A MODEL FOR THE TERTIARY STRUCTURE OF tRNA

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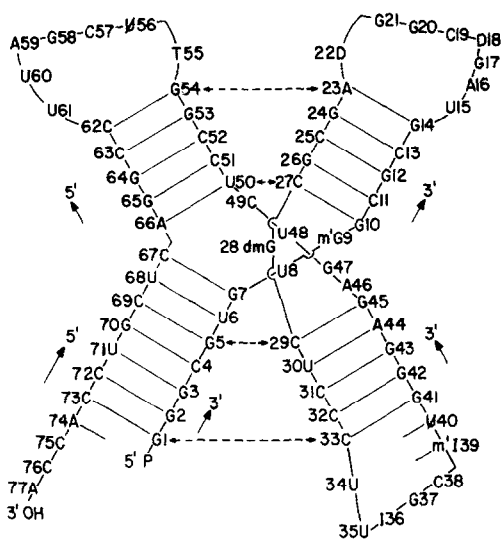
SUMMARY: A model for tRNA is described which has as its basic structural mode a four stranded RNA helix, formed by pairing two stems respectively from the four ubiquitous arms of the clover-leaf secondary structure. The relation of the model to biochemical and crystallographic data on tRNA is considered.

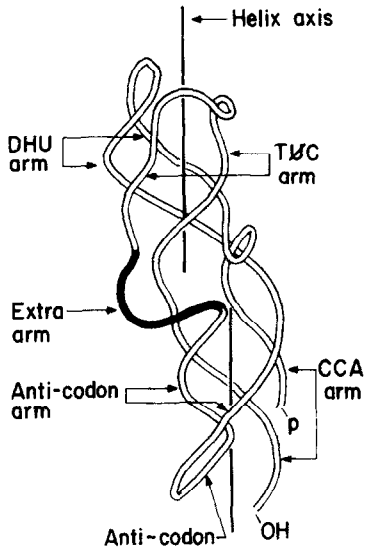
A knowledge of tertiary structure of tRNA is of great importance in molecular biology, and much effort has been put into its elucidation over the last decade (1,2) by various chemical, enzymatic, physical-chemical, X-ray diffraction and model building investigations (1-25). In this report, we describe a new model for the tertiary structure of tRNA which was conceived in the course of attempting to correlate currently available chemical, genetic (1-14) and X-ray crystallographic data (15-19).

The model differs substantially from previous ones (20-25) in its basic structural mode, which is a four-stranded RNA helix (26), formed by pairing two stems respectively from the four ubiquitous arms of the clover-leaf secondary structure. A Kendrew wire model (27), a space-filling CPK model (28), and a computer generated model (29) have been constructed for the sequence of Baker's yeast $tRNA^{Ala}_{1a}$ (30).

Figure 1-a shows the clover-leaf secondary structure in which close base-base interactions in the tertiary structural model are indicated by the dotted lines (residue numbers given in the legend). Figure 1-b shows the back-bone arrangement. This model utilizes hydrogen bonding by the exocyclic hydrogens of the base-paired residues in the wide groove of the helices of the clover-leaf. In this manner two four-stranded helical segments are formed, running in the same direction, but with their axes displaced by 7.5 Å.



a



b

Figure 1-a. Sequence of yeast $tRNA^{Ala}_{1a}$ folded into clover-leaf secondary structure. Close base-base tertiary structural interactions involve residues: 74-40, 73-41, 2-32, 72-42, 3-31, 71-43, 4-30, 70-44, 69-45, 66-10, 51-26, 65-11, 62-14, 61-16, 60-17, 58-19, 57-20, 56-21. Note that the conventional format for the presentation of the clover-leaf has not been used. Rather the adjacency of arms has been changed to enable a spatial arrangement that displays the wide grooves on the surface presented to the viewer. The model is folded in three dimensions by the following operations. First the DHU arm is raised out of the plane of the paper and then is rotated 180° so that the narrow groove faces up. Then, the anticodon arm is similarly rotated to bring its narrow groove facing up. By these motions these wide grooves can be brought into interaction with the wide groove side of the adjacent TØC and CCA stems. Some abbreviations are: T, ribothymidine; Ø, pseudouridine; I, inosine; $\bar{1}$mG, 1-methylguanosine, $\bar{1}$mI, 1-methylinosine; dmG, N^2-dimethylguanosine.

