

Mechanism of peptide bond formation on ribosomes

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In spite of extensive studies carried out on structure and function of ribosomes during the last four decades or so, the crucial information on the mechanism of peptide bond formation was missing. However, with the very recent elucidation of crystal structures of 30S, 50S and 70S ribosomes¹⁻⁴, it is being conjectured that the solution of the riddle is in sight⁵. The term 'peptidyl transferase' was coined for the elusive enzyme which was expected to be a protein and most likely a ribosomal protein. When no such enzyme could be pin-pointed, the concept that several proteins constitute the peptidyl transferase centre gained ground. With the discovery of ribozymes^{6,7}, the notion started changing and it was thought that ribosomal RNA might catalyse the peptide bond formation. It was reported by Burma *et al.*⁸ as early as in 1985 that the complex of 16S and 23S RNA is capable of catalysing polyphenylalanine synthesis to very small, but significant extent, with the help of a few ribosomal proteins. Seven years later, Noller and his co-workers⁹ demonstrated that ribosome of *tetrahymena* stripped of 80–90% protein by protease digestion retain high biological activity, indicating that ribosomal RNA acts as peptidyl transferase. It was shown recently by Nitta *et al.*¹⁰ that ribosomal RNA without any proteins acts as peptidyl transferase, but this claim was subsequently retracted¹¹.

Experiments like crosslinking, foot printing, etc. carried out in various laboratories indicated the positioning of the three tRNAs at the three sites in the cavity between 30S and 50S ribosomes, where the peptide bond formation takes place. Actually, in the domain V of 23S RNA, the so-called peptidyl transferase centre is located. A and P loops of domain V, responsible for binding amino acyl tRNA and peptidyl tRNA were also clearly identified. This is also confirmed by cryoelectron microscopic studies^{12,13} as well as X-ray diffraction studies³. Cryoelectron microscopic studies first provided direct evidence of conformational changes of 30S and 50S ribosomes which were predicted earlier by Burma *et al.*¹⁴

and later by other laboratories. X-ray diffraction pictures helped to trace the phosphate backbone of all the three RNAs, which could not be done earlier. The known crystal structures of proteins could be fitted easily into the three-dimensional structures of ribosomes. But the most exciting clue has been obtained about the mechanism of peptide bond formation by the collaborative effort of Peter Moore and Thomas Steiz and their co-workers¹⁵. Yarus and their co-workers¹⁶ had designed an interesting inhibitor of peptide bond formation. This was synthesized by coupling 3'-OH of CCdA (terminal bases of tRNA) to the amino group of the *O*-methyl tyrosine residue of puromycin through a phosphate group (Figure 1 *a* and *b*). This is an analogue of tetrahedral intermediate expected to be formed around carbon atom of carboxyl group of amino acid and NH

group of the others. Tetrahedral carbon is replaced with tetrahedral phosphate. CCdA is expected to bind to the P site and puromycin to the A site and thus block protein synthesis. Crystal of large ribosomal subunit from *Haloarcula marismortui* were soaked with the Yarus inhibitor and positions of the two components were determined. Another construct was made by coupling of 3'-OH of C-terminal of minihelix (a suitable substrate for amino acyl tRNA synthetase) to the 5'-OH of the N6 dimethyl moiety of puromycin by a phosphodiester bond (Figure 1 *c*). This was found to bind to A site, as expected. Yarus inhibitor binding sites were also identified as A and P sites. Both analogues are contacted exclusively by ribosomal RNA residue from domain V of 23S RNA. There were no protein side chain residues close to the site of peptide bond formation. The resi-

