'CLOVERLEAF' CONFORMATION FOR 5S RNAs

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The most attractive feature of the now generally accepted "cloverleaf" secondary structure for tRNAs lies in the fact that it clearly delineates potential functional sites (in the various "loops") and permits the study of functional homologies between the various species of tRNA molecules. The purpose of the present communication is to propose "cloverleaf" structures for the two 5S ribosomal RNAs of known nucleotide sequence, from E. coli and from mammalian KB cells, which point out structural homologies between these two RNAs and suggest possible functional sites on the molecule.

The primary nucleotide sequence for E. coli 5S ribosomal RNA was worked out by Brownlee, Sanger and Barrell (1967) who at the same time suggested a rather open ringlike secondary structure for this molecule, containing three short helical "stems". It has been pointed out by Cantor (1967) and by Boedtker and Kelling (1967) that structures can be constructed having a much larger number of base pairs than originally suggested by Brownlee et al. In the structure presented by Cantor 80% of the bases were paired, whereas in Boedtker's structure the figure was 62%, commensurate with her various measurements which indicated a minimum base pairing content of 60%.

The primary structure of 5S RNA from mammalian KB cell ribosomes was worked out by Forget and Weissman (1967) and although the authors suggested that there was extensive opportunity for base pairing, no secondary structure has yet been published.

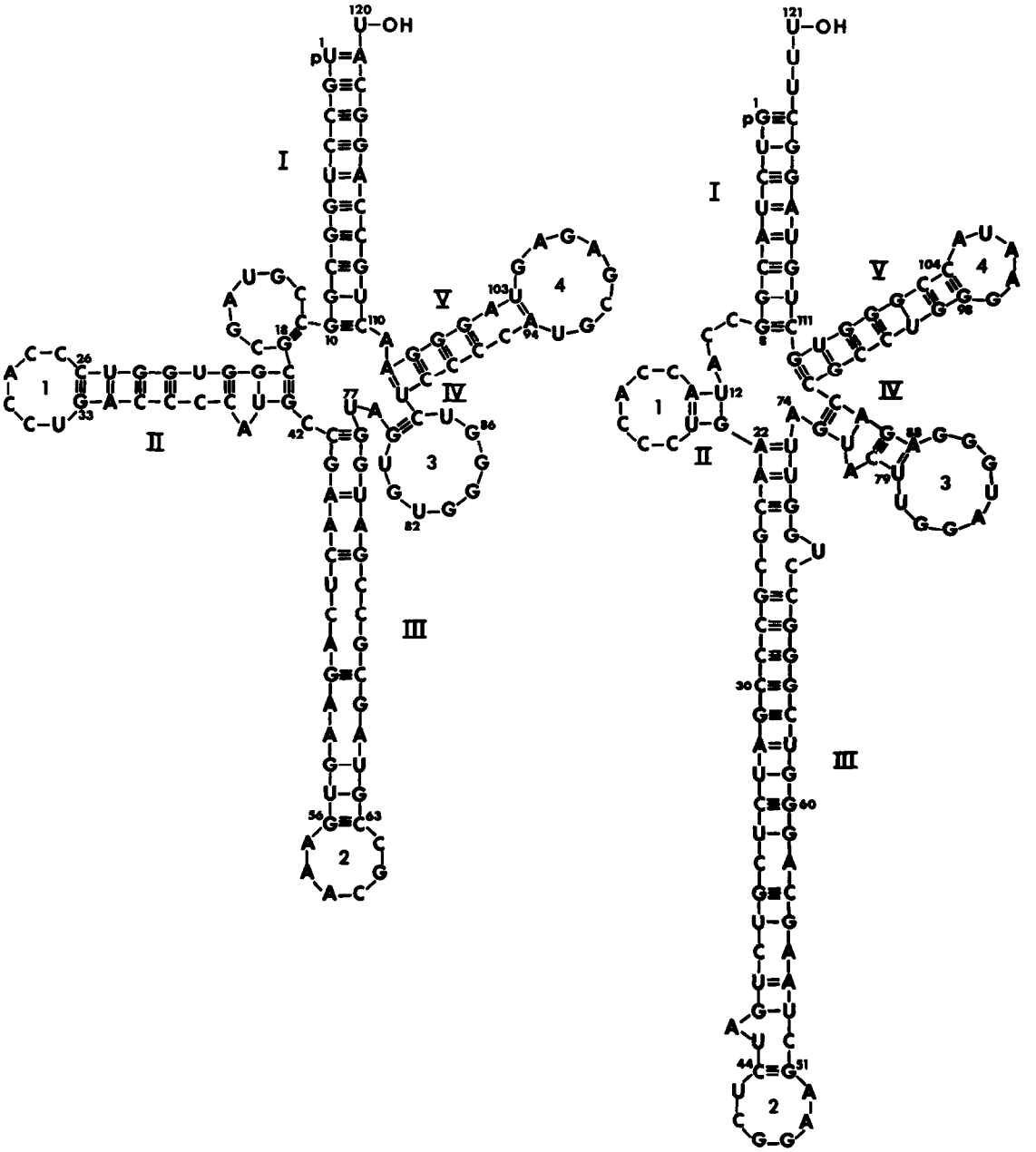

Fig. 1. Hypothetical secondary configurations for the 5S RNAs of E. coli and KB cells.