Figure 1-b. Schematic presentation of the backbone of the model.

One of the standard pairing schemes in such a four-stranded helix is shown in Figure 2-a (31-33). It should be noted that a full complement of hydrogen bonds between all the base pairs of the double helices to form base tetrads of the kind shown in Figure 2-a is not a requirement of the model; but a compatible steric arrangement with equivalent Van der Waal's contacts for such base tetrads without hydrogen bonds is a requirement. This four-stranded helical arrangement not only brings together the non-helical loops and terminus so that they can form acceptable interactions, but also keeps the molecule very compact (34,35). This arrangement also provides

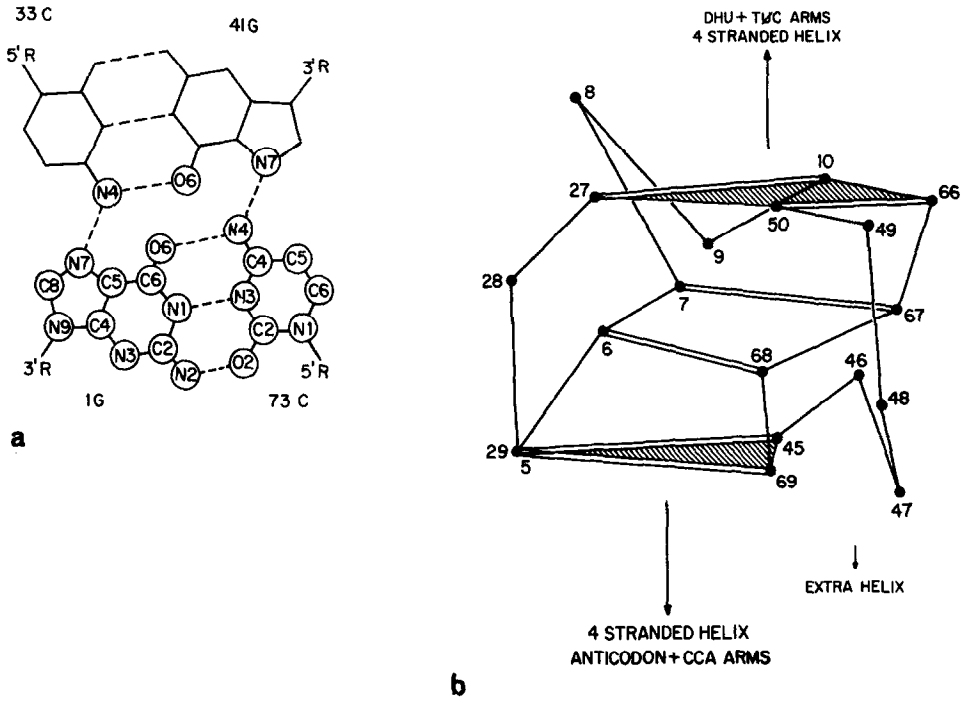


Figure 2-a. Base pairing scheme of four stranded helix. The arrangement of the antiparallel arms is such that all four chains are antiparallel. Wide grooves are completely removed from solvent by this H-bonding scheme, which ought to provide extra stability to the Watson-Crick base pairs.

Figure 2-b. A schematic presentation of the folding of the central part of the model. The 1-carbon of each residue is shown by a dot. Double lines indicate Watson-Crick base pairs. A shaded area represents a base tetrad plane (similar to that in Figure 2-a). The 1-C of residue 29 is under that of residue 5. Note that this central region is less dense than the helical regions.

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for more even distribution of the mass in the molecule, which seems desirable in view of the packing requirements (36) of the molecule into known unit cells of tRNA crystals (18,37-39). The molecule has dimensions of 24 x 32 x 80 Å. However, due to the high degree of hydration of tRNA in aqueous solution (about 0.8 g of water per g of tRNA (40)), the apparent dimensions including the solvent shells could be about 40 x 90 Å.

