Effect of Initiation Factor 3 Binding on the 30S Ribosomal Subunits of *Escherichia coli**

(ribosomes/conformation/protein synthesis/initiation/aminoacyl-tRNA binding)

CYNTHIA L. PON AND CLAUDIO GUALERZI

Max-Planck-Institut für Molekulare Genetik, Abt. Wittmann, Berlin-Dahlem, Germany

Communicated by Luigi Gorini, September 23, 1974

ABSTRACT Under certain conditions, initiation factor 3 (IF-3) can cause the release of aminoacyl-tRNA bound to 30S ribosomal subunits of *E. coli*. It is shown that this IF-3-induced aminoacyl-tRNA release cannot be attributed to either nucleolytic attack or competition between IF-3 and aminoacyl-tRNA for the same ribosomal binding site. It was found that the 30S-aminoacyl-tRNA-codon complexes formed in the absence of IF-3 are intrinsically different from those prepared in the presence of IF-3. In the absence of IF-3, the ribosomal binding of aminoacyl-tRNA is a virtually irreversible process, since the bound aminoacyl-tRNA can neither be spontaneously released upon dilution nor exchanged for unbound aminoacyl-tRNA. In the presence of IF-3, the binding of one molecule of IF-3 per 30S ribosome renders the binding of aminoacyl-tRNA reversible upon dilution and promotes exchange between bound and unbound aminoacyl-tRNA. It is suggested that this difference is due to a conformational transition of the 30S ribosomal subunit induced by the binding of IF-3. The possible implications of this finding in relation to the mechanism of action of IF-3 and its functional role in the cell are discussed.

Initiation factor IF-3 binds to a specific site of the 30S ribosomal subunit (1-4) and is necessary for the initiation of protein synthesis (5-9). Nothing is known, however, about the molecular basis of its action.

Since one of the characteristics of IF-3 is the ability to induce the release of aminoacyl-tRNA (with the exception of the initiator fMet-tRNA) from their codon-specific complexes with the 30S ribosomal subunit (10), the properties of this reaction have been investigated to understand the molecular basis for this activity. Our findings indicate that the binding of IF-3 to the ribosome triggers a conformational transition of the 30S particle, which is responsible for the dissociation of the aminoacyl-tRNA from the 30S subunit and can probably account for other IF-3 functions as well.

MATERIALS AND METHODS

Buffers: A—10 mM Tris·HCl, pH 7.2, 15 mM magnesium acetate, 100 mM NH₄Cl, 20 mM KCl. B—70 mM Tris·HCl, pH 7.7, 16 mM magnesium acetate, 35 mM KCl, 2 mM 2-mercapto ethanol. C—50 mM Tris·HCl, pH 7.2, 13 mM magnesium acetate, 25 mM KCl.

The preparation of 30S ribosomal subunits (11), radioactively labeled Phe-tRNA (12), and *N*-acetylPhe-tRNA (13) were performed as previously described.

IF-3 was purified (with the omission of the heating step) as reported earlier (10). This procedure involves an assay (for

details, see legend of Fig. 1) based on the breakdown of the 30S-poly(U)-Phe-tRNA complex which is "atypical" in comparison with other published methods (7, 14, 15). It results in a protein, however, showing homogeneity in both one- and two-dimensional electrophoresis, having a molecular weight of 22,500 as determined by sedimentation and diffusion (Paradies, Gualerzi, and Pon, unpublished) and displaying biological properties identical to those published for IF-3 (refs. 7-9 and 14-17).

RESULTS

To determine the amount of IF-3 required for the breakdown of the 30S-poly(U)-*N*-acetyl[¹⁴C]Phe-tRNA complex, fixed amounts of this complex were diluted into tubes containing

