Hypothesis

Location of tRNA on the ribosome

Alexander S. Spirin

Institute of Protein Research, USSR Academy of Sciences, 142292 Poustchino, Moscow Region, USSR

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Electron microscopy results of Lake [J. Mol. Biol. (1976) 105, 131–159] and Vasiliev et al. [FEBS Lett. (1983) 155, 167–172] suggest that the 70 S ribosome has an open pocket or a cavity at the base of the L7/L12 stalk of the 50 S subunit, on the side of the 30 S subunit opposite to its bulge or platform. It is this pocket that is proposed here to be the site for binding and retention of two L-shaped tRNA molecules on the ribosome. The model proposed is consistent with the facts about interactions of the protein L7/L12 with the elongation factors (EF-Tu and EF-G) involved in tRNA binding and translocation, as well as with the data available on the participation of proteins S3, S5, S10, S14 and S19 in the formation of tRNA sites.

Ribosome shape *Ribosomal subunit coupling* *tRNA site* *Elongation factor* *L7/L12 protein stalk* *tRNA site protein*

1. INTRODUCTION

Electron microscopy studies of ribosomes and their subunits, in particular of bacterial ribosomes, have led to elucidation of their morphological features [1–14]. The models of the 30 S subunit, the 50 S subunit and the 70 S couple proposed in [2,4,13] now seem to be the best approximation to the real shapes of the particles. A drawing of the 70 S ribosome model proposed in [4] and specified in [14] is presented in fig.1. According to the Lake–Vasiliev model, the two ribosomal subunits are coupled in such a way that the head of the 30 S subunit joins the head (central protuberance) of the 50 S subunit and the side ledge (platform) of the 30 S subunit contacts with the side lobe (L1 ridge [15]) of the 50 S subunit; the L7/L12 stalk of the 50 S subunit [8] sticks out from the ribosome [14]. The concave (interface) side of the 50 S subunit is found to be covered by the partner 30 S subunit just partially, a rather large region of the 50 S subunit surface at the base of the L7/L12 stalk being free (fig.1).

2. THE MODEL PROPOSED

Thus, the association of the two ribosomal subunits in the way mentioned results in the formation of an open pocket, or cavity restricted from three sides:

(i) By the 50 S subunit surface including the base of the L7/L12 stalk and a part of the head (central protuberance);
(ii) By the surface of the 30 S subunit head where the proteins S14 and S19 are located [16–18];
(iii) By the surface of the 30 S subunit body with the protein S5 located nearby [17,18].

Both the main groove separating the head and the body of the 30 S subunit and the groove between the head and the rest of the 50 S subunit open into this pocket; since the two grooves are opposite each other, the pocket has a through passage or a channel to the other side of the ribosome; i.e., to the region of the side lobes (the region of platform and L1 ridge) of the subunits.

I propose that the pocket or cavity mentioned is the site for binding and retention of two L-shaped

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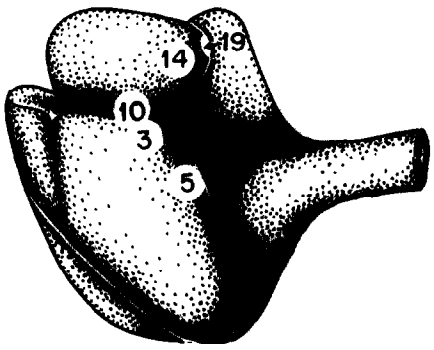

Fig.1. Drawing of the 70 S ribosome model after Lake (1976) and Vasiliev et al. (1983). View from the 30 S subunit. Positions of the 30 S subunit proteins supposed to be in the region of tRNA-binding sites are given by numerals in circles (proteins S3, S5, S10, S14 and S19, respectively).

tRNA molecules on the ribosome (fig.2). Indeed, the pocket seems to be strikingly 'complementary' to the L-shaped tRNA molecules. The scale model of the pair of tRNA molecules with stacked anticodons [19] (see also tRNA pair model C in [22]) perfectly fits in this region of the 70 S ribosome model, the anticodons being towards the 30 S subunit, the acceptor ends to the interface of the 50 S subunit and the corners outside (fig.2). According to the model, the ribosomal A site for tRNA is formed by the surface of the 30 S subunit head with proteins S14 and S19 [16–18] and partly by the 50 S subunit head with the 5 S RNA–protein complex [20]. The P site for tRNA is formed by the surface of the 30 S subunit body with protein S5 [17,18] and the surface of the 50 S subunit at the base of the L7/L12 stalk [8]. The anticodons of the two tRNAs are found in the groove separating the head and the body of the 30 S subunit on the side opposite the subunit interface (the region of proteins S3 and S10 [18,21]). The acceptor ends of the tRNAs get into the region of the groove separating the head (central protuberance) from the rest of the 50 S subunit at the subunit interface; correspondingly, this region is suggested to be the peptidyl transferase center of the ribosome.

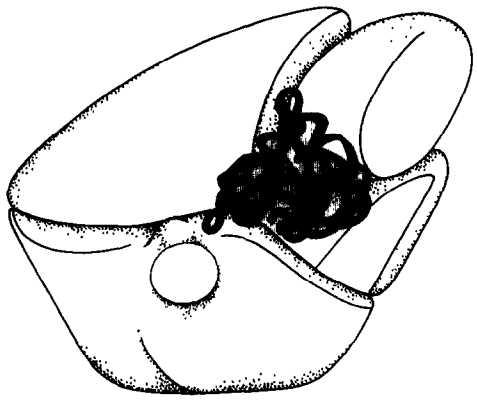

Fig.2. Proposed location of two tRNA molecules in the A (right) and the P (left) sites on the 70 S ribosome.

