Protection Patterns of tRNAs Do Not Change during Ribosomal Translocation*

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The translocation reaction of two tRNAs on the ribosome during elongation of the nascent peptide chain is one of the most puzzling reactions of protein biosynthesis. We show here that the ribosomal contact patterns of the two tRNAs at A and P sites, although strikingly different from each other, hardly change during the translocation reaction to the P and E sites, respectively. The results imply that the ribosomal micro-environment of the tRNAs remains the same before and after translocation and thus suggest that a movable ribosomal domain exists that tightly binds two tRNAs and carries them together with the mRNA during the translocation reaction from the A-P region to the P-E region. These findings lead to a new explanation for the translocation reaction.

Ribosomes contain three tRNA binding sites, the A, P, and E site, viz. the A site where the decoding takes place, the P site, where the peptidyl-tRNA is located before peptide bond formation, and the E site, which is specific for deacylated tRNA (1–6). During elongation of the nascent peptide chain, each tRNA passes through the ribosomal binding sites in the sequence A → P → E. To elongate the nascent peptide chain by one amino acid, the ribosome goes through a cycle of reactions, the so-called elongation cycle. The three basic reactions of an elongation cycle are 1) occupation of the A site by an aminoacyl-tRNA according to the corresponding codon at the A site, 2) peptidyl-tRNA bond formation, which transfers the already synthesized peptidyl residue to the aminoacyl-tRNA so that the resulting peptidyl-tRNA, prolonged by an amino acid, now resides at the A site, and 3) the translocation reaction, which moves the peptidyl-tRNA to the P site and the deacylated tRNA to the E site.

Cross-linking and footprinting studies have identified components of the ribosome that interact with the tRNAs in the specific sites (reviewed in Refs. 7 and 8). Recently, a technique developed by Eckstein and co-workers (9) has been used to identify these components. Cross-linking and footprinting studies have identified components of the ribosome that interact with the tRNAs in the specific sites (reviewed in Refs. 7 and 8). Recently, a technique developed by Eckstein and co-workers (9) has been used to identify these components.

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used for footprinting experiments followed that described in Dabrowski et al. (10) except that the iodine cleavage was started by adding to the mixture with the thiocapped tRNAs 1/50 volume of 50 mM iodine in ethanol solution (2% ethanol final concentration). After 1 min at 0 °C, the nonbound tRNA was removed from the ribosomal complexes by gel-filtration over a Sephacryl-S300 cDNA Spin column (2 min, 4 °C, 1500 rpm, HB4 rotor). We included in Fig. 1A (left panel) the cleavage pattern of nontreated, transcribed tRNA in the presence of iodine as a control. Practically no cleavage was observed with thiocapped tRNA bound to ribosomes in the absence of iodine (Fig. 1B, right panel). We also tried to add I2 after the gel-filtration step instead of before. However, the resulting cleavage pattern was weaker. A possible explanation is that the reaction time of iodine was well terminated by the gel-filtration if added before, thus preventing secondary reactions.

Processing of the Data—The relative intensities of the free tRNAs in solution have been determined previously by vertical scanning relative to a set of control bands (Table I in Ref. 10). The intensities of all As are given relative to that of A9. Likewise G10, C11, and U12 were the reference bands for all Gs, Cs, and Us, respectively. The control bands have an intensity comparable with most of the other bands and do not seem to be affected by the tertiary structure of the tRNAs in solution. The data shown in Table I were obtained in the following way. The values of the horizontal comparison (the intensity of a band of a bound tRNA relative to the corresponding band of a tRNA in solution) were multiplied by the relative intensities of the corresponding bands derived from the vertical scanning of free tRNA. The result of the multiplication gives the intensity of a band of ribosomal bound AcPhe-tRNA or tRNApho relative to the respective control band of AcPhe-tRNA or tRNApho in solution, respectively (vertical comparison). For example, U16 of AcPhe-tRNA in solution has an intensity of 0.24 relative to U12 of AcPhe-tRNA in solution (vertical scanning); U16 of AcPhe-tRNA in the PPost site has a relative intensity of 4.33 compared with U16 of AcPhe-tRNA in solution (horizontal comparison). The product of both numbers, 0.24 × 4.33 = 1.04, gives the intensity of U16 of AcPhe-tRNA in the PPost site relative to U12 of AcPhe-tRNA in solution (vertical comparison). U16 of AcPhe-tRNA shows an enhanced accessibility when bound to the ribosome, because iodine cleavage at U16 of the free AcPhe-tRNA is restricted because of the conformation of the tRNA. A conformational change of AcPhe-tRNA upon binding relieves this restriction.