The TψC and DHU stems are associated at one end and the CCA and the anticodon stems at the other. Interactions between the DHU and the TψC loops result in mutual shielding (3,4,6,7,9,41,42); the same is partially true for

the CCA end and the anticodon loop (3,4,6,7,9,14,41-46). Thus, the molecule has no exposed region except for the anticodon and the CCA terminal. In considering alternative schemes for pairing of the helical segments of the clover-leaf, account has been taken of the report that in denatured (but highly structured) $tRNA^{Leu}_3$ from yeast, the anticodon and TΨC stems may be linked under conditions where effective charge neutralization is not achieved. In this case, it was suggested that upon appropriate site binding of cations, a stabilized DHU helix interacts with the TΨC helix to give rise to the biologically active conformation (47).

The central connecting region between the two helical sections enables the polynucleotide chains to complete the necessary folding. A schematic diagram of this section is shown in Figure 2-b. This scheme appears to be universally applicable to all tRNA sequences since in this region they differ significantly only in the region of the "extra arm", which plays a minor role (7,42,48). The extra helix can be accommodated close to the anticodon arm without significantly modifying the gross molecular structure (see Figure 1-b and 2-b) (49).

To maximize the number of non-clover-leaf hydrogen bonds throughout the molecule, various non-Watson-Crick base-pairing arrangements (50), previously observed from single crystal structure analysis (51), have been utilized. Thus, the molecule contains twenty-four more hydrogen bonds than in the clover-leaf structure (52,53). Bases are also continuously stacked through the central folding region. The roles of unusual bases and the variable length of the extra arms have been examined also in connection with the stability of tRNA tertiary structure and its resistance to nuclease attack (54-64).

All the dihedral angles are close to the standard values (65-67). There are six "syn" conformations(residues 8,16,17,28,40 and 69) (64,68). For the non-helical regions bond angles and bond lengths have been calculated to be close to the standard values (65) from the measured coordinates of the Kendrew wire model (average bond length = 1.5 Å, S.D. = 0.14 Å, average bond

angle = 111.0°, S.D. = 9.0°), and non-bonded interatomic distances have been also checked with those coordinates (average of distances shorter than 3.3 Å = 2.8 Å) (69) and at the level allowed by the space-filling CPK models. For helical regions, all of these parameters have standard values. These calculations indicate that the model is stereochemically sound. Close phosphate-phosphate distances less than 6.0 Å located in the central folding region, are following: Residues 5-6 (5.77 Å), 7-8 (5.99), 7-28 (5.95), 8-9 (4.60), 8-28 (4.42), 45-46 (5.86), 48-68 (5.26), 49-68 (5.40), 68-69 (5.21). Twenty to thirty weak divalent cation binding sites (or double that many monovalent ones) (70) are required in the four-stranded region of the helix (the closest interchain P-P distance is 5.33 Å) in addition to a few strong cation binding sites in the central region (70).

Synthetase recognition in this model could involve one whole surface of the molecule, the backside of the model in Figure 1-b which consists largely of the CCA and DHU arm (71-74). Since the gross structure would be similar for all sequences, primary structure on the surface involved could provide the main source of recognition. This model would not require gross change for aminoacyl tRNA (75). In this model, the recognition of codon message and peptide formation are made in one end of the molecule while at the other end the role of DHU and TΨC loops is suggested in the involvement of peptidyl site binding (9,76-78). The model is consistent with photo-dimerization (the distance between C13 and U8 is 4-5 Å) (79) and fluorescence data (80,81). The molecule can "breathe", and even assume larger dimensions in response to changing ionic environment (53,82), by merely opening the two ends of the structure. In such cases, the central region would be quite slim.

The placement of the CCA end near the anticodon loop does not preclude a reasonable distance on the ribosome between an amino acid residue on one tRNA and a peptidyl group on the adjacent tRNA (83). This arrangement of CCA end and anticodon loop makes possible an anticodon conformation that suggests a new mechanism of wobble base-pairing (84). This novel arrangement

consists of one single stranded helix (residues 36,37 and 38) with its axis perpendicular to the molecular axis and two bends at residues 35-36 and 38-39 (see Figure 1-a and 1-b). Owing to disruption of the helical conformations, and the flexibility of the polynucleotide chain, residues 36 and 38 but not 37 can wobble simply by adopting a variety of allowed ribose and backbone conformations.

