Phe-tRNA binding to 70 S ribosomes in the presence of various messenger oligonucleotides. The binding assay was carried out as described under “Materials and Methods.” Incubation was for 3 min at 37°C.

upon magnesium concentration. Fig. 1 shows that Phe-tRNAPh, with the anticodon 6’GAA", is bound stably to the A site when it is programmed with the cognate codon UUU. While the binding is qualitatively much weaker with the altered A-site codons, significant differences in the behavior of these codons must be noted.

GUU, requiring a sterically unfavorable purine-purine interaction at position 1 of the codon, gives an almost negligible degree of binding, whereas UUG (altered at the “wobble” position 3) and UCU (changed so as to require a sterically neutral A-C pair at position 2 of the codon) show some weak binding at higher magnesium concentrations.

In contrast, CUU, which by these criteria should behave in a manner similar to that of UCU, shows binding which saturates at about 30% of the UUU level and in the same magnesium concentration range. The behavior of CUU is surprising. We do not ascribe it to contamination of the messenger AUGCUU with AUGUUU, since (i) the amount of contaminant required to account for the CUU curve in Fig. 1 (approximately 10%) is ruled out by the oligonucleotide analysis (see “Materials and Methods”) and (ii) this level of contamination would be incompatible with the very low yield of dipeptide from AUGUUU (see (c) below).

The time course of aa-tRNA binding at the standard assay concentration of magnesium (11 mM) is shown in Fig. 2A. For all five modified codons, a plateau is reached within <3 min; for the unmodified codon UUU (not shown) a plateau at 0.7 Phe-tRNA molecule per ribosome is reached in the same time. The fact that the time taken to reach maximal binding is independent of the level of maximal binding suggests strongly that the binding is a steady state phenomenon involving dissociation and recycling of aa-tRNA. In agreement with Fig. 1, the codon GUU shows the lowest binding level while UUG and UCU give a higher level and, again, the level for CUU is anomalously high. The shortened codon UU (7, 8) behaves exactly as UUG and UCU (Figs. 1 and 2A).

FIG. 2. Time course of reactions of the Phe ternary complex with 70 S ribosomes bearing different A-site codons. A, enzymic binding of Phe-tRNAPh; B, EF-Tu-dependent GTP hydrolysis; and C, fMet-Phe dipeptide formation. Assays were carried out, as described under “Materials and Methods,” in the presence of 11 mM magnesium and different oligonucleotide messengers as indicated. In A, the behavior of AUGCUU, AUGUUG, and AUGUU was identical.

GTP Hydrolysis—The turnover of Phe-tRNAPh in the A site with a mismatching codon leads to an increase in GTP consumption, i.e. to catalytic (rather than stoichiometric) GTP hydrolysis (Fig. 2B). In contrast, the correctly matching codon UUU gives an initial consumption of GTP which levels off as the A site becomes fully occupied and thereafter rises only slowly, if at all. This we attribute to relatively stable
binding of aa-tRNA in the correctly programmed A site and labile binding, with dissociation and recycling of aa-tRNA, when the A site carries a codon containing a single mismatching base. The shortened codon UU behaves similarly.

The extent of GTP hydrolysis may be correlated with the extent of binding by the following consideration. While the altered codons UUG, UCU, and UU behave in an identical manner, the codons GUU and CUU both give less GTP hydrolysis. The low hydrolysis induced by GUU is not surprising, since, as seen already, GUU gives the worst codon-anticodon fit and may thus be expected to discriminate the most strongly among incoming ternary complex in the pre- hydrolytic binding step. (In the same way, the severely truncated tetrancleotide messenger AUGU (7) gives only very little GTP hydrolysis; Fig. 2B). The reduced hydrolysis induced by CUU has clearly a different origin: since CUU gives stronger binding of Phe-tRNA^AA than do UUG, UCU, and UU, the codon CUU induces the lowest rate of dissociation of mismatched Phe-tRNA^AA and thus the rate of catalytic hydrolysis of GTP is also lowered.

