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A Model for Protein Synthesis Involving the Intermediate Formation of Peptidyl-5S RNA

(peptidyl transferases)

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ABSTRACT **A model for protein synthesis is proposed in which the donor for the peptide elongation reaction is peptidyl-5S RNA. Space-filling models show that peptide bond formation between peptidyl-5S RNA and aminoacyl-tRNA is eminently feasible from a stereochemical point of view. The peptide is transferred to 5S RNA, while at the same time the deacylated tRNA is exchanged by a new aminoacyl-tRNA acceptor. Two peptidyl transferases are required by the model, both of which have sites for binding the termini of both aminoacyl-tRNA and peptidyl-5S RNA. The model makes detailed predictions about the properties of the transferases.**

In a current model of protein synthesis (1–4), it is assumed that peptide bond formation occurs by transfer of the growing peptide chain from peptidyl-tRNA to an aminoacyl-tRNA. While there is no doubt that the latter is the acceptor in the peptide elongation reaction, and that the growing peptide remains esterified to an RNA throughout the synthesis of the protein, there is in fact no evidence that the donor is a peptidyl-tRNA. There is, however, evidence to the contrary. For example, it is known that ribosomes containing active peptidyl transferase are completely devoid of peptidyl-tRNA hydrolase activity (5, 6), and I have recently found (7) that neither is there peptidyl-tRNA hydrolase activity in the isolated ribosomal proteins, even though the chloramphenicol-binding activity indicative of the presence of ribosomal peptidyl transferase (8) is intact (manuscript submitted to *J. Mol. Biol.*). Since all peptidyl transferases become hydrolases in the absence of their proper acceptor (9), it follows that peptidyl-tRNA is not the substrate for the ribosomal peptidyl transferase.

This, and evidence based on the structures of some antibiotics (ref. 10, and unpublished data) have pointed the way to a different model of protein synthesis, in which a peptidyl-5S RNA is the donor for the elongation reaction. Around this central feature a model of protein synthesis has been constructed that is as consistent with all the known experimental facts as is the translocation model (1, 2). Only the mechanism of the reactions taking place on the 50S subunit will be considered.

Protein synthesis is a cyclic process, necessitating the repeating of certain steps a large number of times. At the beginning of each cycle of reactions leading to the formation of a peptide bond, the ribosome has to return to the same initial configuration. At the conclusion of peptide bond synthesis, the immediate product of the reaction, the peptidyl-tRNA, is in the site of the acceptor of the growing peptide chain (1, 2). In order to reestablish conditions conducive to donating the peptide in the elongation reaction, the growing peptide somehow has to be transferred to a donor position, and a new aminoacyl-tRNA acceptor must take the place of the deacylated tRNA. In the translocation model (1–4), this is done by translocating the entire peptidyl-tRNA from the acceptor to the donor site on the ribosome. As I mentioned above, there is evidence against the peptidyl donor being esterified to a tRNA, and moreover, there are mechanistic problems in translocating a molecule as large as a tRNA esterified to a peptide the size of a protein, especially since this complex is bound to the ribosome, both at the 30S (11, 12) and at the 50S subunit (13–16), and only one enzyme, the G factor (17) is thought to effect this complicated task. It can also be demonstrated by means of Corey-Pauling-Koltun (18) space-filling models that for two amino acids to form a peptide bond, their amino groups have to face in opposite directions. Hence, the RNAs to which the amino acids (or peptides) are esterified, have to approach in an antiparallel way. While the reading of the codons requires tRNAs on the ribosome to be parallel to each other, there is an easy way to accomplish the required antiparallel juxtaposition if the acceptor aminoacyl-tRNA were to approach a donor RNA built into the ribosome in the required way.

I have previously pointed out (19) that 5S RNA can assume a cloverleaf conformation similar to that of tRNA. Particularly, both have "stems" 11 nucleotides long, forming one complete turn of an RNA helix (20), which can be bound together by Mg^{++} bridges, causing the 3′-OH groups to lie close together. I therefore propose that the donor in the peptide elongation reaction is a peptidyl-5S RNA.

