Acta Biochimica Polonica

Vol. 48 No. 2/2001

485–493

QUARTERLY

Elements of thermodynamics in RNA evolution★✪

El¿bieta Kierzek, Ewa Bia³a and Ryszard Kierzek✉

Institute of Bioorganic Chemistry, Polish Academy of Sciences, Poznañ, Poland

Received: 14 February, 2001; revised: 9 May, 2001; accepted: 23 May, 2001

Key words: ribonucleic acids, thermodynamics

The paper presents some aspects correlating thermal stability of RNA folding and the occurrence of structural motifs in natural ribonucleic acids. Particularly, the thermodynamic stability of 2′-5′ and 3′-5′ linked RNA and the contribution of unpaired terminal nucleotides (dangling ends) in secondary (2D) and tertiary (3D) structures of RNA are discussed. Both examples suggest that during evolution nature selected sequences and structures of RNA which are the most thermally stable and efficient for their biological function.

The world of a living organism has changed since life began on the Earth. Ribonucleic acids (RNA), deoxyribonucleic acids (DNA) and proteins are among those biomolecules which evolution used to adapt to new environments. The commonly used term – RNA world – is related to the hypothesis that RNA was the first among this triad of molecules. During evolution, DNA and proteins have become more stable and biologically more efficient and adopted most functions of RNA [1].

The biological functions of RNAs are dependent on their structure. The transcription experiments in which the length of RNA is extended demonstrate that structural elements present in shorter transcripts are conserved in longer RNA. It presumably means that folding of RNA is driven thermodynamically [2]. Moreover, the most thermodynamically stable structures of RNA are very often the same as those found from phylogenetic analysis. Occasionally, however, phylogenetic structures

★Presented at the International Conference on "Molecular Architecture of Evolution, Primary and Secondary Determinants" Poznañ, Poland, October 29–31, 2000.

✪This work was supported by NIH grant 1 R03 TW1068-01 to R.K. and D.H. Turner (University of Rochester).

✉Ryszard Kierzek, Institute of Bioorganic Chemistry, Polish Academy of Sciences, Z. Noskowskiego 12/14, 60-704 Poznañ, Poland, tel.: (48 61) 852 8503 ext. 143; fax: (48 61) 852 0532; e-mail: rkierzek@ibch.poznan.pl

Abbreviations: CD, circular dichroism; NMR, nuclear magnetic resonance; 2D, two dimensional; 3D, three dimensional.

can be identical to thermodynamically suboptimal structures, a structures that is different in free energy by 5% from the most stable structure [3].

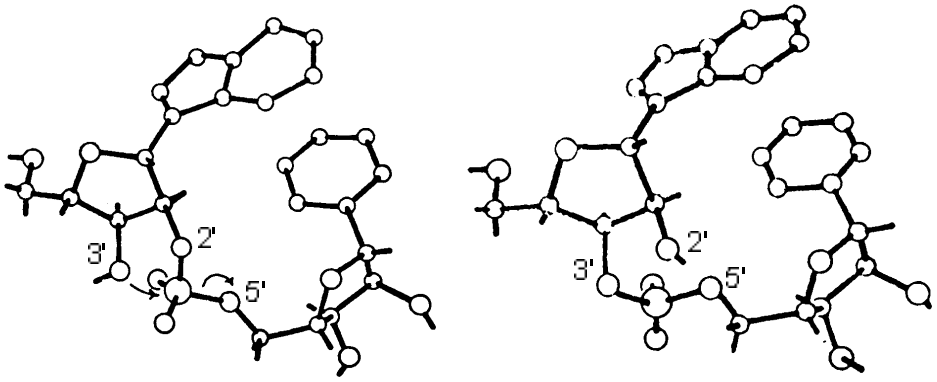
In this paper we discuss the relations between thermodynamic stability of 2′-5′ and 3′-5′ linked RNA as well as the contribution of unpaired terminal nucleotides (dangling ends) in secondary (2D) and tertiary (3D) structure of RNA. Sequential analysis of some structural motifs in RNA and their contribution to the thermal stability suggest that the most thermodynamically stable motifs occur in RNA more often. It allows to assumption that, during evolution, RNA changed in a way to adopt, at once, the most thermodynamically stable and the most biologically efficient structure.