The average difference between the intensities of any corresponding bands of two patterns were calculated in the following way: The absolute values of the differences for all corresponding bands were taken, and the mean of these values was calculated. For example, U9 of AcPhe-tRNA in solution had an intensity of 0.07 relative to U12 of AcPhe-tRNA in solution (vertical scanning); U9 of AcPhe-tRNA in the PPost site has a relative intensity of 3.76 compared with U9 of AcPhe-tRNA in solution (horizontal comparison). The product of both numbers, 0.07 × 3.76 = 0.24; the average intensity of U9 of AcPhe-tRNA in the PPost site relative to U12 of AcPhe-tRNA in solution (vertical comparison) is 0.24.

RESULTS

Some Remarks to the Methods Applied—Phosphorothioated tRNAs were the product of an in vitro transcription. About four phosphorothioates were randomly incorporated at either the A, G, U, or C positions during transcription. The resulting tRNAs could be easily acylated with phenylalanine and were active in binding to ribosomal binding sites and poly(Phe) synthesis (10). The thiocapped decylated tRNApho and AcPhe-tRNA were bound to poly(U)-programmed ribosomes to either P or A sites, respectively, and the corresponding contact patterns were analyzed before and after translocation.

After forming the ribosomal complexes, the thiocapped (5'- 32P)tRNA was cleaved with iodine as described previously, and the RNA was applied to a denaturing polyacrylamide sequencing gel (10). The band intensity of each position was assessed by scanning and compared with the corresponding position of a tRNA in solution (see “Experimental Procedures”).

A weaker band intensity resulting from binding to the ribosome over restriction of the cleavage reagent was less because of steric hindrance, thus impairing the access of I2 so that a close ribosomal contact can be assumed at this position of the tRNA. An enhancement of the band intensity of a ribosome-bound tRNA compared with the one in solution indicates a conformational change of the tRNA upon binding to the ribosome; phosphates in bent regions of tRNA seem to be more accessible to iodine (12).

There is still another possibility why a band intensity could be weakened. Thioates at certain positions could hinder the binding of the tRNA to the ribosome so that these tRNAs are removed by gel-filtration and are not applied to the sequencing gel. Control experiments have demonstrated that decylated tRNApho could not be bound to ribosomes when thiocapped at position A9; the same was true for AcPhe-tRNA when it was thiocapped at position U8 (10). These two positions and those in gel extremes (~−1~5 ~−69~76) could not be identified (because of their localization) and were classified as “not determinable” in Table I and Figs. 1 and 2.

Ribosomal Contact Patterns of AcPhe-tRNA and tRNApho—The elongating ribosome contains at least two tRNAs, either at the A and P sites before the translocation reaction or at the P and E sites after (for review see Ref. 13). Only the initiating ribosome contains a single tRNA at the P site. Therefore we distinguish the initiating ribosome with one tRNA at the P site (for initiation) and the elongating ribosome with tRNAs at the A and the PPre sites (PRE for pre-translocational state) or at the PPost and the E sites (POST for post-translocational).

PRE complexes were prepared with a thiocapped AcPhe-tRNA at the A site or a thiocapped tRNApho at the P site. The second tRNA in the complex was a native tRNApho or AcPhe-tRNA, respectively. According to the puromycin reaction, 100% of the bound AcPhe-tRNA was present at the A site. The cleavage patterns of both complexes were analyzed before and after an elongation factor-G-dependent translocation. More than 70% of the AcPhe-tRNA bound to the A site was translocated to the PPost site as indicated by the puromycin reaction. Furthermore, the cleavage pattern of AcPhe-tRNA and tRNApho was analyzed in the P site. An autoradiogram of such a footprinting experiment for AcPhe-tRNA in the three investigated sites (A, PPost, and P) is shown in Fig. 1A. The cleavage patterns for tRNApho in PPost, E sites, and P site is shown in Fig. 1B.

