POSSIBLE RELATIONSHIP OF PEPTIDYL TRANSFERASE BINDING SITES, 5S RNA AND PEPTIDYL-tRNA

STANISLAV CHLADEK

Detroit Institute of Cancer Research,
Division of the Michigan Cancer Foundation,
Detroit, Michigan 48201

Received August 23, 1971

SUMMARY

A possible role of the 5S RNA in the peptide chain elongation process is suggested. This role consists of an interaction of the Ψ loop of peptidyl tRNA at the P-site of peptidyl transferase with the single stranded region of the 5S RNA. The interacting region may also be one of the sites for peptidyl transferase binding to the 5S RNA, moreover, the resulting "release" of peptidyl transferase from its binding site on the 5S RNA (due to binding of the Ψ loop of peptidyl tRNA to the 5S RNA) could cause conformational changes in the enzyme leading to an exposure of its "CCA binding site" for peptidyl tRNA.

Peptidyl transferase, a peptide bond-forming enzyme (or complex of enzymes) is considered to be an integral part of the 50S subunit of bacterial ribosomes[1]. The two-binding site model of peptidyl transferase is now widely accepted. Aminoacyl transfer RNA is believed to be bound in the acceptor (A) site and peptidyl (or N-acyl-aminoacyl) tRNA in the peptidyl (P) or donor site of peptidyl transferase prior to peptide bond formation[2,3]. The acceptor (CCA) ends of both tRNAs must be in juxtaposition to accomplish peptide bond formation by transferring the peptide residue from tRNA at the P-site to the amino group of AA-tRNA at the A-site.

Relatively simple compounds such as AA- (or N-acyl-AA) nucleosides or short (2-6 units) oligonucleotides derived from the 3'-terminus of AA-tRNA (or N-acyl-AA-tRNA) can replace whole molecules of the corresponding tRNAs and interact with peptidyl transferase as substrates for either A- or P-site, i.e., to act as an acceptor or donor* of the peptide chain[4]. Nevertheless, special conditions have to be used to elicit the acceptor or donor activity of the "short fragments": (a) higher Mg^{++} concentration and (b) for binding to the

* This has become known as the fragment reaction.

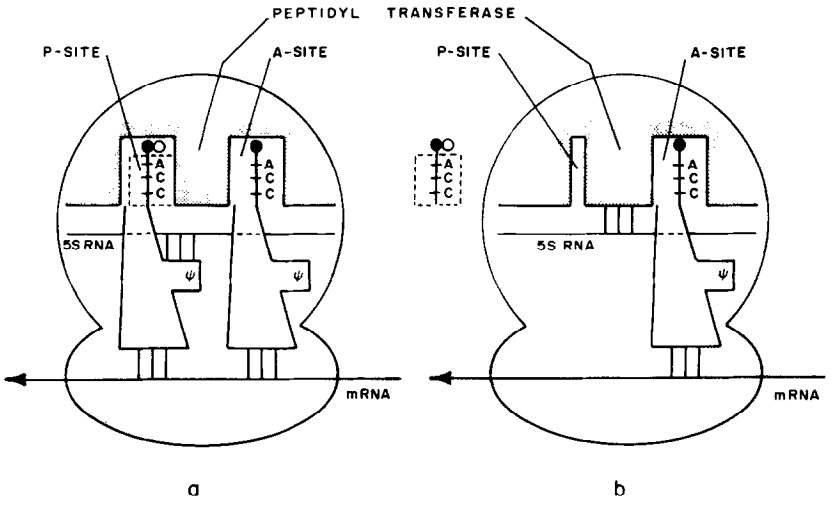

Fig. 1. Simplified model of peptidyl transferase and 5S RNA binding sites.

(a) Peptidyl-tRNA is locked in 5S RNA site (by interaction of its $GT\Psi C\frac{A}{G}$ loop) and the P-site is accessible for the CCA end of peptidyl-tRNA. The situation is depicted prior to the peptide bond formation.

(b) The 5S RNA binds to the peptidyl transferase site, the P-site is then closed for the entry of the peptidyl-CCA derivative (alcohol is not present). Peptide bond formation does not take place.