FIG. 1. Stoichiometry of IF-3 induced release of *N*-acetyl-¹⁴C-Phe-tRNA from 30S ribosomal subunits. One pmol of the 30S ribosomal subunits used throughout this study bound no less than 0.4-0.5 pmol of aa-tRNA when incubated for 30 min at 22° in the presence of saturating amounts of aa-tRNA. However, for the assay of releasing activity, the complex was routinely prepared using a limiting amount of aa-tRNA to obtain conditions in which a large proportion (about 70%) of the added tRNA became ribosome-bound. In a typical binding reaction, 13.3 A₂₆₀ units (890 pmol) of 30S ribosomal subunits, 200 μg of poly(U), 500 μg of *E. coli* unfractionated tRNA containing 125,000 cpm (180 pmol) *N*-acetyl[¹⁴C]Phe-tRNA were incubated for 25 min at 22° in 1 ml of buffer A. After incubation, aliquots of the complex containing 75 pmol of 30S ribosomal subunits which had bound 11.7 pmol of *N*-acetyl[¹⁴C]Phe-tRNA were diluted in 5 ml of buffer B containing the indicated amounts of purified IF-3 (1 μg = 44 pmol). After mixing, the tubes were incubated for 5 min at 22° and the contents were filtered through Millipore filters (pore size, 0.45 μm). After washing of the tubes and filters with 5 ml of buffer C, the filters were dried and the amount of *N*-acetyl-¹⁴C-Phe-tRNA remaining bound to ribosomes was determined.

* This is paper no. 97 on "Ribosomal Proteins." The preceding paper is ref. 23.

TABLE 1. *Rebinding to ribosomes of N-acetyl[¹⁴C]Phe-tRNA released by IF-3*

Step	N-acetyl[¹⁴ C]Phe-tRNA	cpm
1	Originally bound to 30 S	28,755
2	Remaining on filters	4,804
3	Recovered in EtOH precipitate	29,966*
4	Incubated with poly(U) and 30 S	22,155 (100%)
5	Rebound to 30 S	20,045 (90.4%)

An aliquot of complex prepared as described in Fig. 2 containing 6 A_{260} units of 30 S, 150 μ g of poly(U) and 150 μ g of unfractionated tRNA containing 35,000 cpm (50 pmol) N-acetyl[¹⁴C]Phe-tRNA (of which approximately 80% was ribosome bound—step 1) was diluted with 25 ml of buffer B containing 8 μ g of IF-3. After 5 min at 37°, the incubation mixture was divided into five equal portions which were filtered through Millipore filters. The amount of N-acetyl[¹⁴C]Phe-tRNA remaining bound to ribosomes was determined (step 2); 2 M potassium acetate, pH 5.2, (0.1 volume) and ethanol (2 volumes) were added to the combined filtrates containing the released N-acetyl[¹⁴C]Phe-tRNA. After 2 hr at -30°, the RNA was collected by centrifugation and dissolved in 0.5 ml of H₂O (step 3). An aliquot of 0.4 ml of this N-acetyl[¹⁴C]Phe-tRNA was incubated in 1.0 ml of Buffer A containing 25 A_{260} units of 30 S and 200 μ g of poly(U) (step 4). After 45-min incubation at 37°, the bound N-acetyl[¹⁴C]Phe-tRNA was determined by Millipore filtration (step 5).

* The aa-tRNA recovered in the eluate is about 25% higher than the N-acetyl[¹⁴C]Phe-tRNA released by IF-3. This is due to the presence of a fraction of unbound N-acetyl[¹⁴C]Phe-tRNA present in the incubation mixture as explained in the legend to Fig. 1.

buffer B and the amounts of IF-3 as indicated in Fig. 1. The amount of complex remaining in each tube after 5 min of incubation at 22° was determined by Millipore filtration. A plateau of N-acetylPhe-tRNA release was reached when a stoichiometric amount of 1 pmol of IF-3 per pmol of 30 S present in each assay (i.e., the sum of the 30 S participating in complex formation and the free 30 S) was attained.

In the experiment shown in Table 1, the biological activity of the aa-tRNA released from the complex was tested. As seen in the table, when the N-acetyl[¹⁴C]Phe-tRNA released from

TABLE 2. *Simultaneous ribosomal binding of [methyl-¹⁴C]IF-3 and [³H]Phe-tRNA*

Additions				[methyl- ¹⁴ C]IF-3		[³ H]Phe-tRNA	
30 S	poly-(U)	IF-3	Phe-tRNA	cpm	(pmol)	cpm	(pmol)
+	+	+	-	12,972	(90.6)	—	—
+	+	+	+	12,998	(90.6)	6,464	(10.7)
+	+	-	+	—	—	6,062	(10.1)

The reaction mixtures contained in 0.5 ml of buffer A: 1.5 A_{260} units of 30S ribosomal subunits, 100 μ g of poly(U), and where specified, 20 μ g of [methyl-¹⁴C]IF-3 (2). After 15 min incubation at 37°, 400 μ g of unfractionated tRNA containing 150 pmol of [³H]Phe-tRNA were added as indicated in the table. After an additional incubation at 22°, the contents of each tube were loaded onto 10–30% sucrose density gradients (3). After 2 hr of centrifugation at 42,000 rpm at 2° in a Spinco SW 50.1 rotor, the gradients were fractionated and the ³H and ¹⁴C radioactivities in each fraction were determined.