**Dipeptide Formation**—It is generally accepted that dipeptide formation can be taken as a criterion of complete and conformationally correct binding of tRNA in the A site. When the A site is programmed with the incorrect codons CUU, GUU, UCU, UCG, and UU, then rapid dissociation of the Phe-tRNA^AA from the A site after GTP hydrolysis must lead to only a small fraction of the Phe-tRNA^AA being formylmethionylated. Since the dissociation is followed by recycling, whereas dipeptide formation is irreversible, a linear increase in the amount of dipeptide may be expected, and this increase should continue after the plateau of Phe-tRNA^AA binding has been reached. This behavior is indeed seen in Fig. 2C. For each mismatching codon, the amount of dipeptide formed increases linearly with time, in contrast to the plateau of Phe-tRNAPhe binding (Fig. 2A).

There are two differences between Fig. 2, A and C which deserve comment. (i) As expected, the mismatched codon GUU gives the lowest rate of dipeptide formation. This is consistent with its low induction of aa-tRNA binding (Fig. 2A) and of GTP hydrolysis (Fig. 2B). However, the equivalence of the three codons UUG, UCU, and UU, which behaved identically in Figs. 1 and 2, A and B, is now lifted and UUG is seen to lead to rather more dipeptide formation and UCU to rather less. The yield of dipeptide from UUG is about twice that from CUU; this difference cannot be explained by experimental error. (ii) The behavior of the mismatched codon UCU as shown in Figs. 1 and 2, A and B, is now clarified by postulating that, after GTP hydrolysis, CUU binds Phe-tRNA^AA more strongly than do the other codons. However, this does not suffice to explain Fig. 2C, which shows clearly that the dipeptide yield from CUU is not higher but significantly lower than that from UUG, UCU, and UU. (A trivial explanation for the stronger binding of Phe-tRNA^AA by CUU without peptide formation would be an unexpectedly strong binding of the ternary complex without subsequent GTP hydrolysis. However, this explanation is disposed of by the data of Table I: the AUGCUU-dependent binding of Phe-tRNA^AA is fully suppressed by the replacement of GTP with its nonhydrolyzable analogue quanyl-5′-yl imidodiphosphat.)

Our results therefore lead us to suggest that even after GTP hydrolysis the rate of conversion of A-site-bound Phe- tRNA^AA to the dipeptidy-1-tRNA is still dependent upon the correctness of the codon-anticodon matching.

**Effect of the Antibiotics Sparsomycin and Viomycin**—Sparsomycin prevents correct binding of aa-tRNA to the A site after GTP hydrolysis; its action is directed at the acceptor terminus of the aa-tRNA molecule (9) and results in a complete inhibition of peptide transferase activity and in a lowering of the overall binding energy of the aa-tRNA to the A site, thus stimulating the effect of a single-base omission (7) or mismatch (this work) in the codon. Viomycin has the opposite effect: by increasing the affinity of the aa-tRNA for the A site it prevents translocation (10) and, in a test in vitro, compensates for codon shortening or interruption (7, 8).

As Table II shows, viomycin restores, as expected, the binding of Phe-tRNA^AA to various extents. Thus, none of the mismatch interactions causes a sufficiently severe displacement of the tRNA to abolish the normal reaction of viomycin. The rather greater restoration of the binding by UU, compared with that for CUU, UUG, UCU, and GUU, is presumably due to the comparative steric freedom of the shortened codon vis-à-vis the altered ones; likewise, the lowest stimulation by viomycin is seen for the most poorly matching codons GUU and UCU.