P-site (donor) substrate a significant concentration of an aliphatic alcohol must be present[4]. It has been suggested that ethanol promotes binding of the substrate to the P-site, perhaps changing the ribosomal conformation in some way[4,5]. Since there is no requirement for ethanol (or higher Mg^{++} concentration in some instances) for the binding of a complete tRNA (AA or peptidyl) to the appropriate peptidyl transferase sites, and since the transfer activity of AA (or peptidyl or N-acyl-AA) derivatives of fragments are lower than those of corresponding tRNA derivatives themselves[4], it could be a lack of recognition sites on fragments which necessitates these modified conditions for binding or peptide bond formation. It is generally assumed that the ribosomal complex containing intact tRNAs and messenger RNA is more stable than that of the end fragments, due to other binding sites in intact molecules of tRNAs in the addition to the CCA sequence. The CCA sequence is nevertheless

considered as the only recognition site in the tRNA molecule to interact directly with peptidyl transferase[4].

"The common sequence" $GT\Psi C^{A}_{G}$[6] has been found in all tRNAs sequenced thus far[7] and the tetranucleotide TΨCG has been shown to inhibit non-enzymatic binding of AA-tRNA to both A- and P-sites[8,9]. Therefore, the $GT\Psi C^{A}_{G}$ sequence has been suggested as a binding site of tRNA to 50S ribosomes, possibly to 5S RNA[10]. Indeed, the complementary sequence of CGAAC has been located in a single stranded region of 5S RNA[11] and thus the idea that $GT\Psi C^{A}_{G}$ binding is actually the binding to the 5S RNA does not seem unreasonable.

I would like to propose that binding of the $GT\Psi C^{A}_{G}$ sequence of peptidyl tRNA (N-acyl-AA-tRNA) occurs in the neighborhood of the A- or P-site and further causes conformational changes in these sites. This results in an increase in the exposure of both sites for binding the CCA parts of tRNA molecules. In other words, the "CCA binding sites" of peptidyl transferase (particularly the P-site, which is known to be less accessible[4]) are normally dormant and their exposure may require prebinding of the $GT\Psi C^{A}_{G}$ sequence to some closely related site. The tRNA would then be locked at two main loci on the 50S subunit and an additional one on the 30S subunit (anticodon-codon). If we consider that the binding of the $GT\Psi C^{A}_{G}$ portion of tRNA is to 5S RNA, we can further suppose a close relationship exists between 5S RNA and peptidyl transferase. I would further propose that the single-stranded part of the 5S RNA (the sequence CGAAC[11] complementary to $GT\Psi C^{A}_{G}$ of the tRNA) is also a binding site for peptidyl transferase. Conformational changes in the enzyme due to the binding of tRNAs $GT\Psi C^{A}_{G}$ sequence to 5S RNA template can thus be roughly classified as an allosteric-like effect. It would then seem possible that the "release" of peptidyl transferase from at least one binding site on 5S RNA (which would result from the binding of tRNA) could cause pronounced conformational changes in the enzyme leading to an exposure of CCA binding sites.

Recently, it has been suggested that the ribosomal complex passes

through a cycle of contraction and expansion during the process of translation[12]. The conformational changes undergone by peptidyl transferase, as suggested here, could contribute to the overall process.

Even though not essential for the proposed model, additional conformational changes could also occur in substrate tRNA molecules, e.g., unfolding of tertiary structure of tRNA as a result of an exposure of $GT\Psi C^{A}_{G}$ to a ribosomal site[8,9]. G is usually considered to take part in helical pairing while T, Ψ and C may bind to the A and G residues in the dhU loop[13].

