Mechanism of peptide-bond formation by ribosome revisited

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A sensation in X-ray crystallography as well as ribosomology was created when the crystallographic analyses of ribosomes were reported by three different laboratories[1–3] in 1999. Microcrystals of 30S and 50S ribosomes were, however, obtained and studied by Yonath and co-workers[4] earlier. Detailed information about 30S ribosomes was reported later by the same group[5]. The group at New Haven headed by Moore and Steitz[6], subsequently reported the atomic structure of the large subunit from *Haloarcula marismortui* at 2.4 Å resolution. The study of the complex containing three RNAs and more than 50 proteins in the two subunits (30S and 50S ribosomes) revealed the positions of practically all the proteins and three-dimensional orientation of RNAs in the protein-synthesizing machinery. It was suspected for a long time that the peptide-bond formation, following the assembly of the amino acids in proper sequence was effected by ribosomal RNAs rather than proteins[7,8]. Therefore, it was encouraging to observe from the crystallographic data that the site of peptide-bond formation in the cavity, formed due to the association of the two subunits where aminoacyl tRNAs are assembled, is composed of RNA alone and no protein was found in the vicinity. The mechanism of peptide-bond formation appeared to be solved by one of the laboratories[9] by studying the crystal structure of 50S ribosomes complexed with Yarus inhibitor[10], which is an analogue (tetrahedral carbon replaced with phosphorus) of tetrahedral intermediate expected to be produced prior to the formation of the peptide bond. A particular base of 23S RNA (A_{2486} of *H. marismortui* which is equivalent to A_{2451} of *E. coli*) was found to be nearest to the tetrahedral carbon (P in this case), and assumed to act as acid/base catalyst as histidine in the serine protease. It was suggested that the peptide-bond formation takes place by a mechanism which is opposite to that of the proteolytic breakdown. However, the problem is the pk_a of adenine (N1 or N3 atom), which is in the acidic range. pk_a of the particular adenine at the catalytic centre was found to be 7.6 by dimethyl sulphate modification[11]. It was assumed that the neutral pk_a was the effect of the nearby base G_{2482} (G_{2447} of *E. coli*), and due to the environment surrounding the adenine base. Details of this mechanism have been presented earlier[12]. Thus the problem of peptide-bond formation finally appeared to be solved after a period of about three decades.

However, soon after the mechanism was proposed it faced criticisms from several laboratories, basically on two grounds. Substitution of the particular adenine base (A_{2451}) with other bases did not drastically affect the peptidyl transferase activity[13,14]. The other point of controversy is the method of determination of the pk_a of adenosine by dimethyl sulphate treatment[11]. The change in pk_a of adenine was observed only in inactive ribosomal preparation[15]. Further, G_{2457} (*E. coli*), a base proposed to be critical for neutral pk_a of adenine was found to be inessential. Another concern was the relevance of understanding the mechanism of peptide-bond formation from the crystal structure[16]. This was refuted by the fact that even 50S ribosomes in crystalline state were capable of peptide-bond formation[17], and the rate measurements are not reliable under any circumstances[18]. It was also argued on the same ground that the base-substitution data[13,14] may also not be reliable. Katunin *et al.*[19], who developed a rapid method of determination of peptide-bond formation, observed that the substitution of A_{2451} with uracil has strong inhibitory effect on peptide-bond formation. However, Moore and Steitz's group has stated that 'the chemical reactivity data that appeared to support the concept that A_{2486} (Ad_{2451}) acts as a general acid/base catalyst do not speak of its role in protein synthesis'[17,18]. This was due to several objections raised by Muth *et al.*[20]. First, A_{2451} in ribosomes from *H. marsimortui* displays an inverted pH profile and second, in yeast C_{2452} rather than A_{2451} is modified in a pH-dependent manner. Third, it appears that the base A_{2451} is not accessible for pH-dependent dimethyl sulphate modification without structural rearrangement. This is also supported by the observation of Bayfield *et al.*[15] which showed that the base is modified in an inactive but not in an active preparation of ribosome. Therefore the group headed by Moore and Steitz[17,18] re-examined the crystal structure of 50S ribosomes substituting the Yarus inhibitor with analogues of aminoacyl tRNA and peptidyl tRNA individually, and superimposed the two structures to derive the most probable structure of 50S ribosomes at the time of peptide-bond formation. There was an indication that the Yarus inhibitor must be distorting the physiological structure at the site of peptide-bond formation. Further, Muth *et al.*[20] hinted at the contribution to overall catalysis of general acid/base and/or conformational catalysis involving a group at the active site. Yonath (pers. commun.) is of the opinion that the conformational change of ribosome takes place prior to the peptide-bond formation and that the change is pH-dependent and may also depend on a protein. It may be mentioned in this connection that a similar suggestion (conformational change) was made as early as in 1985 (ref. 21), which was supported by cryoelectron microscopic studies in Frank's laboratory[22]. This was also observed by various laboratories from their own studies (references cited in ref. 22).