However, sparsomycin does not depress the level of binding found with CUU. This is important for the following reason: CUU obviously affects the bound tRNA in the first instance, at its anticodon. Sparsomycin is known, however, to affect the tRNA at its acceptor terminus. Yet the C-A mismatch in position 3 of the anticodon clearly immunes the A-site Phe- tRNA^AA against any further destabilizing action of the spar- somycin. This can only be explained by the postulate (see "Discussion") that the effect of the mismatch at the anticodon is transmitted through the tRNA structure to the acceptor terminus.

**DISCUSSION**

The experiments presented here show that the effects of any single alteration in the A-site codon are qualitatively the same: the binding energy of the aa-tRNA to the A site is reduced, the aa-tRNA dissociates and is recycled, and thus aa-tRNA binding to the ribosome is reduced while GTP hydrolysis is greatly stimulated. The similar effect of a single omission in the A-site codon provides an experimental justification for our earlier assumption that the only effect of a mismatch (this work) in the codon. Viomycin has the opposite effect: by increasing the affinity of the aa-tRNA for the A site it prevents translocation (10) and, in a test in vitro, compensates for codon shortening or interruption (7, 8).

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Quantitatively, the effects of alterations are not all identical, since not all base pair mismatches are equally favorable or unfavorable. For example, the third codon position is generally accepted to tolerate a mismatch more readily than the first two and, in accordance with this, the altered codon UUG and the shortened codon UU showed the smallest effect of alteration (e.g. Fig. 2C). Changes in the 1st and 2nd codon positions proved more important, especially for the codon GUU, which, with its purine base at position 1, discriminates efficiently against the incoming Phe ternary complex.

Our explanation of the observed degrees of Phe-tRNA^AA binding and GTP hydrolysis may be stated formally in the following way. The affinity of Phe-tRNA^AA for the A site, after GTP hydrolysis, increases for different codons in the order U < GUU < UU ≈ UCU ≈ UUG < CUU < UUU. At the left-hand end, the interaction in the initial recognition step is too weak to induce GTP hydrolysis. At the right-hand end the Phe-tRNA^AA is stably bound after GTP hydrolysis and stoichiometric hydrolysis of GTP is observed. Between these two extremes some GTP hydrolysis occurs (depending on the quality of matching in the initial recognition step) and the bound Phe-tRNA^AA then dissociates more or less rapidly (depending on the quality of matching after GTP hydrolysis) so that the rate of catalytic GTP hydrolysis must pass through...
a maximum. Experimentally, the maximum corresponds to the binding strength of UU, UUG, and UCU, so that the rates of GTP hydrolysis in the steady state (i.e., after 3 min) fall in the order U ≈ UUU < GUU ≈ CUU < UU ≈ UCU ≈ UUG.

As mentioned above (cf. Figs. 1 and 2A), binding of Phe-tRNA<sup>Phe</sup> to ribosomes programmed with the A-site codon CUU was somewhat stronger than expected. This effect is not large, since the behavior of CUU resembles much more closely that of UCU, UU etc. than that of UUU. We attribute this effect to the non-ideality of the six-base-pair-long region of double helix comprising the A- and P-site messenger and anticodons, allowing some distortion and resulting in an unusually stable A-C base pair. As pointed out by Ninio and Claverie (22), the total energy of the codon-anticodon interaction is not just the sum of the hydrogen bond energies.

However, in spite of the slight stabilization of A-site-bound Phe-tRNA<sup>Phe</sup> by CUU, the yield of dipeptide for CUU was low. Both this observation and the observed differences in the group UU/UCU/UUG imply that the rate of dipeptide formation, given that aa-tRNA is bound in the A site, is not independent of the correctness of codon-anticodon matching. This would mean that the codon-anticodon interaction is important for at least three distinguishable stages in the elongation cycle, not only for the preliminary recognition step before GTP hydrolysis and for the rate of dissociation of the charged aa-tRNA from the A site after GTP hydrolysis, but also for the rate of peptide bond formation. If correct, this is of interest because, while the involvement of the codon-anticodon interaction in the first two steps can be formalized as a contribution to the overall binding energy that varies with the correctness of the matching, the effect of the codon-anticodon interaction in the third step can only be visualized as the result of a direct structural interplay between the anticodon and acceptor regions of the tRNA molecule in the A site: steric mismatch at the anticodon causes misalignment at the peptidyltransferase center and thus lowers the rate of the peptidyl transfer reaction. This long range effect would also explain the observed pattern of inhibition of Phe-tRNA<sup>Phe</sup> binding by sparsomycin (cf. Table II and “Results”).