The gels were scanned, and the relative intensities of the bands were compared with the corresponding bands of AcPhetRNA or tRNApho in solution. The error spread between independent experiments was around ±15% as in previous experiments (10). Fig. 2 shows a summary of the results. Strongly protected nucleotides (intensity < 30% of that of the corresponding band of the tRNA in solution) and nucleotides with enhanced accessibility (intensity > 130%) are indicated within the tertiary model of the tRNA.

Comparison of the Protection Patterns—Previously we have shown that the protection patterns of AcPhe-tRNA in the A site and decylated tRNApho in the P site are different, whereas there is hardly a difference between tRNApho in the P and PPost sites (10). The surprising result in this study is that there is a striking difference between P site-bound AcPhe-tRNA (PPost or P1) and P site-bound tRNApho (PPost or P1), whereas the three protection patterns of AcPhe-tRNA in the various states seem to be very similar. The same is true for the three protection patterns obtained with tRNApho. All are very similar to each other (Fig. 2, A–F). In all three states of tRNApho (P, PPre, E sites) the protection is much stronger than the protection patterns observed with AcPhe-tRNA. tRNApho shows more than 30 strongly protected positions in each ribosomal binding state, in contrast to the maximal 9 positions found in the AcPhe-tRNA (A and PPost). On the other hand, only a few positions of enhanced accessibility (maximum 3) are found with tRNApho, whereas for AcPhe-tRNA these positions are numerous (minimum 13). The stronger protection of the decylated tRNA at the P and E sites probably reflects a more compactly folded structure. The data obtained by horizontal comparison (intensity of a position relative to the corresponding band of the tRNA.
in solution, Fig. 2) indicate the changes of the reactivities upon binding to a ribosomal site compared with the reactivities of the tRNAs in solution. We have shown previously that tRNAPhe and AcPhe-tRNA in solution show a different cleavage pattern, probably because there is a conformational change in the tRNA upon aminoacylation (10). An alternative explanation, namely that the differences are caused by selection by the aminoacyl-tRNA synthetase, is unlikely because (i) it was not observed in binding studies of thioated tRNAs to the corresponding synthetase (9), and (ii) the levels of aminoacylation of native tRNAs and phosphorothioated tRNA were almost the same (10) but should be severely reduced with phosphorothioated tRNA if such a selection would take place. Indeed, a conformational change caused by aminoacylation was directly observed in flu-