The secondary structures I wish to propose for E. coli and KB 5S RNA are shown in Fig. 1. These structures have the following features:

1. There are 32 base pairs (53%) in E. coli 5S RNA, and an additional 18 bases (not counting looped-out bases) are in helical regions, but not hydrogen-bonded. The total helical content is thus 68%. These figures agree well with the amount of helicity determined by optical and other methods by Boedtker and Kelling (1967) and by Cantor (1968). In the KB 5S RNA model there are 37 base pairs (61%) and 12 additional bases in helical regions (70%).

2. The stem of the cloverleaf is 11 nucleotides long, the same as in all tRNAs known so far (Holley et al, 1965; Madison, Everett and Kung, 1966; Zachau et al, 1966; Rajbhandary et al, 1967). This most likely corresponds to one complete turn of the helix in RNA (Arnott, Wilkins, Fuller and Langridge, 1967).

3. Loop 1 in both structures contains the unique sequence -C-C-A-C-C-C(U)-, not found anywhere else in the 5S RNAs, nor in any of the known tRNAs. This is especially remarkable, since there is very considerable duplication of nucleotide sequences in the two 5S RNAs (Brownlee et al, 1967; Raacke, unpublished). Furthermore, 5S RNA from yeast also has a T_1-nucleotide consistent with the sequence U-A-C-C-A-C-C-U-U-G (Hindley, 1967).

4. The deployment of the arms and loops is exactly the same as in the known tRNAs. (In the structure proposed by Boedtker and Kelling (1967) the arrangement of the arms is reversed). By building space-filling models it can be shown that these arms are very flexible, and can be folded in various configurations. This was also discussed for the cloverleaf models of tRNA by Lake and Beeman (1968), who by X-ray scattering studies have determined that the arms of the tRNA cannot be splayed out, but must be folded either two up and two down, or three one way and one another. Therefore in 5S RNA the arms are probably also folded, although Boedtker and Kelling's (1967) hydrodynamic studies indicated that 5S RNA was more asymmetric than tRNA.

5. Loop 2 is 6 nucleotides long in both structures, and rich in purines, but otherwise species-specific.

6. Loop 3 is 8 nucleotides long in both structures and contains the common sequence -U-G-G-G-. In E. coli 5S RNA this is a unique sequence not found

anywhere else in the molecule, although in KB 5S RNA there is a second -U-G-G-G- sequence in arm V, but this is hydrogen-bonded. There are no -U-G-G-G- sequences as such in the known tRNAs, although yeast $tRNA_{ala}$ has a -PSU-G-G-G- sequence (Holley et al, 1965), yeast $tRNA_{ser}$, -OMU-G-G-G- (Zachau, et al, 1966) and yeast $tRNA_{phe}$, -DiHU-G-G-G- (Rajbhandary et al, 1967).

7. Loop 4 is also species-specific, as it is very different in the two kinds of 5S RNA.

The most obvious functional implication of the present models is that loop 1 is the site for binding the 5S RNA to the 50S ribosomes, since this is a property presumably shared by all 5S RNAs (Rosset, Monier and Julien, 1964; Comb and Sarkar, 1967; Knight and Darnell, 1967). If this is correct, it would mean that 5S RNA from any source can bind to any kind of 50S ribosome.

Since the anticodon loop of tRNAs (loop 2) interacts with mRNA on the 30S ribosome, it is tempting to speculate that arm III and loop 2 of 5S RNA also extend from the 50S ribosome to the 30S, and that this part of the molecule functions in holding 30S and 50S sub-units together. A rough calculation of the lengths of the tRNA and 5S RNA molecules in the present model, based on the data of Arnott et al (1967) on helical RNA, indicates that the length of tRNA is about 75 Å, that of E. coli 5S RNA about 105 Å, and that of KB 5S RNA about 140 Å. Assuming the 3'OH terminus to lie at about the equator of the 50S ribosome, the anticodon of tRNA would just reach the 30S sub-unit, and presumably the mRNA bound to it. Because of the exact equivalence of the stems of the cloverleaves in tRNA and 5S RNA it is tempting to speculate that a tRNA and a 5S RNA lie side by side on the ribosome. In this case the 5S RNA in E. coli would extend 30 Å beyond the tRNA and it would therefore presumably cross the messenger. Interaction could be avoided, however, if the 5S RNA would lie in a groove in the 50S and extend into, rather than onto the 30S particle. It is thought that one of the functions of 5S RNA is actually to hold the two ribosomal sub-units together (Aubert, Monier, Reynier and Scott, (1968), but this point has not yet been proven specifically.

Since loop 2 is different in the two 5S RNAs, it would be expected that the interaction of 30S and 50S ribosomes is species-specific. While I have not been able to find any data on this particular point, it is a reasonable assumption.