At present, a general consensus appears to be emerging that some kind of multiple check mechanism operates in protein biosynthesis at the level of aa-tRNA interaction with the A site, and that this can provisionally be interpreted in terms of a “proofreading” model (1). Thompson and his colleagues, using various tRNA species (3, 4), were the first to show a relation between the GTP turnover and the strength of the codon-anticodon interaction; later, by replacing GTP with its more slowly hydrolyzable analogue guanosine-5′-[(γ-thio)triphosphate, they were able to demonstrate (23) that the performance of a ribosome, both in accuracy and in speed, is the result of a delicate balance between different rate constants, as first suggested by Ninio (2). The Uppsala group has emphasized the essential part played by kinetic factors in protein biosynthesis under normal (6) and starvation (24) conditions.

While we have stressed the role of binding energy, this does not imply a contradiction in interpretation; it follows from our different experimental approach, which has concentrated upon the measurement of stoichiometric ratios and their dependence upon the identity of the A-site codon for a given tRNA, rather than upon the determination of rate constants. It is clear that a complete molecular description of the protein elongation cycle, as of any reaction, will ultimately have to be formulated in terms which allow for the basic interconvertibility of rate constants on the one hand and energy levels, including those of intermediate and transition states, on the other.

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REFERENCES

1. Hopfield, J. J. (1974) Proc. Natl. Acad. Sci. U. S. A. 71, 4135-4139
2. Ninio, J. (1974) J. Mol. Biol. 84, 297-313
3. Thompson, R. C., Dix, D. B., Gerson, R. B., and Karim, A. M. (1981) J. Biol. Chem. 256, 81-86
4. Thompson, R. C., and Stone, P. (1977) Proc. Natl. Acad. Sci. U. S. A. 74, 198-202
5. Donner, D., Vilems, R., and Kurland, C. G. (1978) Proc. Natl. Acad. Sci. U. S. A. 75, 3192-3195
6. Ruusila, T., Ehrenberg, M., and Kurland, C. G. (1982) EMBO J. 1, 741-745
7. Horning, H., Woolley, P., and Lührmann, R. (1983) FEBS Lett. 156, 311-315
8. Lührmann, R. (1980) Nucleic Acids Res. 8, 5813-5824
9. Cundliffe, E. (1980) in Ribosomes (Chamblas, G., Craven, G. R., Davies, J., Davis, K., Kahan, L., and Nomura, M., eds.) pp. 555-581, University Park Press, Baltimore
10. Cañas, M. J., and Model, J. (1980) Biochemistry 19, 5411-5416
11. Noll, M., Hapke, B., Schreier, M. H., and Noll, M. (1973) J. Mol. Biol. 75, 281-294
12. Traub, P., Mizuhashi, S., Lowry, C. V., and Nomura, M. (1971) Methods Enzymol. 20, 391-407
13. Hershey, J. W. B., and Thach, R. E. (1967) Proc Natl Acad. Sci. U. S. A. 57, 759-766
14. Arat, K.-I., Kawakita, M., and Kaziro, Y. (1972) J. Biol. Chem. 247, 7029-7037
15. Mohr, S. C., and Thach, R. E. (1969) J. Biol. Chem. 244, 6566-6576
16. Schetters, H., Gassen, H. G., and Matthaei, H. (1972) Biochim. Biophys. Acta 272, 549-559
17. Endland, T. E., and Uhlenbeck, G. C. (1978) Biochemistry 17, 2069-2076
18. Jay, E., Bambara, R., Padmanabhia, R., and Wu, R. (1974) Nucleic Acids Res. 1, 331-353
19. Volekaert, G., and Fiers, W. (1977) Anal. Biochem. 83, 228-239
20. Nirenberg, M., and Leder, P. (1964) Science 145, 1399-1409
21. Parmeggiani, A., and Sander, G. (1981) Mol. Cell. Biochem. 35, 129-158
22. Ninio, J., and Claverie, P. (1971) J. Mol. Biol. 65, 63-82
23. Thompson, R. C., and Karim, A. M. (1982) Proc. Natl Acad. Sci. U. S. A. 79, 4922-4926
24. Kurland, C. G. (1983) Biochim. Biophys. Acta 74, 1-9
SUPPLEMENTARY MATERIAL TO: DECODING AT THE RIBOSOMAL A SITE