| Position | AcA | AcP_post | deP_pre | deE | deA |
|----------|-----|----------|---------|-----|-----|
| G1–C4   | ND  | ND       | ND      | ND  | ND  |
| G5      | 0.1 | 0.3      | 0.0     | 0.0 | ND  |
| G6      | 0.4 | 0.4      | 0.1     | 0.1 | ND  |
| A7      | 0.5 | 0.5      | 0.1     | 0.2 | ND  |
| U8      | ND  | ND       | 0.0     | 0.1 | ND  |
| A9      | 0.3 | 0.2      | 0.1     | 0.0 | ND  |
| G10     | 0.2 | 0.2      | 0.1     | 0.0 | 0.3 |
| C11     | 0.1 | 0.1      | 0.0     | 0.0 | 0.2 |
| U12     | 1.0 | 1.0      | 0.2     | 0.2 | 0.5 |
| C13     | 1.1 | 1.0      | 0.2     | 0.3 | 0.4 |
| A14     | 1.2 | 1.1      | 0.4     | 0.7 | 0.2 |
| G15     | 0.6 | 0.6      | 0.1     | 0.1 | 0.3 |
| U16     | 0.7 | 1.0      | 0.2     | 0.2 | 0.4 |
| C17     | 1.2 | 1.5      | 0.7     | 0.5 | 0.5 |
| G18     | 1.3 | 1.3      | 0.8     | 0.8 | 1.0 |
| G19     | 0.9 | 0.8      | 0.4     | 0.3 | 0.5 |
| U20     | 0.3 | 0.6      | 0.2     | 0.2 | 0.2 |
| A21     | 0.6 | 0.7      | 0.3     | 0.4 | 0.1 |
| G22     | 0.3 | 0.4      | 0.1     | 0.1 | 0.2 |
| A23     | 0.2 | 0.3      | 0.1     | 0.1 | 0.1 |
| G24     | 0.4 | 0.5      | 0.1     | 0.1 | 0.3 |
| C25     | 0.8 | 0.9      | 0.2     | 0.3 | 0.3 |
| A26     | 0.4 | 0.3      | 0.3     | 0.5 | 0.2 |
| G27     | 0.2 | 0.2      | 0.1     | 0.1 | 0.2 |
| C28     | 0.4 | 0.9      | 0.3     | 0.2 | 0.4 |
| G29     | 0.5 | 0.5      | 0.2     | 0.2 | 0.5 |
| G30     | 0.8 | 0.5      | 0.2     | 0.3 | 0.7 |
| A31     | 0.6 | 0.3      | 0.0     | 0.1 | 0.2 |
| U32     | 0.2 | 0.2      | 0.0     | 0.0 | 0.6 |
| U33     | 0.2 | 0.3      | 0.2     | 0.3 | 0.7 |
| G34     | 0.1 | 0.1      | 0.1     | 0.3 | 0.2 |
| A35     | 0.2 | 0.2      | 0.6     | 0.7 | 0.5 |
| A36     | 0.3 | 0.3      | 0.5     | 0.6 | 0.6 |
| A37     | 0.5 | 1.0      | 0.6     | 0.7 | 0.7 |
| A38     | 0.4 | 0.5      | 0.8     | 1.0 | 0.8 |
| U39     | 0.5 | 0.6      | 0.3     | 0.3 | 0.6 |
| C40–C43 | 1.2 | 1.4      | 0.4     | 0.5 | 0.5 |
| G44     | 0.4 | 0.6      | 0.2     | 0.2 | 0.4 |
| U45     | 0.5 | 0.9      | 0.2     | 0.2 | 0.7 |
| G46     | 0.9 | 0.8      | 0.7     | 0.6 | 0.4 |
| U47     | 1.9 | 2.5      | 0.7     | 0.6 | 1.2 |
| C48     | 1.1 | 1.3      | 0.8     | 0.7 | 1.7 |
| C49     | 1.2 | 1.5      | 0.4     | 0.5 | 1.3 |
| U50     | 0.3 | 0.7      | 0.1     | 0.1 | 0.9 |
| U51     | 1.3 | 1.5      | 0.3     | 0.3 | 1.2 |
| G52     | 0.4 | 0.3      | 0.1     | 0.1 | 0.5 |
| G53     | 0.4 | 0.8      | 0.3     | 0.3 | 0.6 |
| U54     | 0.7 | 0.8      | 0.1     | 0.2 | 0.7 |
| C56     | 0.1 | 0.2      | 0.1     | 0.1 | 0.3 |
| G57     | 0.3 | 0.2      | 0.1     | 0.1 | 0.3 |
| A58     | 0.1 | 0.1      | 0.0     | 0.1 | 0.1 |
| U59     | 0.2 | 0.4      | 0.1     | 0.1 | 0.8 |
| U60     | 0.3 | 0.5      | 0.1     | 0.1 | 0.8 |
| C61–C62 | 0.9 | 1.4      | 0.4     | 0.3 | 0.9 |
| G63     | 0.4 | 0.4      | 0.2     | 0.2 | 0.2 |
| A64     | 0.3 | 0.5      | 0.1     | 0.2 | 0.2 |
| G65     | 0.3 | 0.5      | 0.2     | 0.1 | ND  |
| U66     | 0.4 | 0.4      | 0.1     | 0.1 | 0.7 |
| C67–A76 | ND  | ND       | ND      | ND  | ND  |

**Table I**

Accessibilities of the phosphates of the tRNAs in the various states: AcPhe-tRNA in the A (AcA) and PPost (AcP_post) sites and deacylated tRNA\(^{\text{Pse}}\) in the Ppre (deP_pre), E (deE) and A (deA) sites.

The numbers give the intensity relative to the corresponding control band (A9, G10, C11, or G12) of the respective tRNA in solution (vertical comparison; see "Experimental Procedures"). The intensities of AcPhe-tRNA in the A and deacylated tRNA\(^{\text{Pse}}\) in the Ppre sites have been published previously relative to the corresponding bands of the unbound tRNAs (Table II in Ref. 10; horizontal comparison; see "Experimental Procedures"); the data were recalculated according to the vertical comparison and are included to facilitate a comparison. ND, not determined.
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orescence studies (14, 15) and revealed by electrophoresis through perpendicular denaturing gradient gels (16). Therefore, the data derived from the horizontal comparison might not properly reflect the differences between the patterns of AcPhe-tRNA and tRNAPhe. To eliminate the influence of the conformation of the free tRNA, the reactivities of the bound tRNAs were normalized to a fixed set of reference bands of the corresponding free tRNAs, namely A9, G10, C11, or U12 (see “Experimental Procedures”). These values are given in Table I (vertical comparison) and were used for the difference calculations (Fig. 3).