RESULTS AND DISCUSSION

Thermal stability of 2′-5′ RNA and 3′-5′ RNA

Naturally occurring RNA and DNA contain 3′-5′ oriented internucleotide bonds. The only exemption is 2′-5′ pppApApA whose synthesis is indirectly promoted by α and β-interferons [4]. There can be several reasons why RNA is linked *via* 3′-5′ phosphodiester bonds. It could be connected to differences in the rate of formation and chemical stability of RNA containing 2′-5′ and 3′-5′ internucleotide bonds. A second reason could be the inability of 2′-5′ RNA to form biologicallyactive structures.

The oligomerization of activated 5′-phosphates, such as 5′-phosphoimidazolates of ribonucleosides, in conditions similar to prebiotic and in the presence of complementary polyribonucleic acid results in the formation of various sizes of RNA [5]. Analysis of the internucleotide bonds orientation within the oligoribonucleotides formed by transcription of various templates demonstrated that 2′-5′ internucleotide bonds are formed predominantly or at least in equivalent amounts to 3′-5′ internucleotide bonds. This suggests that the formation rate of the internucleotide bond is not a selective factor. An alternative element which could cause domination of RNA with 3′-5′ over 2′-5′ internucleotide bonds is chemical stability. Chemical cleavage of RNA proceeds *via* an in-line mechanism and requires placing in line the functional groups participating in the process [6]. The orientation of the 3′-hydroxyl, the phosphorus atom and the leaving 5′-oxygen in 2′-5′ RNA indicates that they are placed almost in line (Fig. 1) [7]. A different arrangement occurs in 3′-5′ RNA. It is necessary to break stacking in-



2' - 5' RNA 3' - 5' RNA

Figure 1. The arrangement of phosphodiester bonds in 2′-5′ and 3′-5′ RNA.

The arrows indicate the shift of the electrons during in-line hydrolysis of phosphodiester bonds.

teractions of adjacent nucleotides and rearrange the ε and ξ bonds within the internucleotide bond to place the 2′-hydroxyl, phosphorus and the leaving 5′-oxygen in line. However, a kinetic study of the cleavage of RNA containing 2′-5′ and 3′-5′ internucleotide bonds demonstrates that the stability of

was measured by the UV melting method in 1 M sodium chloride, 10 mM sodium phosphate and 0.5 mM Na_2EDTA at pH 7.0 [11]. The melting of self-complementary 2′-5′ oligoribonucleotides containing G-C base pairs demonstrated typical melting curves, however, the transition was broad and the hyperchromism

Table 1. Comparison of thermodynamic parameters for duplex formation by 2′-5′ and 3′-5′ oligoribonucleotides.

Sequences	(2′-5′) oligomers				(3′-5′) oligomers				(3′-5′) – (2′-5′) difference data			
	$-\Delta H°$	$-\Delta S°$	$-\Delta G°_{37}$	T_m	$-\Delta H°$	$-\Delta S°$	$-\Delta G°_{37}$	T_m	$-\Delta\Delta H°$	$-\Delta\Delta S°$	$-\Delta\Delta G°_{37}$	ΔT_m
	kcal/mol	eu	kcal/mol	°C	kcal/mol	eu	kcal/mol	°C	kcal/mol	eu	kcal/mol	°C
$(AG)_5 \times (CU)_5$			12 and 35		91.2	250.2	13.9	66.5				
$(CGGCGCCG)_2$	43.5	118.0	6.9	46.0	76.8	199.6	14.8	79.3	33.3	81.6	7.9	33.3
$(GGCGCC)_2$	29.7	79.4	5.1	30.9	67.8	182.0	11.3	65.2	38.1	102.6	6.2	34.3
$(GCGCGC)_2$	28.3	76.6	4.5	25.0	66.0	178.5	10.6	62.1	37.7	101.9	6.1	37.1
$(GCCGGC)_2$	31.7	88.4	4.3	23.8	62.7	166.0	11.2	67.2	31.0	77.6	6.9	43.4
$(UGCGCA)_2$	28.7	81.1	3.5	15.2	51.5	139.7	8.2	53.1	22.8	58.6	4.7	37.9

both phosphodiester bonds is similar in 1 M aqueous ethylenediamine, pH 8, at 40°C [8]. In the presence of a complementary strand, the oligoribonucleotide containing 2′-5′ phosphodiester bonds is cleaved faster.