By means of models, it is possible to show that a tRNA molecule can lie in close apposition to a 5S RNA, with Mg^{++} bridges between phosphates of arms I and III of the molecules. Furthermore, if the 5S RNA is rotated by 180°, so that the 3' ends of the two molecules are juxtaposed, arms II, IV and V of both molecules can be folded up in such a way that the common sequence -U-G-G-G- in loop 3 of 5S RNA is in a position to pair in an anti-parallel manner with the sequence -rT-PSU-C-G- found in loop 4 of all tRNAs.

Several investigators (Gilbert, 1963; Cannon, Krug and Gilbert, 1963; Kurland, 1966) have described a non-enzymatic binding site for tRNA on the 50S ribosome. There is some disagreement on whether or not tRNA can bind to 50S subunits alone. The present model suggests that it is the 5S RNA which is bound to the 50S ribosome, and that it, in turn, binds the tRNA through specific base pairing and Mg^{++} bridges. If the 50S ribosomes are prepared by a method which conserves the 5S RNA, then binding of tRNA to isolated 50S particles should be observed; whereas if the isolation of the 50S resulted in loss of 5S RNA, no such binding should be observed. This aspect is amenable to experimental verification.

The sequences -C-G-A-A- or -U-G-A-A- and -A-A-G-C- or -A-A-G-U- could also bind the common tRNA sequence in an anti-parallel fashion (depending on whether the tRNA and 5S RNA molecules are apposed in a parallel or anti-parallel configuration), and they would provide stronger hydrogen binding than the proposed sequence -U-G-G-G-. There is a -C-G-A-A- (residues 43-46) sequence in E. coli, but not in KB 5S RNA; whereas both E. coli (residues 55-58) and KB (residues 20-23) have a -U-G-A-A- sequence. Furthermore, there are two -A-A-G-C- (residues 54-57 and 48-51) sequences in KB 5S, whereas in E. coli there is one -A-A-G-U- (residues 52-55) sequence. All of these sequences, however, are in hydrogen-bonded regions. Moreover, I have not been able to construct a structure in which any of these sequences would appear in anatomically cognate parts

in the two types of 5S RNA molecules.

In summary, the proposed secondary structures specifically suggest three experimentally testable functions for 5S RNA, namely: 1. the binding of 5S RNAs to 50S ribosomes by means of a unique and universal base sequence, -C-C-A-C-C-C(U)-; 2. the joining of 30S to 50S ribosomes in a species-specific manner; and 3. the binding of tRNA by Mg^{++} bridges between the phosphates in the backbones of the helical regions of the two molecules and by anti-parallel base pairing between the common sequences -U-G-G-G- and -rT-PSU-C-G-. The verification of these structures and their common features can only come from comparative data and must await the elucidation of nucleotide sequences of additional 5S RNAs.

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REFERENCES

1. Arnott, S., Wilkins, M.H.F., Fuller, W., and Langridge, R., J. Mol. Biol. 27, 535 (1967).
2. Aubert, M., Monier, R., Reynier, M., and Scott, J.F., personal communication (1968).
3. Boedtker, H., and Kelling, D.G., Biochem. Biophys. Res. Comm. 29, 758 (1967).
4. Brownlee, G.G., Sanger, F., and Barrell, B.G., Nature 215, 735 (1967).
5. Cannon, M., Krug, R., and Gilbert, W., J. Mol. Biol. 7, 360 (1963).
6. Cantor, C.R., Nature 216, 513 (1967).
7. Cantor, C.R., Proc. Natl. Acad. Sci. U.S. 59, 478 (1968).
8. Comb, D.G., and Sarkar, N., J. Mol. Biol. 25, 317 (1967).
9. Forget, B.G., and Weissman, S.M., Science 158, 1695 (1967).
10. Gilbert, W., J. Mol. Biol. 6, 389 (1963).
11. Hindley, J., J. Mol. Biol. 30, 125 (1967).
12. Holley, R.W., Apgar, J., Everett, G.A., Madison, J.T., Marquisee, M., Merrill, S.H., Penswick, J.R., and Zamir, A., Science 147, 1462 (1965).
13. Knight, E., Jr., and Darnell, J.E., J. Mol. Biol. 28, 491 (1967).
14. Kurland, C.G., J. Mol. Biol. 18, 90 (1966).
15. Lake, J.A., and Beeman, W.W., J. Mol. Biol. 31, 115 (1968).
16. Madison, J.T., Everett, G.A., and Kung, H., Science 153, 531 (1966).
17. Rajbhandary, U.L., Chang, S.H., Stuart, A., Faulkner, R.D., Hoskinson, R. M., and Khorana, H.G., Proc. Natl. Acad. Sci. U.S. 57, 751 (1967).
18. Rosset, R., Monier, R., and Julien, J., Bull. Soc. Chim. Biol. 46, 87 (1964).
19. Zachau, H.G., Dütting, D., Feldmann, H., Melchers, F., and Karau, W., Cold Spring Harbor Symposia 31, 417 (1966).