FIG. 2. Effect of IF-3 on the ribosomal binding of [¹⁴C]Phe-tRNA. Each incubation mixture contained in 0.8 ml of buffer A: 6.5 A_{260} units of 30S ribosomal subunits, either 40 or 200 μ g of poly(U), and where specified 10 μ g of IF-3. After 5 min of incubation at 37°, 1 mg of unfractionated tRNA containing 400 pmol of [¹⁴C]Phe-tRNA was added to each tube and the incubation was continued. At the indicated times, 100 μ l were withdrawn from each tube and filtered directly through Millipore filters which were washed with 6 ml of buffer C and dried, and the radioactivity was determined. (Δ) 40 μ g of poly(U), no IF-3; (▲) 40 μ g of poly(U), IF-3 present; (○) 200 μ g of poly(U), no IF-3; (●) 200 μ g of poly(U), IF-3 present.

ribosomes by IF-3 was collected, concentrated by ethanol precipitation and then incubated in the presence of poly(U) and a molar excess of 30S particles, full activity (90%) of the released N-acetyl[¹⁴C]Phe-tRNA in "rebinding" to ribosomes was found. This finding, along with the fact that purified IF-3 did not show any appreciable nucleolytic activity (not shown) strongly argues against the possibility of attributing the breakdown of the 30S-N-acetylPhe-tRNA-codon complex to a degradative process (i.e., a nucleolytic attack).

In spite of the fact that the IF-3 and the aa-tRNA binding sites on the ribosome are likely to be different (3), the possibility that the IF-3 induced breakdown of the 30 S-aa-tRNA complex could be due to a competition between IF-3 and aa-tRNA for the same ribosomal binding site was examined. If IF-3 competes with the aa-tRNA for the same ribosomal binding site, one would expect IF-3 to inhibit the binding of aa-tRNA to 30S particles and to be absent from 30S ribosomes carrying an aa-tRNA. Neither of these conditions, however, was experimentally found (see Fig. 2 and Table 2). That IF-3

FIG. 3. Effect of complex dilution on the IF-3-dependent release of *N*-acetyl [^{14}C]Phe-tRNA. The 30S-poly(U)-*N*-acetyl- ^{14}C]Phe-tRNA complex was prepared as described in Fig. 1. Aliquots containing approximately 1 A_{260} unit of 30S ribosomes binding 12.5 pmol of *N*-acetyl ^{14}C]Phe-tRNA were diluted with buffer B (O) or buffer B containing 2 μg of IF-3 (●) as indicated in the figure.

does not inhibit the ribosomal binding of [^{14}C]Phe-tRNA is seen in Fig. 2, where the time course of poly(U)-directed binding of [^{14}C]Phe-tRNA to 30S ribosomes was studied at two different concentrations of poly(U) in the presence and in the absence of IF-3. As seen in the figure, under the chosen binding conditions, IF-3 slightly stimulated the initial rate of binding

FIG. 4. Effect of IF-3 on exchange between 30S bound- ^{14}C -Phe-tRNA and free ^3H -Phe-tRNA. The 30S-poly(U)- ^{14}C -Phe-tRNA complex was prepared as described in Fig. 1. Equal aliquots of the complex containing 360 pmol of 30S ribosomal subunits were diluted 5-fold in: buffer B (O), buffer B with 10 μg of IF-3 (●), buffer B with 2 mg of unfractionated tRNA containing 750 pmol of ^3H -Phe-tRNA (Δ , ^{14}C cpm; $\square$, ^3H cpm), and buffer B with 2 mg of unfractionated tRNA containing 750 pmol of ^3H -Phe-tRNA and 10 μg of IF-3 ($\blacktriangle$, ^{14}C cpm; $\blacksquare$, ^3H cpm) and the tubes were incubated at 22°. At the indicated times, aliquots corresponding to 36 pmol of 30S ribosomal subunits were filtered through Millipore filters and the ^3H and ^{14}C radioactivities on each filter were determined. Under the counting conditions of this experiment, 1 pmol of ^{14}C -Phe-tRNA gave approximately 140 cpm and 1 pmol of ^3H -Phe-tRNA gave approximately 600 cpm.

at both poly(U) concentrations and the final level of binding only at the lower poly(U) concentration.