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REFERENCES:

1. D. H. Gauss, F. von der Haar, A. Maelicke, and F. Cramer, Annual Review of Biochem., Vol. 40, 1045 (1971) and references therein.
2. S. Arnott, Progr. in Biophys. and Mol. Biol. Vol. 22, 179 (1971) and references therein.
3. S. W. Brostoff and V. M. Ingram, Science, 158, 666 (1966)
4. F. Cramer, H. Doepner, F. V. D. Haar, E. Schlinme, and H. Seidel, Proc. Natl. Acad. Sci., 61, 1384 (1968)
5. R. Thiebe & H. G. Zachau, Eur. J. of Biochem. 5, 546 (1968)
6. M. Yoshida, Y. Kaziro & T. Ukita, Biochim. et Biophys. Acta, 166, 646 (1968)
7. A. R. Cashmore, D. M. Brown & J. D. Smith, J. Mol. Biol., 59, 359 (1971)
8. N. K. Kochetkov & E. I. Budowsky, Progr. Nucl. Acid Res. Mol. Biol., Vol. 9, 403 (1969)
9. T. Seno, M. Kobayashi & S. Nishmura, Biochim, et Biophys, Acta, 190, 285 (1969)
10. A. D. Mirzabekov, D. Lastity, E. S. Levina & A. A. Bayev, Nature, 229, 21 (1971)
11. B. F. C. Clark, S. K. Dube & K. A. Marker, Nature, 219, 484 (1968)
12. J. A. Nelson, S. C. Ristow & R. W. Holley, Biochim. et Biophys. Acta, 149, 590 (1970)
13. O. C. Uhlenbeck, J. Baller & P. Doty, Nature, 225, 508, (1970)
14. J. Eisinger, B. Feuer & T. Yamane, Nature New Biology, 231, 126 (1971)
15. R. D. Blake, J. R. Fresco & R. Langridge, Nature, 225, 32 (1970)
16. S. H. Kim, G. Quigley, F. L. Suddath & A. Rich, Proc. Natl. Acad. Scie., 68, 841 (1971)
17. T. Sakurai, S. T. Rao, J. Rubin, M. Sundaralingam, Science 172, 1234 (1971)
18. S. H. Kim,G. Quigley, F. L. Suddath, A. McPherson, J. J. Kim, D. Sneden, J. Weinzierl & A. Rich, 9th Internatl. Congr. of Crystallography, Collected Abstracts, p. s. 42 (1972).
19. Unpublished work of Princeton laboratory.
20. M. Levitt, Nature, 224, 759 (1969) and references therein.
21 D. J. Abraham, J. Theor. Biol. 30, 83 (1971)
22. T. T. Wu, Bull of Math. Biophysics, 31, 395 (1969)
23. A. Danchin, FEBS letters, 13, 152 (1971)
24. W. Guschlbauer, Nature 209, 258 (1966)