3. DISCUSSION

The following experimental data are consistent with the above proposal:

(1) The chemical cross-linking results of Ofengand [22] show that the central part of the tRNA in the A site contacts with the ribosome namely by its *right* side, if viewed from the corner of its L-shaped molecule. Moreover, the central part of the tRNA in the A site is found to be specifically cross-linked with protein S19 [22] located on the 30 S subunit head. Then the left side of the tRNA molecule could serve for the interaction with EF-Tu, as demonstrated in [23]. On the other hand, the central part of tRNA in P site contacts with the ribosome only by its *left* side, if viewed from the molecule corner [22].

(2) The ribosomal proteins of the 30 S subunit bordering the pocket under consideration include the proteins S3, S5, S10, S14 and S19, according to direct immuno-electron microscopy data [24] and to our results on the synthesis of chemical, physico-chemical, electron-microscopic and neutron-scattering data [18]. From early reconstitution experiments it is known that namely these proteins, while not requisite for particle assembly, are necessary for the restoration of the tRNA-binding activity of the particles [25,26]. The addition of proteins S3 and S14 to routinely isolated ribosome preparations stimulates the RNA-binding activity, seemingly due to the restoration of the A sites in defective particles [27]. Antibodies against any of the 5 proteins listed inhibit the tRNA-binding activity of the ribosome [28]. Ribosome-bound tRNA protects proteins S3, S14, S19 from trypsin [29]. Among the proteins reported by different groups to be chemically

cross-linkable with tRNA or its codon, the same proteins S3, S5, S10, S14 and S19, as well as adjacent proteins S7, S9 and S13, are the most often mentioned [30–38] (reviews [22,39]). (Proteins S18 and S21 localized on the other side of the 30S subunit, namely on its side lobe or platform, are also reported to be cross-linkable with tRNA or its codon; however, the survey in [39] warns about the especially high nucleophilicity of these proteins and calls for scepticism about the affinity labeling results on them.)

(3) There is a lot of evidence that the binding sites of elongation factors are localized near to the L7/L12 stalk of the ribosome. Thus, functions of both EF-Tu and EF-G strongly depend on the presence of the L7/L12 protein complex on the ribosome (review [40]). Antibodies against protein L7/L12, and only they, inhibit the interaction of EF-Tu and EF-G with the ribosome (review [41]). EF-Tu and EF-G can be chemically cross-linked with protein L7/L12 [42,43]. Finally, localization of EF-G in the region of the 50 S subunit adjacent to the L7/L12 stalk has been directly demonstrated by immuno-electron microscopy [44]. At the same time, it is evident that the elongation factors are to be in the vicinity of the A and P sites of the ribosome since they are involved in tRNA binding and displacements. Indeed, it has been experimentally shown that EF-G directly interferes with aminoacyl-tRNA binding [45–47] and that it can be cross-linked with the proteins S19 and S3 [43,48] which are reported to be neighbours of tRNA [22,30,32,34,36,37]. All this suggests that the A and P sites of the ribosome are not far from the L7/L12 stalk.

Earlier Lake [49] proposed the model where the A and P sites of the 70 S ribosome are positioned on the other side of the 30 S subunit, in the region of its side lobe (platform). To explain the participation of some outside proteins of the 30 S subunit in tRNA binding, as well as the involvement of EF-Tu as a protein contacting simultaneously both with tRNA and with some L7/L12 stalk region, Lake speculated that there is also a site for preliminary tRNA binding on the way to A site, the so-called R site, situated on the outside surface of the 30 S subunit. (According to the model proposed, switching of tRNA from the R site to the A site is accompanied by the conformational rearrangement of the tRNA anticodon from the 5′-stacked conformation in the R site to the normal 3′-stacked one in the A site.)

As to the R site, contacts (photo-chemical cross-links) of the ribosome with aminoacyl-tRNA before and after GTP cleavage and EF-Tu removal (R site and A site binding, respectively) were reported as similar in [50]. This result disagrees with the idea of a significant rearrangement of tRNA on the ribosome when switching from the preliminary binding in the 'R site' to the final fixation in the A site.

Anyhow, the location of the A and P sites of the 70 S ribosome in the region of the side ledge (platform) of the 30 S subunit or in its neighbourhood [49] remains to be considered seriously. This idea has been supported by the communication [51] on the localization of tRNA anticodon in the side bulge region of the 30 S subunit using the immuno-electron microscopy technique, as well as by reports on the localization of the peptidyl-transferase center of the 50 S subunit in its side lobe (L1 ridge) region or between it and the head (central protuberance) [52,53]; i.e., in front of the side lobe (platform) of the 30 S subunit. Correspondingly, in the models of Ofengand [22] and Olson et al. [53] the tRNA sites are also placed in the side bulge (platform) region of the 30 S subunit. At the same time, this position of the tRNA sites is not consistent with the facts mentioned above in (2) and, especially, (3).

Here I propose the alternative model (fig.2) where the pair of tRNA molecules is placed on the other side of the 30 S subunit, thus filling and well fitting the natural cavity of the 70 S ribosome. The elongation factors performing the 'accommodation' of tRNA to the A site (EF-Tu) and the translocation of tRNA from the A site to the P site (EF-G) can also be placed in the same cavity. The mobile L7/L12 stalk [54] can be supposed to be involved in interactions of the ribosome with the elongation factors and tRNAs.

I hope that direct experiments will be stimulated in order to choose from the two alternative models.

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