We consider differences between the intensities of two positions as significant if they are different by at least a factor of two and if the higher value is not below 0.3. Furthermore, we calculated an average deviation between two patterns (see “Experimental Procedures”) to be independent from arbitrarily chosen criteria of differences. If AcPhe-tRNA in the A site is compared with AcPhe-tRNA in the PPost site (Fig. 3A) or the Pi site (not shown), 8 of 61 positions are different. The average deviation of a band from an AcPhe-tRNA at the PPost site to that at the A site is only 0.16. Practically no difference is observed when the patterns of tRNAPhe in the PPre and E site, respectively, are compared (Fig. 3C), and the average deviation is 0.05. It follows that the protection patterns of AcPhe-tRNA are very similar before and after translocation. The deacylated tRNA before and after translocation at the PPre and the E sites, respectively, shows practically an identical pattern.

In contrast, when PPost-site bound AcPhe-tRNA is compared with PPre-site bound tRNAPhe, 40 of 60 positions differ in their protection pattern (Fig. 3B), and the average deviation (0.44) of a band is much larger. Similarly, a comparison of the pattern of AcPhe-tRNA with that of tRNAPhe in the Pi sites reveals a large number of differences (35 of 60; not shown); the average deviation is 0.40. Therefore, the tRNAs seem to interact with different binding sites, although conventional understanding assigns them to the same binding site, the P site.

A pattern specific for deacylated tRNAPhe was found regardless of whether the tRNAPhe was present at the P or E sites. Similarly, a pattern specific for AcPhe-tRNA was found when bound to either A or P sites. Do the two distinct contact patterns depend on the charging state of the tRNA rather than reflect different features of ribosomal binding sites?

We analyzed this problem in the following control experiment. Ribosomes were programmed with the MF-mRNA (17), which is a heteropolymeric mRNA of 46 nucleotides and carries the two unique codons AUG-UUC in the middle. A deacylated and thioated tRNAPhe was bound either to the A site after prefilling the P site with a deacylated tRNAfMet or to the Pi site. When the thioated tRNA Phe was present at the Pi site, a pattern known for deacylated tRNA was observed (not shown). In contrast, tRNA Phe in the A site showed a different pattern (Table I). 30 of 51 positions differed in their intensities as compared with tRNAPhe in the PPre site (Fig. 3D). 19 of these 30 positions approach a reactivity of the corresponding positions of AcPhe-tRNA in the A site. It seems that tRNAPhe in the A site adopts a protection pattern similar to that of AcPhe-tRNA in the same site. Some nucleotides still show a protection state typical for the tRNAPhe pattern (especially the anticodon, e.g. A35, A36). In contrast, many others show a typical AcPhe-tRNA protection (Table I), for example nucleotides within the anticodon stem (G26, G30, U39), the extra arm (G44, U45), and the TψC stem loop (C49, U51-U55, C61-G63). A calculation of
the average difference between the corresponding nucleotides of two patterns revealed the following. The pattern of a deacetylated tRNA^{Phe} at the P_{Pre} site is very different to that of a deacetylated tRNA^{Phe} at the A site (average difference 0.31). The difference between P_{Post} site-bound AcPhe-tRNA and P_{Pre} site-bound tRNA^{Phe} (0.44) is significantly larger than the corresponding value of A site-bound tRNAs (0.26), i.e. the difference between AcPhe-tRNA and tRNA^{Phe} bound to the A site. Thus, the pattern of a tRNA^{Phe} at the A site deviates sharply from that of a tRNA^{Phe} at the P site but approaches that of an AcPhe-tRNA at the A site, although significant differences are observed. It is clear that both the charging state of a tRNA as well as the features of a ribosomal binding site severely affect the corresponding protection pattern of the tRNA.

DISCUSSION

It has been shown previously that strikingly different contact patterns were observed with deacetylated tRNA^{Phe} at the P site and AcPhe-tRNA at the A site of ribosomes before translocation (10). Here we demonstrate that the distinct pattern of either tRNA hardly changes during translocation to the P and E sites, respectively.