Models show that when the peptide is on the tRNA, at the end of the previous elongation step, the 3′-OH of 5S RNA is in a stereochemically perfect position for executing a nucleophilic attack on the carbonyl of the peptide, thereby effecting its transfer from tRNA to 5S RNA. This can be seen by the space filling models in Fig. 1. On the other hand, when the peptide is on the 5S RNA, the amino group of the acceptor aminoacyl-tRNA, in turn, is in a stereochemically perfect position for a nucleophilic attack on the carbonyl of the peptide, causing its transfer to the aminoacyl-tRNA, and synthesis of a new peptide bond. This can be seen by the models of Fig. 2. The chemical representation of the two transfer reactions is shown in Fig. 3. The space-filling models also show that in the transfer back and forth between the tRNA and 5S RNA, the peptide chain suffers no lateral translocation at all, only a vertical extrusion motion, as amino acids are inserted at the bottom.

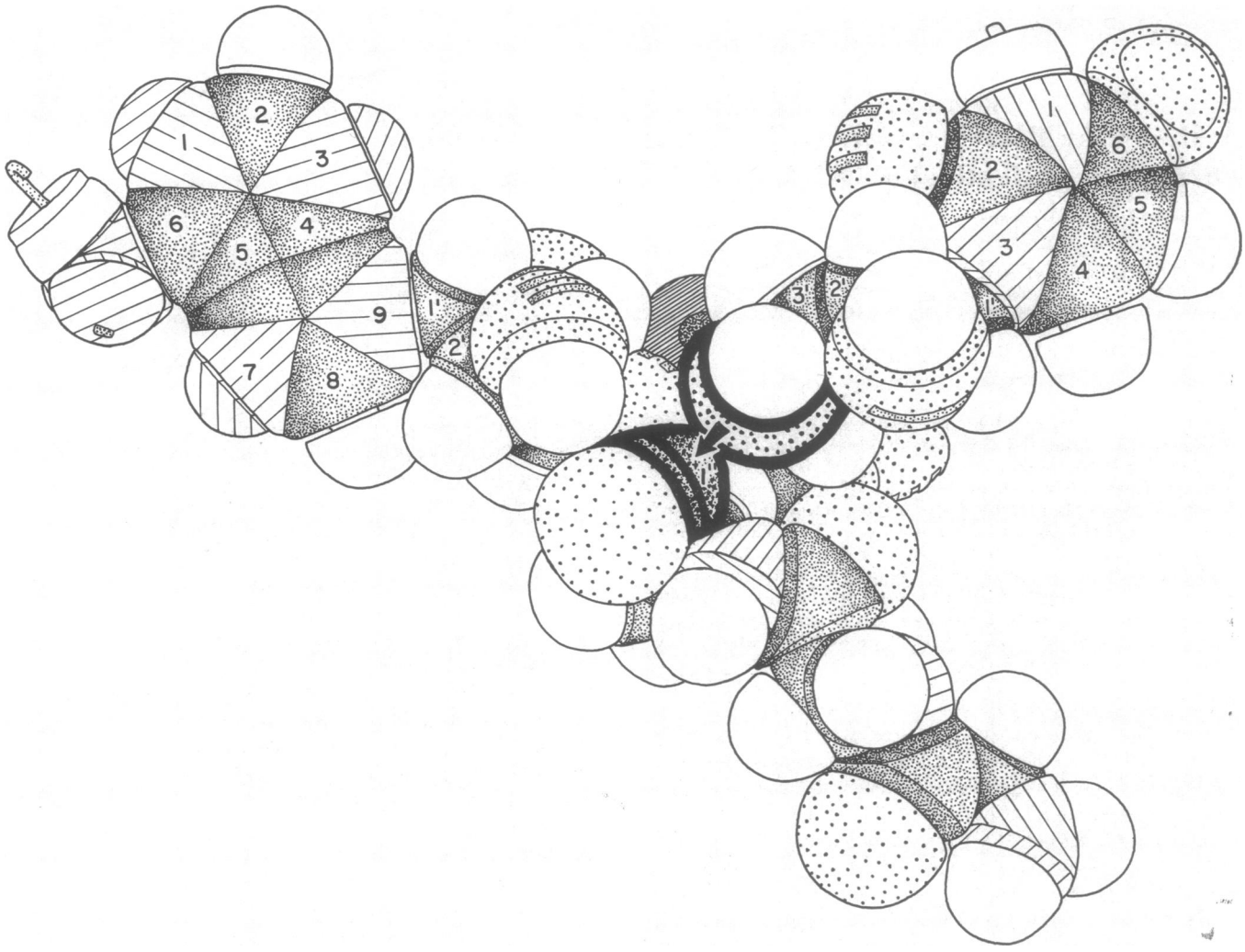

FIG. 1. Representation by means of Corey-Pauling-Koltun space-filling models of the synthesis of peptidyl-5S RNA. Reaction between Gly-Gly-Ala-tRNA and 5S RNA to yield Gly-Gly-Ala-Ala-5S RNA. 5S RNA and tRNA are represented by their 3'-alanyl termini, uridylic and adenylic acids, respectively. The backbones of the two RNAs are joined by a Mg^{++} atom (the *closely hatched* atom in the drawing). The view is perpendicular to the axes of the molecules. The peptide should be perpendicular to the paper, but was distorted so it could appear more clearly. The *arrow* shows the nucleophilic attack of the hydroxyl group on the carbonyl carbon of the peptide. The two atoms involved in the reaction are *heavily outlined*. The *white* atoms are H; the *finely stippled ones*, C; the *coarsely stippled* ones, double-bonded O, *coarsely stippled with slits*, single-bonded O; the *slatted ones*, N. The numbers correspond to the numbers in the chemical formulae in Fig. 3 and are meant only to serve as a guide.