The observations concerning formation and cleavage of RNA containing 2′-5′ and 3′-5′ internucleotide bonds suggest that neither of the factors discussed above is responsible for the domination of 3′-5′ RNA. Perhaps the arrangement of the internucleotide bond improves the ability of 3′-5′ RNA to form thermally more stable and biologically active RNA structures.

To get information about thermal stability of 2′-5′ RNA duplexes chemical synthesis of 2′-5′ oligoribonucleotides was performed [9]. Protected ribonucleoside-2′-phosphoramidites were used for synthesis on solid support. Deprotection and purification were performed in the same way as for 3′-5′ RNA [10]. The sequences of the 2′-5′ oligoribonucleotides used in the experiments are listed in Table 1. Thermal stability of 2′-5′ RNA duplexes was about half of that observed in 3′-5′ RNA. Analysis of the correlation between the concentration of oligoribonucleotides and melting temperatures demonstrated a linear dependence. This means that melting of 2′-5′ oligoribonucleotides proceeds *via* a two state transition similar to 3′-5′ RNA. The thermodynamic parameters: enthalpy (ΔH^0), entropy (ΔS^0) and free energy (ΔG^0_{37}) as well as melting temperature (T_m) are collected in Table 1. Analysis of this data demonstrated that 2′-5′ oligoribonucleotides were much less stable than 3′-5′ oligoribonucleotides. The value of free energy (ΔG^0_{37}) of 2′-5′ RNA was less than 50% of the free energy of 3′-5′ RNA or 1 kcal/mol less per each G-C base pair present in the duplex. The melting temperature was 30 to 40°C lower than for 3′-5′ RNA. This means that, at 37°C and physiological concentration, 2′-5′ oligoribonucleotides were in a single stranded form and did not form stable RNA helixes.

Additional information about the structure of 2′-5′ duplexes is provided by CD and NMR

spectra. CD spectra demonstrate that 2′-5′ RNA forms an A-form RNA helix at low temper a ture [12]. As shown in Fig. 2, the CD spectra of 1.1×10^{-4} M 2′-5′ CGGCGCCG dem onstrated A-form RNA, but increasing the tem-

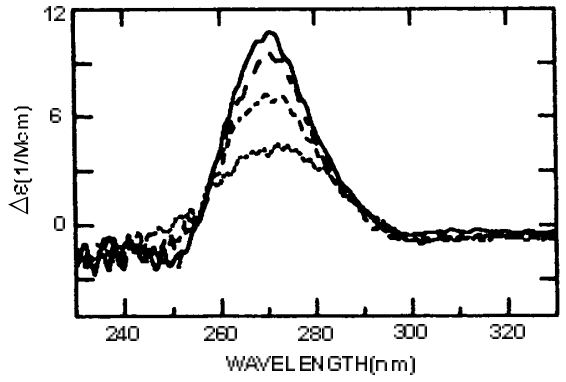


Figure 2. Temperature dependent CD spectra of 1.10×10^{-4} M of 2′-5′ CGGCGCCG at 0°C (–), 20°C (– – –), 40°C (- - -) and 60°C (· · ·).