Two ways were used to calculate and compare the relative intensities of a band. 1) The intensity of a position of a bound tRNA was calculated relative to the corresponding band of the tRNA in solution (horizontal comparison). 2) The intensities of, for example, all A nucleotides of a bound tRNA were related to the reference band A9 of the corresponding tRNA in solution (vertical comparison, see “Experimental Procedures,” “Processing of the Data”). Both methods of data processing clearly reveal that the protection patterns of AcPhe-tRNA in the investigated binding sites (A, P_{Post}, P_{i}) are similar as well as those of tRNA^{Phe} in the P_{Pre}, E, and P_{i} sites, whereas both types of patterns are strikingly different. The intensities of the corresponding nucleotide positions of an AcPhe-tRNA at either the A or the P_{post} site differ by 0.16 on average. A difference of only 0.05 is found, if correspondingly, the patterns of deacetylated tRNA^{Phe} at P_{Pre} and E sites are compared. However, the average difference is about 0.4 between an AcPhe-tRNA pattern and that of a tRNA^{Phe}.

The type of pattern observed with AcPhe-tRNA at the A site before and at the P site after translocation is termed \( \alpha \) (\( \alpha \)), because only the \( \alpha \)-like pattern is found at the A site. Correspondingly, we define the type of pattern seen of deacetylated tRNA at the P and the E sites before and after translocation, respectively, as the \( \epsilon \) pattern (\( \epsilon \), because only the \( \epsilon \)-like pattern is observed at the E site). A few positions at the 5' and the 3' ends could not be judged in our analysis, but at least the CCA-3' end has been tested already by chemical probing. The protection pattern at the CCA end did not change during translocation from the A to the P site (18), in agreement with our findings.

Although the conformation of both tRNA species in solution seems to be different, there is evidence that binding to the ribosome equalizes the conformations of deacyl-tRNA and AcPhe-tRNA (10). Furthermore, a control experiment demonstrated that the protection pattern is not mainly determined by the charging state of the tRNA, because tRNA^{Phe}, when bound to the A site, definitively shows not an \( \epsilon \) pattern (Fig. 3D) but approaches the \( \alpha \) pattern. Therefore we prefer the view that most of the protections of the ribosome-bound tRNAs are because of direct contacts with the ribosome, and that the differences in the \( \alpha \) and \( \epsilon \) protection patterns reflect interactions with different ribosomal components. This interpretation seems to be justified because the contact patterns of thioated
tRNAs in a complex with synthetase correlates well with x-ray analysis data (12, 19), i.e. the protection pattern is directly related to the binding site. Even if some protections are caused by a conformational change upon binding, this change is caused by interactions with the ribosome, viz. reflects interactions between the tRNA and the binding site.

The data suggest that the micro-environment of the tRNAs does not change during translocation, i.e. components of the ribosome, which are in contact with the tRNAs and are responsible for the protection patterns, are the same before and after translocation. This is surprising, because according to previous models of translocation, differences would be expected between A site- and P site-bound AcPhe-tRNA as well as between P site- and E site-bound tRNAPhe, whereas the patterns of P site-located deacyl-tRNA and AcPhe-tRNA would be expected to be similar.

Our results are supported by other studies. (i) AcPhe-tRNA protects a similar set of nucleotides of 23 S rRNA in the A and in the P site, whereas a different set of nucleotides was protected by P site- or E site-located tRNAPhe (20). (ii) Recently, the 23 S rRNA neighborhood of deacylated tRNAs was analyzed in the P and E sites (21). The tRNAs contained up to five randomly incorporated 4-thiouridines to which a phenanthro-
line was attached. This residue cleaved nucleic acids in the presence of Cu$^{2+}$ ions within about 20 Å of the site of the attachment. 118 cleavage sites were detected, 85% of which were identical at P and E sites, also supporting the view that the ribosomal micro-environment of the tRNAs in the two sites does not change. (iii) Finally, our observation that tRNAPhe and AcPhe-tRNA at the Pi site show highly different patterns is in agreement with results from energy transfer studies using fluorescent probes. The fluorescence signals from deacylated tRNA and AcPhe-tRNA at the Pi position were so different that the authors concluded that these tRNAs must be present at different sites (14, 15). Those results also suggested that AcPhe-tRNA did not change the site when translocated from the A to the P position, again in accord with the findings reported here.