The model also provides for the growing peptide chain to remain firmly bound to the ribosome at all times. Furthermore, since the 5S RNA is buried within the ribosome (21), it must be under the tRNA, presumably bound on the surface of the ribosome. Consequently, since the protein is formed between the two RNAs, this process must also take place inside the ribosome, presumably in a groove. Newly formed peptide bonds would therefore be protected, as shown experimentally by Malkin and Rich (22).

Both peptidyl transfer reactions are well known reactions and do not require energy. The peptide transferase responsible for transferring the peptide from tRNA to 5S RNA will be referred to as transferase II. Its active site recognizes the terminus of peptidyl-tRNA, and it should also bind the terminus of 5S RNA, i.e., uridylate, in order to keep the acceptor group in the proper configuration. The peptide-bond forming enzyme will be referred to as transferase I, and its active site recognizes peptidyl-uridylate, whereas its secondary site binds the terminus of aminoacyl-tRNA. Since the reactants are antiparallel, there is a transferase on each site of the tRNA–Mg–5S RNA complex.

The outstanding characteristics of the peptidyl transferases needed by the 5S RNA model are thus that each recognizes the termini of both tRNA and 5S RNA. Consequently, they should also bind antibiotics that are analogues of these termini. Two such antibiotics are puromycin, the well-known analogue of the terminus of aminoacyl-tRNA (23), and chloramphenicol, recently shown by model studies to be an analogue of peptidyl-uridine (unpublished models). Two such proteins, one ribosomal and one soluble, capable of binding both puromycin and chloramphenicol, have in fact been found in this laboratory (ref. 6, 7, and manuscripts submitted to *J. Mol. Biol.*). Furthermore, the binding of chloramphenicol to the ribosomal protein mimics some properties of peptide-bond synthesis, in that it requires K^+ or NH_4^+, as does the latter (24–26), whereas the binding of puromycin to this protein does not