So lu tion was 1 M so dium chlo ride, 10 mM so dium phos phate, 0.5 mM Na_2EDTA, pH 7.0.

per a ture above 20°C re sulted in a pro gres sive de crease of the am pli tude at 250 nm. The de creas ing am pli tude means that 2′-5′ RNA was melted and the helical structure was lost (melting temperature of 2′-5′ CGGCGCCG at this con cen tration was 46.4°C). Sim i lar be hav ior was ob served for 3′-5′ RNA [13]. ^{1}H NMR spectra indicated that imino protons of guanosines and amino protons of cytidines formed hydrogen bonds with nucleotides in the com ple men tary strand [14]. Fig ure 3 pres ents the ^{1}H NMR spectrum of 1.4 mM 2′-5′ CGGCGCCG at 10°C. The saturation of resonances at 12.59, 12.93 and 13.02 ppm allows their assignment to imino protons of G5, G2 and G3, re spec tively, and at the same time to correlate them to amino protons of cytidines (region 8.3–8.8 ppm).

Evidently, 2′-5′ RNA forms helical structures but their ther mal sta bil ity is much lower than for 3′-5′ RNA. Perhaps this is a reason why nature selected 3′-5′ RNA over 2′-5′ RNA. Re cently, ther mal sta bil ity of 2′-5′ DNA was mea sured. It was found to be sig nif i cantly (about 30°C) lower than 3′-5′ DNA [15, 16]. Moreover, using NMR techniques the structure of 2′-5′ d(CGGCGCCG) was solved and it formed an A-form DNA containing alternating N-type and S-type puckers of 3′-deoxyriboses [17].

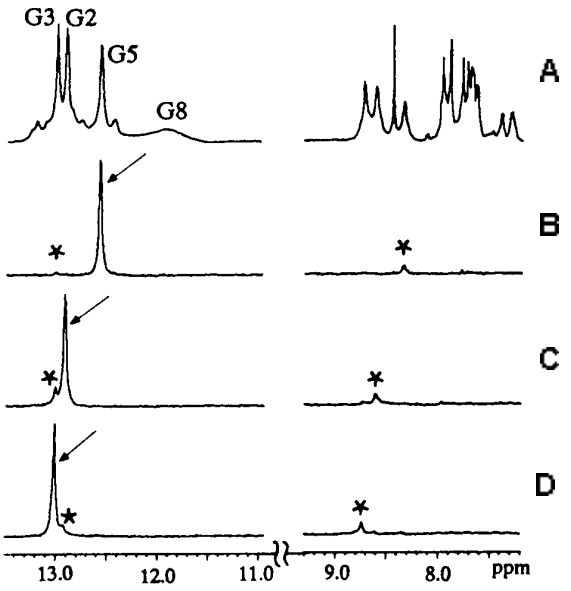


Fig ure 3. 500 MHz pro ton NMR spec trum of 2′-5′ CGGCGCCG.

(**A**) spec trum of 1.4 mM 2′-5′ CGGCGCCG at 10°C in 100 mM sodium chloride, 10 mM sodium phosphate and 0.5 mM Na_2EDTA in 90% H_2O, 10% D_2O, pH 7.0. Dif fer ence spec tra fol low ing 1-s sat u ra tion of (**B**) the resonance at 12.59 ppm, (**C**) the resonance at 12.93 ppm, and (**D**) the res o nance at 13.02 ppm. The sat u-rated res o nances are in di cated by ar rows while the observed NOEs are des ig nated by as ter isks.

Con tri bu tion of un paired ter mi nal nu cleo tides in fold ing of RNA

Unpaired terminal nucleotides (also called dan gling ends) in RNA are very of ten pres ent next to helical regions. They can be 3′- or 5′-dangling ends depending on which side of the du plex the un paired nu cleo tides are present. A study of many he li cal RNA mod els dem on strated that the pres ence of a dan gling end can stabilize an RNA duplex and change its free energy by up to 1.7 kcal/mol (Table 2)

[18]. The presence of 3′-dangling ends increases the stability (ΔG^0_{37}) of duplexes by 0.1 to 1.7 kcal/mol, whereas 5′-dangling ends stabilize duplexes by up to 0.5 kcal/mol. Moreover, this stability depends on the sequence of the dangling end, as well as the sequence and orientation of the adjacent terminal base pair. In large RNA molecules, the definition of dangling end is extended to include unpaired nucleotides in hairpin loops, bulge loops, internal loops and multibranch loops adjacent to helical fragments. Stacking interactions between unpaired terminal nucleotides and terminal base pairs in the helix are responsible for stabilizing the duplex. An analysis of the secondary structure of $tRNA^{Phe}$ from yeast indicates that 12 nucleotides can be considered as 5′- and 3′-dangling ends [19]. Based on the crystal structure of $tRNA^{Phe}$, it can be seen that some dangling ends stack on adjacent helixes whereas some do not [20]. This observation raises interesting questions concerning the general character of this phenomenon.