On one hand, we find that the micro-environment of both tRNAs on the elongating ribosome does not change during translocation. On the other hand, it is known that the mRNA moves three nucleotides through the ribosome in the course of translocation (22), and also the mass centers of gravity of both the mRNA (23) and the two tRNAs move 12 Å within the ribosome (24), in good agreement with the length of one codon. Most important, the two tRNAs turn by an angle of at least 20° during translocation (25, 26). Such a turn means that the elbows of the two L-shaped tRNAs change their positions considerably and move even more than 12 Å during translocation. This paradox can be resolved if one assumes that a domain of the ribosome moves together with the tRNAs and the mRNA during the translocation reaction. This assumption gives a simple explanation for the translocation reaction: the tRNAs do not dissociate from A and P sites to diffuse to P and E sites, respectively; instead the tRNAs are tightly bound to the movable conveyor before, during, and after the translocation reaction. Translocation can be achieved by the ribosome via a movement of this conveyor from the A and P positions to the P and E positions, respectively; thereby the bound tRNAs are translocated. The movable ribosomal domain that binds two tRNAs tightly and transports them during the translocation reaction is termed $\alpha e$ domain according to the corresponding protection patterns.

According to this model the critical step of the elongation cycle is not the translocation as in previous models but rather the A site occupation. During this reaction, the conveyor has to be moved back to A and P positions, whereas the peptidyl-tRNA has to stay at the P site, and the deacyl-tRNA has to be released from the E site. The view that the A site occupation is characterized by a major rearrangement between the (tRNA)$_7$-mRNA complex and the ribosome is consistent with the observations that the occupation of the A region and not the translocation reaction is the rate-limiting step of the elongation cycle (27, 28).

A candidate for a movable $\alpha e$ domain is the so-called “bridge 2,” which is the most massive connection between the ribosomal subunits going from the decoding region of the small sub-
unit to the region of the peptidyltransferase on the large ribosomal subunit (29). Indeed, there are close contacts between P site-bound fMet-tRNA$_{\text{Met}}$ and this intersubunit bridge, as revealed by cryo-electron microscopy (30). Interestingly, those regions of the AcPhe-tRNA exposed at the P i site, revealed by cryo-electron microscopy (30). Interestingly, those regions of the AcPhe-tRNA exposed at the P site (part of the anticodon stem and the D loop, see Fig. 2C) seem to be also freely accessible in the cryo-electron microscopy analysis, whereas higher densities of protection sites are found at regions of contacts between the fMet-tRNA and the ribosomal matrix (30). Therefore, a good correspondence between protection and contact sites exists with tRNA-ribosome complexes as has been already observed with tRNA-synthetase complexes mentioned above.

The conclusions drawn from the protection patterns are not easy to reconcile with hybrid states of tRNA binding. The hybrid-site model suggests that in the PRE state, AcPhe-tRNA is bound in an A/P and deacyl-tRNA in a P/E hybrid state (20). After translocation, the AcPhe-tRNA should be bound in the P/P state, respectively, are scattered over the whole molecule (Fig. 3A) and are not clustered at the anticodon domain. In contrast, we observe that the few differences between the patterns of AcPhe-tRNA before and after translocation at the A and P site, respectively, are scattered over the whole molecule (Fig. 3A) and are not clustered at the anticodon domain. In contrast, the experimental data on which the hybrid-site model is based can be reconciled with an elongation model that incorporates the concept of a movable, ribosomal α-ε domain translocating the tRNAs, i.e. the α-ε model (for discussion see Refs. 31 and 32).

In summary, our results suggest that the same ribosomal components are in contact with the tRNAs before and after translocation, viz. are moving together with the tRNAs during the translocation reaction, thus providing a new explanation for this reaction. The mRNA are connected with the two tRNAs on the ribosome via codon-anticodon interactions. It seems that the mRNA passively follows the movement of both tRNAs during the translocation reaction, because a similar analysis with thioated mRNA has revealed that no contacts exist between phosphate groups of an mRNA and the ribosome outside the decoding region (33). In this view, essentially the tRNAs are pulling the mRNA through the ribosome, underscoring the importance of maintenance of codon-anticodon interactions before and during translocation (13, 33). It turns out that it is not the translocation reaction but rather the molecular mechanism of A-site occupation that is puzzling.

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