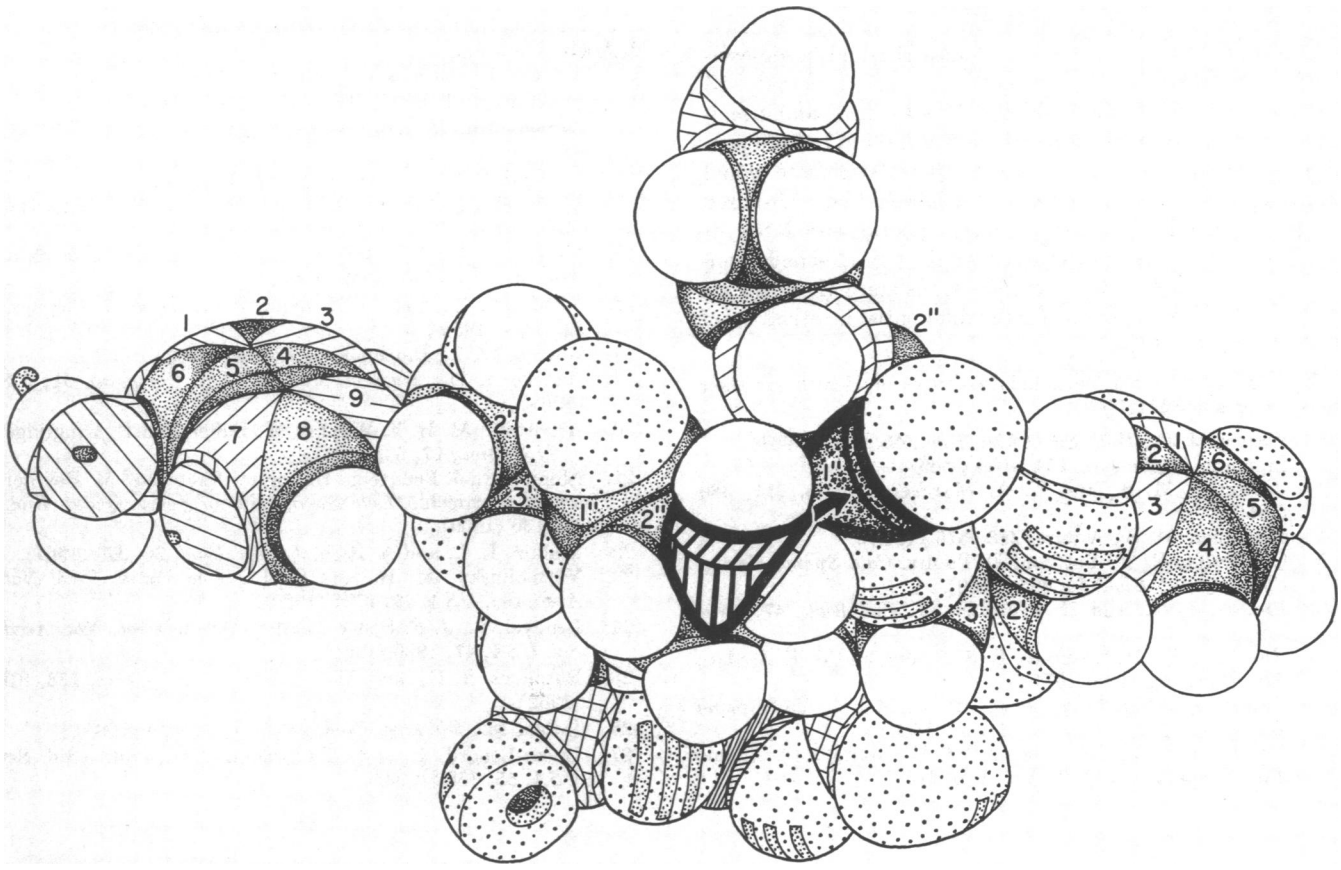

FIG. 2. Representation by means of space-filling models of the formation of a peptide bond. Reaction between glycyl-glycyl-5S RNA and alanyl-tRNA to yield glycyl-glycyl-alanyl-tRNA. The view is along the perpendicular axes of the complex. The *arrow* shows a nucleophilic attack by the amino group of aminoacyl-tRNA on the carbonyl carbon of the growing peptide chain on 5S RNA. The atoms involved in the reaction are heavily outlined. The representation of the atoms is as in Fig. 1, except that the cross-hatched atom is P.