25. G. Melcher, FEBS Letters, 3, 185 (1969)
26. C.-G. Jang, in preparation.
27. Cambridge, Repetition Engineering Ltd. 2 cm = 1Å
28. W. L. Koltun, Biopolymers 3, 665 (1965)
29. Princeton University Computer Graphics Laboratory.
30. R. W. Holley, J. Apgar, G. A. Everett, M. Marquisee, S. H. Merill, J. R. Penswick & A. Zamir, Science, 147, 1462 (1965)
31. E. J. O'Brien, Acta Cryst, 23, 92 (1967)
32. G. Simundza, T. D. Sakore & H. M. Sobell, J. Mol. Biol., 48, 263 (1970)
33. S. Mcgavin, J. Mol. Biol. 55,293 (1971)
34. P. G. Connors, M. Labanuskas and W. W. Beeman Science, 166, 1528 (1969)
35. J. A. Lake & W. W. Beeman, J. Mol. Biol., 31, 1115 (1968)
36. C.-G. Jang unpublished work
37. F. Cramer, Prog. Nucl. Acid Res. and Mol. Biol., 11, 391 (1971)
38. T. Ichikawa and M. Sundaralingam, Nature New Biology 236, 174 (1972)
39. A. D. Mirzabekov, D. Rhodes, J. T. Finch, A. Klug and B. F. C. Clark. Nature New Biology, 237, 27 (1972)
40. I. D. Kuntz, Jr., unpublished.
41. M. Litt, Biochemistry 10, 2223 (1971)
42. O. C. Uhlenbeck, J. Mol. Biol. 65, 25 (1972)
43. G. Zubay and M. Takanami, Biochem. Biophys. Res. Commun., 15, 207 (1964)
44. K. Beardsley, T. Tao and C. R. Cantor, Biochemistry, 9, 3524 (1970)
45. D. B. Miller and R. F. Steiner, Biochemistry, 5, 2289 (1966)
46. B. M. Hoffman, P. Schofield and A. Rich, Proc. Natl. Acad. Sci., 65,39 (1970)
47. O. C. Uhlenbeck, J. G. Chirikjian and J. R. Fresco, Fed. Proc., 31, 422 (1972)
48. M. Staehelin, Experientia 27, 1 (1971)
49. R. D. Blake, J. R. Fresco & R. Langridge, Nature, 225, 32 (1970)
50. Y. Wong, D. Kearn, R. G. Shullman, personal communication.
51. D. Voet & A. Rich, Progr. Nucl. Acid Res. & Mol. Biol. 10, 183 (1970)
52. J. J. Englander, N. R. Kallenbach & S. W. Englander, J. Mol. Biol., 63, 153 (1972)
53. P. Webb & J. R. Fresco, J. Mol. Biol. in press.
54. D. Söll, Science, 173, 293 (1971) and references therein.
55. I. M. Klotz & S. B. Farnham, Biochemistry, 7, 3879 (1968)
56. R. H. Hall, The modified Nucleosides in Nucleic Acids, Columbia University Press, New York 1971.
57. F. Pochon, A. M. Michelson, M. Grunberg-Manago, W. E. Cohn & L. Dondon, Biochem. et Biophys. Acta 80, 441 (1964)
58. E. A. Barnard, Annual Review of Biochem., Vol. 38, 677 (1969)
59. R. W. Holley, G. A. Everett, J. T. Madison & A. Zamir, J. Biol. Chem., 240, 2122 (1965)
60. M. Staehelin, Biochim. et Biophys. Acta Vol. 87, 493 (1964)
61. A. M. Bobst, P. A. Cerutti & F. Rottman, J. Am. Chem. Soc., 91, 1246 (1969)
62. W. Szer, Biochem. Biophys. Res. Commun., Vol. 20, 182 (1965)
63. D. Duetting & H. G. Zachau, Z. Physiol. Chem., 336, 132 (1964)
64. W. Saenger and D. H. Scheit, J. Mol. Biol. 50, 152 (1970)
65. S. Arnott, Progr. in Biophys. and Mol. Biol. Vol. 21, 265 (1970a)
66. S. Arnott and D. W. L. Hukins, Biochem. J., in press.
67. T. Sato, V. Sasisekharan and R. Langridge, in preparation.
68. A. E. V. Haschemeyer and H. M. Sobell Nature 202, 969 (1964)
69. V. Sasisekharan & G. N. Ramachandran, Advances in Protein Chem. Vol. 23, 283 (1968)
70. A. Danchin, Biopolymers 11, 1317 (1972)
71. R. Chambers, Progr. Nucl. Acid Res. & Mol. Biol., Vol. 11, 489 (1971)
72. B. Dudock, C. Diperi, K. Scileppi & Reslbach, Proc. Natl. Acad. Sci., 68, 681 (1971)

73. J. D. Smith, L. Barnett, S. Brenner & R. L. Russell, J. Mol. Biol., 54, 1 (1970)
74. T. Seno, I. Kobayashi, M. Fukuhara & S. Nishimura, FEBS Letters, 7, 343 (1970)
75. L. Chaffin, D. R. Omilianowski & R. M. Bock, Science, 172, 854 (1971)
76. J. Ofengand & C. Henes, J. Biol. Chem., 244, 6241 (1969)
77. J. N. Abelson, M. L. Gefter, L. Barnett, A. Landy, R. G. Russell, & J.D. Smith, J. Mol. Biol., 47 15 (1970)
78. T. S. Stewart, R. J. Roberts, J. L. Strominger, Nature, 230, 6 (1971)
79. M. Yaiv, A. Favre & B. G. Barrell, Nature, 223, 1330 (1969)
80. K. Beardsley & C. R. Cantor, Proc. Natl. Acad. Sci., Vol. 65, 39 (1970)
81. C.-G. Jang, unpublished work.
82. M. Yoshida & T. Ukita, Biochim. et. Biophys. Acta, 157, 466 (1968)
83. C. Woese, Nature, 226, 817 (1970)
84. F. H. C. Crick, J. Mol. Biol. 19, 548 (1966)