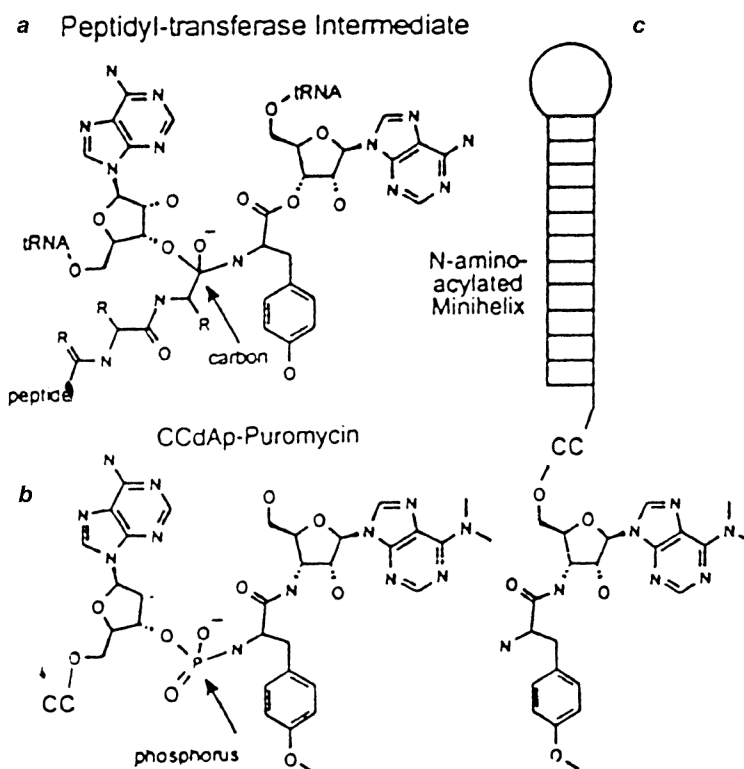


Figure 1. Peptidyl-transferase intermediate and Yarus inhibitor.

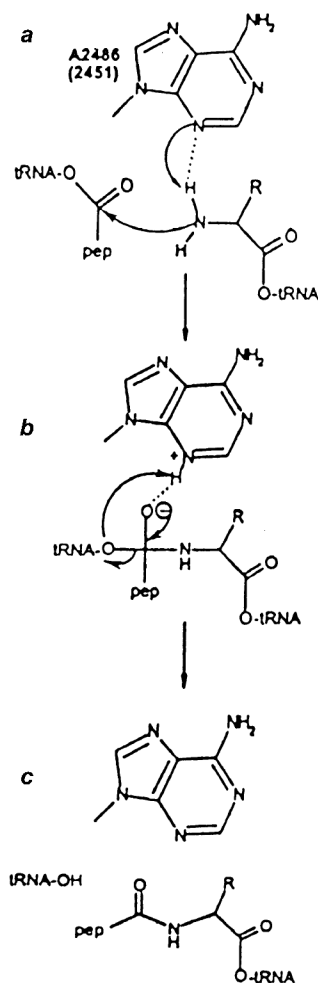


Figure 2. Adenine catalysed peptide bond formation in ribosome.

due of 23S RNA involved in catalysis in the first step, is presumably A2486 (equivalent to A2451 of *E. coli* and located in domain V) whose N3 is about 3 Å from its phosphoramidate oxygen (analogue of tetrahedral carbon of the intermediate). No other base is closer than this. N3 (or N1) has to act as acid-base

catalyst to carry out the desired function. The pK_a of N1 is about 3.5 and that of N3 is two units lower. To act as acid-base catalyst it should be 7 or more. Muth *et al.*¹⁷ measured the pK_a of this particular base in domain V by pH dependence of dimethylsulphate modification and showed that it has pK_a of about 7.6 ± 0.2 . The unusual pK_a may derive in part from the hydrogen bonding to G2482 (G2447 of *E. coli*) which also interacts with a buried phosphate. The mechanism of peptide bond formation as catalysed by adenine (through either N₃ or N₁) is shown in Figure 2. It should be mentioned in this connection that serine proteases act through similar tetrahedral carbon intermediate as in the case of peptide bond hydrolysis. However, histidine (imidazole N) acts as the acid-base catalyst. So the peptide bond hydrolysis (mechanistically) appears to be reversal of peptide bond formation.

Das *et al.*¹⁸ have reported that the tetrahedral intermediate may be converted to six-membered intermediate involving 2'-OH group of peptidyl tRNA which may spontaneously break-down to peptidyl tRNA with the new amino acid and free tRNA. This mechanism cannot be ruled out, although it seems unlikely now on the basis of the mechanism discussed above.

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OPINION

Indian popular science books need popular appeal

Dilip M. Salwi

Popular science books aimed at the young between 6 and 16 are the need of the hour because science and technology is making inroads into their daily life. A society using science and technology in a big way, as is our society now, without making her young aware of science and technology and its effect on society is

heading for suicide. Says the American science writer Seymour Simon, 'If they're (children) not reading books about science by the time they're twelve, you've probably lost them'. In India, writing popular science books for the young has still not caught on among scientists, except in Hindi and some regional lan-

guages like Bengali, Assamese and Marathi. Some science writers have certainly tried their hand at it, both in English and regional languages. But the question is, how good are Indian popular science books *vis-à-vis* those published in the West?

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