The experiment of Table 2 was performed to determine whether the 30S particle can carry an aa-tRNA and an IF-3 molecule simultaneously. In this experiment, either poly(U) alone or poly(U) and ^3H -Phe-tRNA were added to washed 30S ribosomes or to 30S ribosomes which had pre-bound ^{14}C -labeled IF-3. The ^3H and ^{14}C radioactivities associated with 30S ribosomes were then measured following sucrose density gradient centrifugation. As seen from the table, nearly equal amounts of ^3H -Phe-tRNA were found sedimenting with the 30S ribosomes in the presence and absence of ^{14}C -labeled IF-3. Likewise, the same amount of ^{14}C -labeled IF-3 was found to sediment with the 30S particles regardless of the presence of ^3H -Phe-tRNA, suggesting that both molecules can be present simultaneously on the 30S ribosomal subunits.

As seen thus far, IF-3 does not inhibit the binding of aa-tRNA to 30S ribosomes when included in the binding reaction (compare Fig. 2) but induces the release of pre-bound aa-tRNA in our standard assay (compare Fig. 1). The main differences between the two reactions are the volume of the reaction mixture and the order of addition of IF-3 and aa-tRNA.

To solve this apparent discrepancy, the effect of dilution of the complex on IF-3 dependent release of pre-bound aa-tRNA was studied (Fig. 3). When the 30S-poly(U)-*N*-acetyl ^{14}C]Phe-tRNA complex was prepared and diluted from 20- to 400-fold in buffer B containing a fixed amount of IF-3, *N*-acetyl- ^{14}C]Phe-tRNA was released from the complex as a function of the dilution. On the other hand, if the complex was diluted in buffer B containing no IF-3, very little *N*-acetyl ^{14}C]Phe-tRNA was released from the complex, indicating that the binding of *N*-acetylPhe-tRNA is not a reversible reaction.

In the experiment of Fig. 4, a fixed amount of 30S-poly(U)- ^{14}C -Phe-tRNA complex was diluted in tubes containing buffer B with or without IF-3 and ^3H -Phe-tRNA. The extent of dilution, however, was kept small (only 5-fold) to maintain conditions in which very little release should occur (compare Fig. 3). At various times, aliquots were withdrawn from each tube and subjected to Millipore filtration. As expected, pre-bound ^{14}C -Phe-tRNA was neither spontaneously lost from the complex nor exchanged for ^3H -Phe-tRNA in the absence of IF-3 under these conditions. Some ^3H -Phe-tRNA, however, became bound to 30S at a slow rate simply because the ribosomes had not been fully saturated with aa-tRNA (see Fig. 1). When IF-3 was present in the diluting buffer, on the other hand, significant release of ^{14}C -Phe-tRNA was observed only when a molar excess of ^3H -Phe-tRNA was also present in the diluting buffer. This loss of ^{14}C -Phe-tRNA from the complex was paralleled by a rapid uptake of ^3H -Phe-tRNA. Thus, it is clear from this experiment that the observed block of IF-3-induced aa-tRNA release upon *small* dilution of the complex (compare Fig. 3) can be overcome by adding an excess of aa-tRNA to the reaction mixture. To explain the effect of the dilution of the complex on IF-3-dependent aa-tRNA release, we have considered two possibilities: (a) Upon binding of IF-3, the 30S ribosomes undergo a *transient* conformational change which results in the release of pre-bound aa-tRNA. The released aa-tRNA rebinds to 30S ribosomes unless it is diluted in a large volume of buffer or chased by an excess of unbound aa-tRNA. (b) The presence of IF-3 on the 30S ribosomes results in a *permanent* conforma-

tional change of the ribosomes, which renders reversible the binding of aa-tRNA which is otherwise irreversible (compare Figs. 3 and 4). In this case the amounts of ribosome-bound and unbound aa-tRNA would be determined by their relative concentrations according to the law of mass action, and the binding constant would be influenced by temperature.

The first hypothesis implies that the 30S-poly(U)-aa-tRNA complexes formed in the presence or in the absence of IF-3 should be indistinguishable from one another, while the second hypothesis implies that the 30S-poly(U)-aa-tRNA complex formed in the absence of IF-3 is different from the complex formed in the presence of IF-3. To distinguish between these two possibilities, 30S-poly(U)-*N*-acetyl[¹⁴C]Phe-tRNA complexes were prepared at 22° in the presence and in the absence of IF-3 and were subjected to both dilution and temperature shift (Fig. 5). In the absence of any perturbation, the complexes with or without IF-3 are indistinguishable from one another, since both contained the same amount of *N*-acetyl[¹⁴C]Phe-tRNA even after 120-min incubation (not shown). However, when the complexes were diluted with buffer B, or when the temperature was increased from 22° to 40°, the two complexes responded differently, suggesting that the second hypothesis is correct.