Recently, using X-rays and NMR methods, several RNA structures have been solved. Based on the data available in the Nucleic Acid Database and Protein Data bank, an analysis of 124 dangling end interactions in 34 RNA structures was performed [21, 22]. At the beginning it was necessary to define the meaning of stacking terminal unpaired nucleotides. Based on an analysis of many structures, a dangling end considered as stacking must fulfill simultaneously the following requirements: (i) the nearest approach of a non-hydrogen atom of the nucleotide base in the dangling end to a non-hydrogen atom in the terminal pair must be ≤ 4 Å, (ii) the angle between the planes of the nucleotide base in the dangling end and at least one base of the terminal pair must be ≤ 30 Å, (iii) the nucleotide base in the dangling end overlaps the adjacent base pair.

Table 2. Free energy increments (kcal/mol) for unpaired terminal nucleotides

					X				
	A	C	G	U		A	C	G	U
	3′-dangling ends					5′-dangling ends			
→AX U←	–0.8	–0.5	–0.8	–0.6	→XA U←	–0.3	–0.3	–0.4	–0.2
→CX G←	–1.7	–0.8	–1.7	–1.2	→XC G←	–0.5	–0.2	–0.2	–0.1
→GX C←	–1.1	–0.4	–1.3	–0.6	→XG C←	–0.2	–0.3	0.0	0.0
→UX A←	–0.7	–0.1	–0.7	–0.1	→XU A←	–0.3	–0.2	–0.2	–0.2

The next step of analysis was correlating the dangling end and adjacent base pair arrangement in analyzed RNA to the effect of the same dangling end on the stability of model RNA duplexes. The results of the analysis can be collected into three classes. The first group contains the dangling ends which stabilize ($\Delta G^0_{37,\text{stacking end}}$) model RNA duplexes by at least 0.8 kcal/mol. There were 36 such cases among the analyzed dangling ends and in 30 (83%) of them, the base at the dangling end

stacked on the ter mi nal base pair. The re main ing six se quences which are not stacked, were of ten as so ci ated with a strong in ter ac tion im portant for stabilizing the unstacked con for-

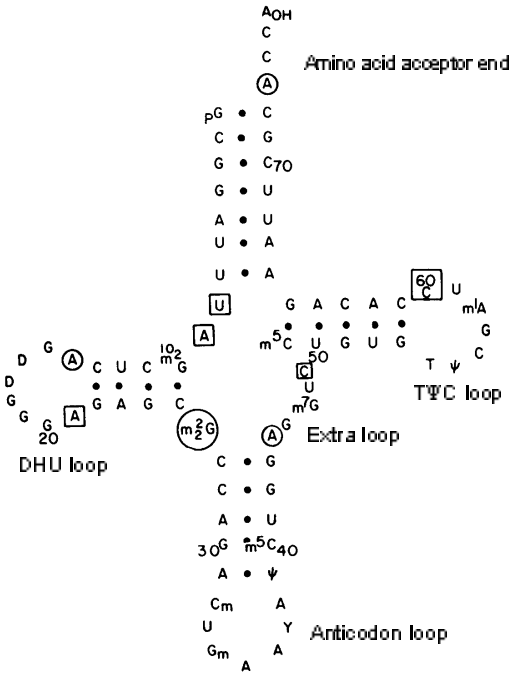


Figure 4. The secondary structure of tRNA^{Phe} from yeast with strongly stack ing nu cleo tides cir cled and weakly stack ing nu cleo tides boxed.

mation. The next group in cludes 32 dan gling ends and in these cases 21 (66%) were stacked. For this group, the sta bil ity of model RNA du plexes ($\Delta G^0_{37,\text{stack ing end}}$) increases by 0.4 to 0.7 kcal/mol. Finally, the last group includes 56 dan gling ends, among them only 19 (34%) are stacked on the adjacent base pair. The dangling ends of this group increase the sta bility ($\Delta G^0_{37,\text{stacking end}}$) of model RNA du plexes by less than 0.3 kcal/mol. These re sults support the hypothesis that dangling ends, which strongly stabilize RNA helices, prefer to stack on top of the ad ja cent he lix and in this way con trib ute to the 3D struc ture of RNA.