About three years ago, a model was proposed for peptide-bond formation in which 2′ OH group of peptidyl tRNA is involved in forming a six-member cyclic intermediate which spontaneously results in the formation of the peptide bond as shown by quantum chemical calculations (Figure 1)[23,24]. For the cyclic intermediate to be formed, the configuration of carbon of the cyclic intermediate has to be preferably in S-configuration from the direction of the nucleophilic attack and the peptide bond that is formed is expected to be in *trans*-configuration (as shown in Figure 1), as present in the natural peptide. Moore (pers. commun.) indicated that 2′ OH group is unlikely to be involved in peptide-bond formation, but now his group has observed that the crucial adenine base A_{2451} may form a second hydrogen bond with 2′ OH of peptidyl tRNA besides the hydrogen bond with the 2′ OH group of A_{2486} (A_{2451})[17]. In our model, the NH_2 group is expected to form a hydrogen bond with

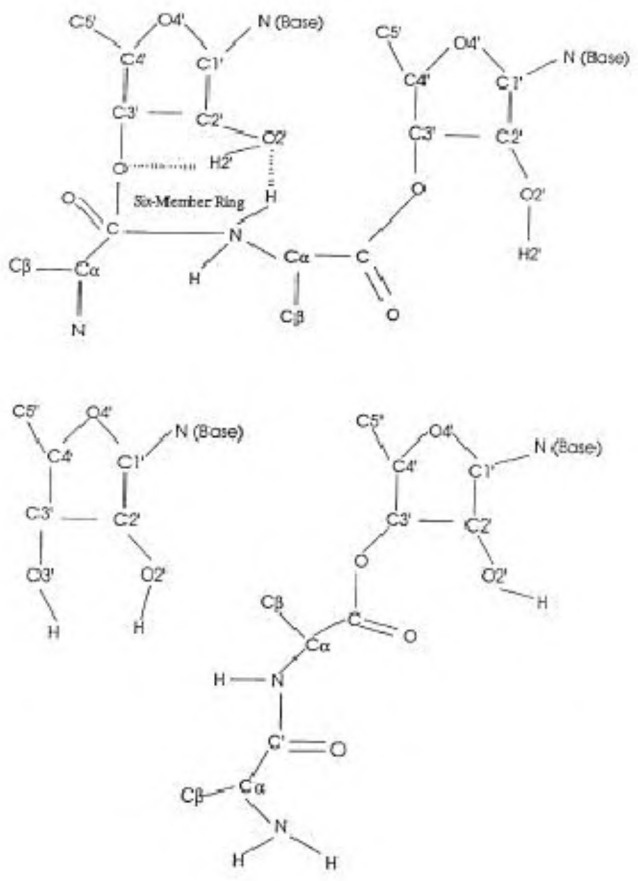

Figure 1.

the 2′ OH group of terminal adenosine of peptidyl tRNA. We argued on the basis of earlier work and quantum chemical data that no additional catalyst is necessary for peptide-bond formation, but peptidyl tRNA itself plays the catalytic role[24]. The strongest evidence in favour of our model is the earlier demonstration in several laboratories[25,26] that the reduction of the 2′ OH group of terminal adenosine of peptidyl tRNA leads to complete loss of peptidyl transferase activity. Thus, the pH-dependent conformational change of 50S ribosomes may be crucial for the final step of peptide-bond formation. X-ray crystallographic data may be of some help in solving the puzzle, and the ribosome as a whole may act as a proper catalyst, simply by properly positioning the activated substrates.