In the last experiment (Fig. 6) the effect of temperature on the level of IF-3-induced aa-tRNA release from 30 S was studied. As seen in the figure, a different plateau of release was reached for each of the three temperatures studied, most likely reflecting the fact that, in the presence of IF-3, a different aa-tRNA binding equilibrium is attained for each incubation temperature.

It should be noted, however, that complete release of aa-tRNA by IF-3 is never attained even at 37° (see Fig. 6) or for the highest dilution of the complex (see Fig. 3). In these cases there is always nearly 20% of the complex which shows no reversibility in the presence of IF-3. To account for this 20%, we must consider that our 30S ribosomal subunits usually contain 2-3% contamination with 50S subunits [as determined by poly(U) assay]. It is conceivable that under our conditions for complex formation (see legend to Fig. 1) these 50S subunits may form 70S couples which, at 15 mM Mg⁺⁺, would bind nearly two molecules of aa-tRNA. Since IF-3 cannot release aa-tRNA from 70S (10), this fact could already give a fraction from 4 to 6% of aa-tRNA virtually unexchangeable or unreleasable by IF-3. If aa-tRNA binding to 70S is slightly favored over binding to 30S subunits under our binding conditions, this figure could further increase. An additional possibility is that, in some cases, an aa-tRNA can associate with 30S-30S dimers. Since IF-3 cannot bind to such dimers (18), this would result in an additional fraction of bound aa-tRNA resistant to IF-3-induced release.

DISCUSSION

It has been shown here that aa-tRNA bound to salt-washed 30S ribosomal subunits cannot be spontaneously released upon dilution of the complex or exchanged with unbound aa-tRNA. This suggests that the "binding" of aa-tRNA results in some change in either 30S and/or aa-tRNA which renders the complex formation virtually irreversible. Similar considerations have led Swan *et al.* (19) to suggest replacement of the word "binding" with the word "uptake." When the 30S-aa-tRNA-codon complex is prepared in the presence of purified IF-3, or the pre-formed complex is incubated in the presence of the factor, however, the binding of aa-tRNA becomes readily

FIG. 5. Effect of dilution and temperature shift on the stability of the 30S-poly(U)-*N*-acetyl[¹⁴C]Phe-tRNA complexes containing or not containing IF-3. The 30S-poly(U)-*N*-acetyl[¹⁴C]Phe-tRNA complexes were prepared at 22° as described in Fig. 2 in the absence and in the presence of 25 μg of IF-3. Each complex was divided into 12 equal aliquots which were diluted 30-fold with buffer B and immediately filtered (hatched area) or diluted 30-fold with buffer B and incubated at the indicated temperatures for the indicated times: (○), no IF-3; (●), IF-3 present. Two samples, which were incubated for 120 min without IF-3, showed no loss of radioactivity (not shown). One hundred percent binding corresponds to 9100 cpm and 8060 cpm of *N*-acetyl[¹⁴C]Phe-tRNA bound in the absence and in the presence of IF-3, respectively.

reversible. This activity cannot be attributed to nucleases contaminating the IF-3 preparation, and no relation was found between this activity and the type of aa-tRNA (with the exception of fMet-tRNA) or the type and amount of template used in complex formation (10). Thus, it seems unlikely that the IF-3-induced aa-tRNA release stems from the RNA-binding property of IF-3 (3, 7, 20). On the contrary, stoichiometric measurements (see Fig. 1) indicate that the target of IF-3 action is the 30S ribosomal subunit. Taken together, these findings strongly imply that the binding of IF-3 induces a conformational change of the 30S ribosomal subunit. Independent support for this idea has also been obtained by comparison of some physical-chemical properties of the 30S ribosomes and the 30S-IF-3 complex, as reported in a separate communication (11).