The con tri bu tion of dan gling ends to the 3D structure of RNA is pretty clear in tRNA^{Phe} from yeast [19]. An analysis of the 2D struc ture of this tRNA indicates the presence of twelve 5′- and 3′-dan gling ends (Fig. 4). They are placed in the anticodon, TΨC and DHU hairpin loops, and at junctions between hair pins. An anal y sis of dan gling end ori en ta tion in the 3D structure of tRNA^{Phe} from yeast confirmed that some dangling ends do stack and some do not stack on ad ja cent he li cal base pairs (Fig. 5) [20]. The following 5′-dangling ends do not stack: A9, A21, C48 and C60, and 3′-dan gling end U8 (Fig. 5A). It is known from model studies that all 5′-dangling ends men tioned, ad ja cent to base pairs in such an ori en ta tion as oc curs in tRNA^{Phe}, destabilize model RNA du plexes by 0.2 kcal/mol while U8 sta bi lizes the duplex by 0.1 kcal/mol [18]. At the same time seven dangling ends (including A14, m^2_2G26, A44, T54 and A73) stack on ad jacent base pairs (Fig. 5B). These dangling ends in model RNA du plexes sta bi lize the he lix by at least 1 kcal/mol. Two re main ing dan gling ends, Cm32 and A38, also stack. How ever, they stabilize model RNA duplexes by 0.5 and 0.3 kcal/mol, respectively [18]. The stack ing of those two dan gling ends can be en hanced by a strong in ter ac tion with Mg^{+2} and wa ter within the anticodon loop [20]. Three of the stack ing nu cleo tides are mod i fied and it is possible that modification additionally en hances the stacking interactions. For exam ple, it was observed that 3N-methyluridine, 5-methyluridine and uridine as 3′-dangling ends stabilize RNA duplexes ($\Delta G^0_{37,\text{stacking end}}$) by 1.51, 1.08 and 0.82 kcal/mol, respec tively.

Very interesting is the example of the un stacked U8 within the 3D structure of tRNA. Particularly noticeable is the correlation be tween the stabilization of the amino acid ac cep tor arm by U8 ad ja cent to var i ous ter mi nal base pairs and the fre quency of oc cur rence of such arrangements. Uridine-8 is one of the conserved nucleotides in tRNA and does not stack on the adjacent base pair at position 7 and 66, but instead forms a reversed Hoogsteen base pair with A14. An anal y sis of 415 tRNA se quences is pres ent in Fig. 6 [23]. There are four possible arrangements of U8 and terminal base pairs at po si tion 7 and 66.