have such requirements. On the other hand, with the soluble protein, it is the binding of puromycin rather than chloramphenicol that mimics properties of protein synthesis, since it requires Mg^{++} and is inhibited by Na^{+} and by Li^{+}, as is protein synthesis (26). Finally, the soluble protein has peptidyl-tRNA hydrolase activity, whereas the ribosomal protein does not (7), exactly as required by the model for transferases II and I, respectively. The isolation of two proteins possessing the rather unusual properties predicted by the model is strong evidence for its correctness. Finally, it was found that protein synthesis is inhibited by uridine, but not by adenosine, also as predicted by the model (unpublished experiments).

For protein synthesis to proceed properly, it is necessary, in addition to the formation of peptide bonds and the cyclical reconstitution of ribosome configuration to have a number of features susceptible to regulation. For example, it is necessary to prevent the transfer of the growing peptide chain to 5S RNA, unless the acceptor aminoacyl-tRNA is also immediately available; due to the hydrolase activity of the transferases in the absence of their proper acceptor (9), there might otherwise be premature chain termination by the peptide–bond forming enzyme. This regulation might be achieved by having allosteric coupling between transferase II and the aminoacyl-tRNA translocase [assumed to be T_u (27) by the present model, the G factor (17) functioning as a translocase for free tRNA] such that transferase II cannot act unless the T_u is acting at the same time. Under natural conditions, then, the peptide would always come to rest on the tRNA. It might,

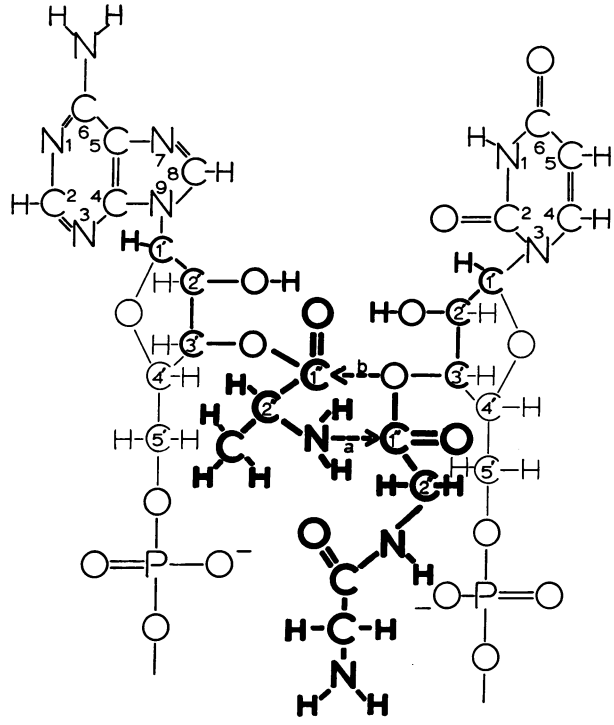

FIG. 3. Chemical representation of the reactions shown in Fig. 1 (*arrow b*) and Fig. 2 (*arrow a*). The numbers serve as guides to the space-filling models.

therefore, be very difficult to isolate the peptidyl-5S RNA intermediate, for even if the transferase II could be desensitized to the action of the translocase, so that a peptidyl-5S RNA could be synthesized without there being an aminoacyl-tRNA in the acceptor site to attack the carbonyl of the peptide and to form a peptidyl-tRNA, the intermediate would still have a very limited life time, for it would be hydrolyzed by transferase I. In order to obtain peptidyl-5S RNA, it would thus be necessary to inactivate the peptide-bond forming enzyme as well as to desensitize transferase II. There are no known agents at present for the differential inactivation of the two transferases.

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