1. Clemens, Jr. W. M., May, J. L. C., Wimberly, B. T., McCutcheon, J. R., Capel, M. S. and Ramakrishan, V., *Nature*, 1999, **400**, 833–840.
2. Ban, N., Nissen, P., Hansen, J., Capel, M., Moore, P. B. and Steitz, T. A., *ibid*, 1999, **400**, 841–847.
3. Cate, J. N., Yusupov, M. M., Yusupova, G. Zh., Earnest, T. N. and Noller, H. F., *Science*, 1999, **285**, 2095–2014.
4. Yonath, A., Saper, M. A. and Wittmann, H. G. in *Structure, Function and Genetics of Ribosomes* (eds Hardesly, H. and Kramer, G.), Springer Verlag, 1985, pp. 112–127.
5. Schluenzen, F. *et al.*, *Cell*, 2000, **102**, 615–623.
6. Ban, N., Nissen, P., Hansen, J., Moore, P. B. and Steitz, T. A., *Science*, 2000, **289**, 905–919.
7. Burma, D. P., Tewari, D. S. and Srivastava, A. K., *Arch. Biochem. Biophys*., 1985, **239**, 427–435.
8. Noller, H. F., Vermita, H. and Zimnak, I., *Science*, 1992, **256**, 1416–1419.
9. Nissen, P., Hansen, J., Ban, N., Moore, P. B. and Steitz, T. A., *ibid*, 2000, 920–930.
10. Welch, M., Chastang, J. and Yarus, M., *Biochemistry*, 1995, **34**, 385–390.
11. Muth, G. W., Ortoleva-Donnelly, L. and Strobel, S. A., *Science*, 2000, **289**, 947–950.
12. Burma, D. P., *Curr. Sci.*, 2001, **80**, 330–331.
13. Polacek, N., Gaynor, M., Yassin, A. and Mankin, A. S., *Nature*, 2001, **411**, 498–501.
14. Thompson, J. *et al.*, *Proc. Natl. Acad. Sci. USA*, 2001, **98**, 9002–9007.
15. Bayfield, M. A., Dahlberg, A. E., Schulmeister, U., Dorner, S. and Barta, A., *ibid*, 2001, **98**, 10096–10101.
16. Yonath, A., *Annu. Rev. Biophys. Biomol. Struct*., 2002, **31**, 257–273.
17. Hansen, J. L., Schmeing, T. M., Moore, P. B. and Steitz, T. A., *Proc. Natl. Acad. Sci. USA*, 2002, **99**, 11670–11675.
18. Schmeing, T. M. *et al.*, *Nature Struct. Biol.*, 2002, **9**, 225–230.
19. Katunin, V. I., Muth, G. W., Strobel, S. A., Wintermeyer, W. and Rodnina, M. V., *Mol. Cell*, 2002, **10**, 339–346.
20. Muth, G. W., Chen, L., Kosek, A. B. and Strobel, S. A., *RNA*, 2001, **7**, 1403–1405.
21. Burma, D. P., Srivastava, S., Srivastava, A. K., Mohanti, S. and Dash, D., see ref. 4, pp. 438–453.
22. Agarwal, R., Lata, K. R. and Frank, J., *Int. J. Biochem. Cell Biol.*, 1999, **31**, 243.
23. Das, G. K., Bhattacharjee, D. and Burma, D. P., *J. Theor. Biol.*, 1999, **200**, 193–205.
24. Bhattacharjee, D., Das, G. K. and Burma, D. P., *Indian J. Biochem. Biophys*., 2001, **38**, 48–52.
25. Quiggle, K., Kumar, G., Ott, T. W., Ryu, E. K. and Chladek, S., *Biochemistry*, 1981, **20**, 3480–3485.
26. Wagner, T. and Sprinzl, M., *ibid*, 1983, **22**, 94–98.

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