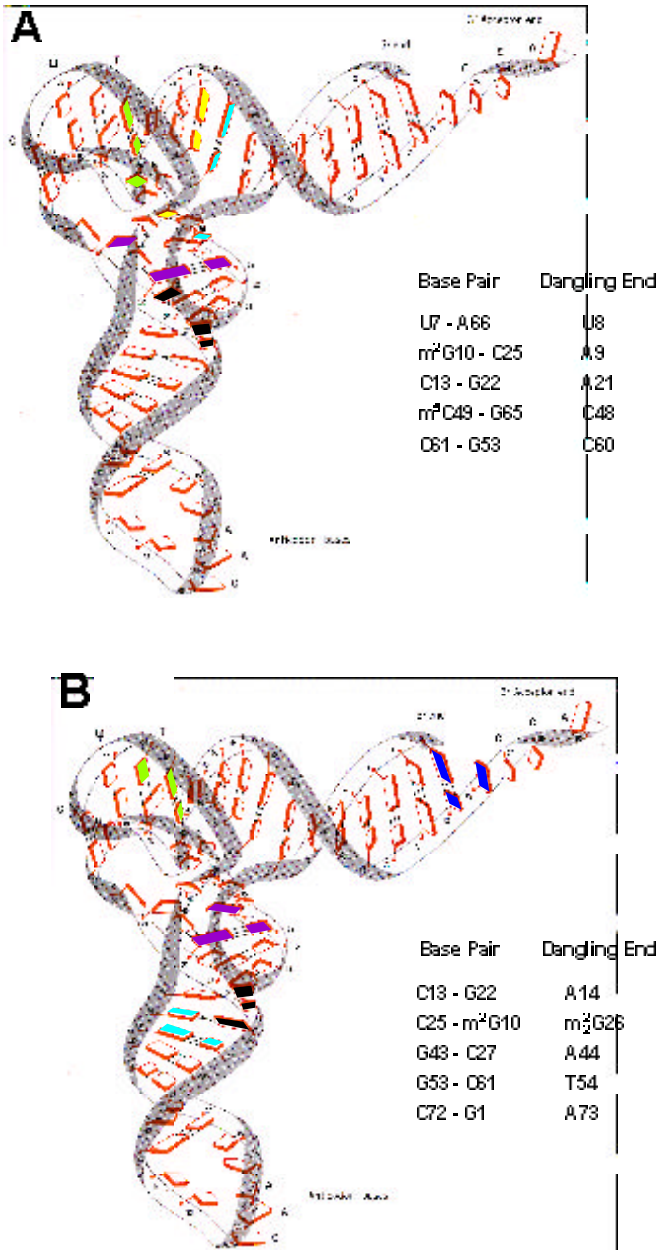


Figure 5. Arrangement of unstacking (A) and stacking (B) dangling ends and adjacent base pairs (marked by the same color) within the 3D structure of $tRNA^{Phe}$ from yeast.

Uridine-8 as a 3′-dangling end stabilizes the RNA duplex by 0.1, 0.6, 0.6 and 1.2 kcal/mol for the arrangements of base pairs shown in segments A–D, respectively. For the first one (Fig. 6A), the stability of U8 as a 3′-dangling end ($\Delta G^0_{37,\text{stacking end}}$) is only 0.1 kcal/mol and this case occurs 90 times (22%). The observed effect is very small, characteristic for unstacked dangling ends. For the arrangements presented in Figs. 6B and 6C, the U8 stacking with A-U and G-C should increase the stability, in both cases, by 0.6 kcal/mol. Both arrangements belong to dangling ends in which stacking was ambiguous and together they occur 305 times (73%) in the tRNAs analyzed. In the last case (Fig. 6D), the stabilization is very large ($\Delta G^0_{37,\text{stacking end}}$ = –1.2 kcal/mol) and U8 should stack with the terminal C-G base pair. Such an arrangement occurs 20 times (5%). The coaxial stacking of amino-acid acceptor and TΨC arms competes with the stacking dangling ends U8 and C48. When the amino-acid acceptor and TΨC arms stack coaxially, as was calculated for $tRNA^{Phe}$,