The finding that IF-3 induces a conformational change of the ribosomes could ultimately provide a clue to understanding the molecular mechanism by which IF-3 exerts its action

FIG. 6. Effect of temperature on IF-3-induced release of *N*-acetyl[¹⁴C]Phe-tRNA. The complex was prepared as described in Fig. 1. Aliquots containing 0.8 A₂₆₀ units of 30S binding 7.7 pmol of *N*-acetyl[¹⁴C]Phe-tRNA were incubated with 1.5 μg of IF-3 for the indicated times at the indicated temperatures and processed as described in Fig. 2.

in crucial biological functions, such as its requirement in ribosomal binding of mRNAs (6, 8) and its DF (dissociation factor) (16, 17) or subunit anti-association (4, 21, 22) activity. Finally, since the possible formation of nonreversible aa-tRNA-30S complexes out of frame or at noninitiation points of the mRNA would likely have a detrimental effect for the cell, the role of IF-3 in maintaining reversibility of aa-tRNA binding to 30S ribosomes could also be regarded as a normal cellular function of this factor.

We would like to express our gratitude to Dr. Akira Kaji, in whose laboratory this work was initiated, and to Dr. H. G. Wittmann for his constant encouragement and interest in this work. The technical assistance of Mrs. Ursel Hundertmark is also gratefully acknowledged.

1. Sabol, S. & Ochoa, S. (1971) *Nature New Biol.* **234**, 233-236.
2. Pon, C. L., Friedman, S. M. & Gualerzi, C. (1972) *Mol. Gen. Genet.* **116**, 192-198.
3. Gualerzi, C. & Pon, C. L. (1973) *Biochem. Biophys. Res. Commun.* **52**, 792-799.
4. Sabol, S., Meier, D. & Ochoa, S. (1973) *Eur. J. Biochem.* **33**, 332-340.
5. Iwasaki, K., Sabol, S., Wahba, A. J. & Ochoa, S. (1968) *Arch. Biochem. Biophys.* **125**, 542-547.
6. Revel, M., Lelong, J. C., Brawerman, G. & Gros, F. (1968) *Nature* **219**, 1016-1021.
7. Sabol, S., Sillero, M. A. G., Iwasaki, K. & Ochoa, S. (1970) *Nature* **228**, 1269-1273.
8. Wahba, A. J., Iwasaki, K., Miller, M. J., Sabol, S., Sillero, M. A. G. & Vasquez, C. (1969) *Cold Spring Harbor Symp. Quant. Biol.* **34**, 291-299.
9. Bernal, S. D., Blumberg, B. M. & Nakamoto, T. (1974) *Proc. Nat. Acad. Sci. USA* **71**, 774-778.
10. Gualerzi, C., Pon, C. L. & Kaji, A. (1971) *Biochem. Biophys. Res. Commun.* **45**, 1312-1319.
11. Paradies, H. H., Franz, A., Pon, C. L. & Gualerzi, C. (1974) *Biochem. Biophys. Res. Commun.* **59**, 600-607.
12. Suzuka, I. & Kaji, A. (1968) *J. Biol. Chem.* **243**, 3136-3141.
13. Haenni, A. L. & Chapeville, F. (1966) *Biochim. Biophys. Acta* **114**, 135-148.
14. Dubnoff, J. S. & Maitra, U. (1971) in *Methods in Enzymology*, eds. Moldave, K. & Grossman, L. (Academic Press, New York), Vol. 20, pp. 248-261.
15. Wahba, A. J. & Miller, M. J. (1974) in *Methods in Enzymology*, eds. Grossman, L. & Moldave, K. (Academic Press, New York), Vol. 30, pp. 3-18.
16. Subramanian, A. R. & Davis, B. D. (1970) *Nature* **228**, 1273-1275.
17. Subramanian, A. R. & Davis, B. D. (1973) *J. Mol. Biol.* **74**, 45-56.
18. Gualerzi, C., Wabl, M. R. & Pon, C. L. (1973) *FEBS Lett.* **35**, 313-316.
19. Swan, D., Sander, G., Bermek, E., Krämer, W., Kreuzer, T., Arglebe, C., Zöllner, R., Eckert, K. & Matthaei, H. (1969) *Cold Spring Harbor Symp. Quant. Biol.* **34**, 179-196.
20. Wickstrom, E. (1974) *Biochim. Biophys. Acta* **349**, 125-130.
21. Kaempfer, R. (1972) *J. Mol. Biol.* **71**, 583-598.
22. Noll, M. & Noll, H. (1972) *Nature New Biol.* **238**, 225-228.
23. Dietrich, S., Schrandt, I. & Nierhaus, K. H. *FEBS Lett.*, in press.