MATERIALS AND METHODS

Materials

E. coli tRNA was purchased from Doehringer, Mannheim, Germany. N. reio nuclear phosphate (EC 3.1.3.1) was from Doehringer, Mannheim, V. Bcr, and Gs were from Sigma, München. Micrococcus luteus polynucleotide phosphorylase (EC 2.7.7.81) and alkaline phosphatase (EC 3.1.3.1) were from Doehringer, Mannheim. T. RHase (EC 3.1.4.3) and V-TRNA ligase (EC 8.6.1.1) were bought from P.L. Biochem. Inc., St. Goar, and Polynucleo-

tide kinase (2.7.7.61) was obtained from Boehringer, Mannheim. Ti and alkaline phosphatase (EC 3.1.3.11) were from Boehringer, Mannheim. 

SUPPLEMENTARY MATERIAL TO: DECODING AT THE RIBOSOMAL A SITE and alkaline phosphatase (EC 3.1.3.11) were from Boehringer, Mannheim. 

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thyrolsynze (sp. act. 18 Ci/mmol) and LY-PITP (sp. act. 35 Ci/

Table 1: Deposition of AMUCo-mediated Phe-tRNA binding by replacement of GTP with the non-hydrolysable analogue GDPNP

Phe-tRNA binding was performed in the presence of EF-Tu/GTP or EF-Ts/GDPNP as described under "Materials and Methods". The reaction mixture contained 11 mM magnesium acetate, 1 nmol of the indicated oligonucleotide, 10 μM GTP or 1 mM GDPNP, 100 pmol EF-Tu and desalted initiator tRNA. Incubation was for 3 min at 37°C.

| Oligonucleotide | Phe-tRNA bound (pmol) |
|-----------------|-----------------------|
| EF-Tu/GTP       | EF-Ts/GDPNP           |
| AUGCUU          | 17.0                  | 4.7                  |
| AUGCCU          | 5.6                   | 1.9                  |
| AUGCCG          | 1.9                   | 1.5                  |

Table 2: Effect of the antibiotic I mercapto and vincomycin on the kinetic of Phe-tRNA to 70S ribosomes programmed with various messenger oligonucleotides

Enzymic Phe-tRNA binding to 70S ribosomes was performed as described under "Materials and Methods". The reaction mixture contained 11 mM magnesium acetate, 1 nmol of the indicated oligonucleotide, 10 μM GTP, desalted tRNA and 50 μM apamin or vincomycin. Incubation was for 3 min at 37°C.

| Oligonucleotide | Phe-tRNA bound (pmol) |
|-----------------|-----------------------|
| Sparsomycin     | 5.4                   | 17.0                  |
| Vincomycin      | 6.0                   | 14.7                  |
| AUGCUU          | 1.9                   | 1.9                  |
| AUGCCU          | 1.5                   | 15.6                  |
| AUGCCG          | 1.7                   | 16.1                  |
| AUGCCG          | 1.0                   | 11.6                  |
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