One could further speculate about the binding of the $GT\Psi C^{A}_{G}$ segment to appropriate loci. From an analogy with the binding of anticodon loop of $tRNA^{Met}_{F}$ to ribosome[14] (in response to coding AUG triplet), it would seem reasonable that the entire Ψ loop or even the adjacent stem region would be required. This is further supported by the fact that all known tRNAs have the same number of nucleotides in the Ψ loop (7)[15] and almost all 5 base pairs in the stem region (for an exception, see ref. 15). It can be also assumed that if peptidyl or N-acyl-AA-tRNA is to be bound to ribosome directly (P-site) without G-factor directed translocation[16] (at about 10 mM Mg^{++} concentration), only gentle distortion of normally closed P-site is necessary. (This distortion would be caused by higher Mg^{++} concentration.) On the other hand, a mere end fragment of tRNA [like CCA (fMet), where the part of the tRNA chain for binding to 5S RNA is missing] requires that more drastic means be used to expose the "CCA locus" of the P-site for the binding of the substrate. This is the case of the "fragment reaction"[4] where ethanol (or other aliphatic alcohol) is part of the reaction environment. As a result of partial distortion of peptidyl transferase binding sites by ethanol, an uncontrolled diffusion of substrate from the A-site to the P-site could take place under the conditions of the "fragment reaction" instead of an enzymatically mediated translocation[17].

Since the P- and A-sites may be considered as allosterically linked, one might even suggest conformational changes in one site induced by a sub-

strate bound to the second site. This assumption would serve to explain the results of Erbe and Leder[18] and Shorey *et al.*[19] which indicate that the peptide bond could not have been formed between N-acyl-AA-tRNA and AA-tRNA unless the former was prebound to the P-site. This suggests that binding of N-acyl-AA-tRNA to the P-site (which is made possible as suggested above) further induces conformational changes in allosterically linked A-site. AA-tRNA is then bound to the proper site and the formation of a peptide bond takes place.

To my knowledge this model constitutes the first attempt to assign the role of the 5S RNA in the peptide chain elongation process** on the basis of its mutual interactions with peptidyl transferase and peptidyl (N-acyl-AA) tRNA.

ACKNOWLEDGMENTS

I wish to thank Dr. J. Zemlicka of the Michigan Cancer Foundation and Dr. H. McAllister of Wayne State University for their helpful discussions and criticisms.

REFERENCES

1 Monro, R. E., Maden, B. E. H., and Traut, R. R., in *Genetic Elements, Properties and Function* (edit. By Shugar, D.), 179 (Academic Press, London, 1967).
2 Watson, J. D., *Bull. Soc. Chim. Biol.*, 46, 1399 (1964).
3 Bretscher, M. S., and Marcker, K. A., *Nature*, 211, 380 (1966).
4 Monro, R. E., Staehelin, T., Celma, M. L., and Vazquez, D., *Cold Spring Harbour Symp. Quant. Biol.*, 34, 357 (1969) and references therein.
5 Silverstein, E., *Biochim. Biophys. Acta*, 186, 402 (1969).
6 Zamir, A., Holley, R. W., and Marquisee, M., *J. Biol. Chem.*, 240 1267 (1965).
7 Zachau, H. G., *Angew. Chem. Internat. Edit.*, 8, 711 (1969).
8 Ofengand, J., and Henes, C., *J. Biol. Chem.*, 244, 6241 (1969).
9 Shimizu, N., Hayashi, H., and Miura, K., *J. Biochem.*, 67, 373 (1970).
10 Pestka, S., *Cold Spring Harbour Symp. Quant. Biol.*, 34, 395 (1969).
11 Jordan, B. R., *J. Mol. Biol.*, 55, 423 (1971).
12 Schreier, M. H., and Noll, H., *Proc. U. S. National Acad. Sci.*, 68, 805 (1971).
13 Levitt, M., *Nature*, 224, 759 (1969).
14 Clark, B. F. C., Dube, S. K., and Marcker, K. A., *Nature*, 219, 484 (1968).
15 Philipps, G. S., *Nature*, 223, 374 (1969).
16 Bretscher, M. S., *Nature*, 218, 675 (1968).

** Very recently, it has been shown[20] that ribosomes reconstituted in the absence of the 5S RNA fail to incorporate 4 (50S) proteins and do not possess peptidyl transferase activity.

17 Monro, R. E., *Nature*, 223, 903 (1969).
18 Erbe, R. W., and Leder, P., *Biochem. Biophys. Res. Commun.*, 31, 798 (1968).
19 Shorey, R. L., Ravel, J. M., and Shive, W., *Nature*, 226, 358 (1970).
20 Erdmann, V. A., Fahnestock, S., and Nomura, M., *Fed. Proc.*, 30, 1203 (1971).