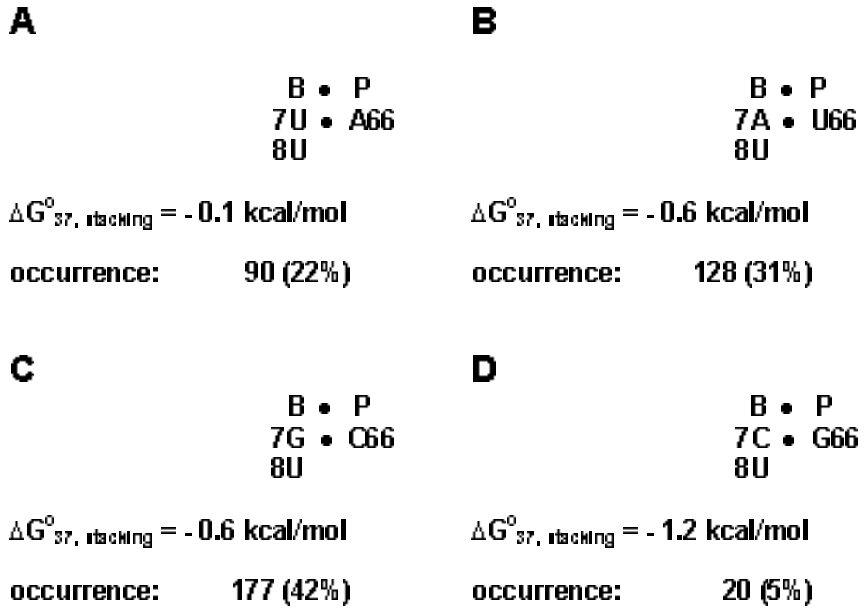


Figure 6. Correlation between arrangements of base pairs at positions 7 and 66 of tRNA and free energy 3′ stacking of U8 (panels A–D) and occurrence of such arrangements in tRNA.

they contribute –2.4 kcal/mol of stability while U8 and C48 as dangling ends together contribute –0.4 kcal/mol [24].

The results discussed above suggest that RNA often selects structural motives which are thermodynamically more stable. However, it is important to remember that interactions compete with each other and these interactions which contribute to the overall thermodynamic stability of RNA are a major determinant. The sequences and structures of RNA at the beginning of evolution are not known but presumably nature selected those RNAs which were the most thermodynamically stable and at the same time the most biologically active.

REFERENCES

1. Gesteland, R.F. & Atkins, J.F. (1993) *The RNA world*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor.
2. Matysiak, M., Wrzesiñski, J. & Ciesio³ka, J. (1999) Sequentional folding of the genomic ribozyme of the hepatitis delta virus: Structural analysis of RNA transcription intermediates. *J. Mol. Biol.* **291**, 283–294.
3. Jaeger, J.A., Turner, D.H. & Zucker, M. (1989) Improved prediction of secondary structure for RNA. *Proc. Natl. Acad. Sci. U.S.A.* **86**, 7706–7710.
4. Baglioni, C., Minks, M.A. & Maroney, P.A. (1978) Interferon action may be mediated by activation of a nuclease by pppA2′p5′A2′p5′A. *Nature* **273,** 684–687.
5. Orgel, L.E. (1987) Evolution of the genetic apparatus: A review. *Cold Spring Harbor Symp. Quant. Biol.* **52**, 123–133.
6. Breslow, R., Anslyn, E. & Huang, D.-L. (1991) Ribonuclease mimics. *Tetrahedron* **47**, 2365–2376.
7. Usher, D.A. (1972) RNA double helix and the evolution of the 3′,5′ linkage. *Nat. New Biology* **235**, 207–208.
8. Usher, D.A. & McHale, A.H. (1976) Hydrolytic stability of helical RNA: A selective advantage for the natural 3′,5′-bond. *Proc. Natl. Acad. Sci. U.S.A.* **73**, 1149–1153.

9. Kierzek, R., He, L. & Turner, D.H. (1992) Association of 2′-5′ oligoribonucleotides. *Nucleic Acids Res.* **20**, 1685–1690.

10. Xia, T., SantaLucia, J., Burkard, M.E., Kierzek, R., Schroeder, S.J., Cox, C. & Turner, D.H. (1998) Parameters for expanded nearest-neighbor model for formation of RNA duplexes with Watson-Crick base pair. *Biochemistry* **37**, 14719–14735.

11. Kierzek, R., Burkard, M.E. & Turner, D.H. (1999) The thermodynamics of single mismatches in RNA duplexes. *Biochemistry* **38**, 14214–14223.

12. Tunis-Schneider, M.J.B. & Maestre, M.F. (1970) Circular dichroism spectra of oriented and unoriented deoxyribonucleic acid films: A preliminary study. *J. Mol. Biol.* **52**, 521–541.

13. Williams, A.P., Longfellow, C.L., Freier, S.M., Kierzek, R. & Turner, D.H. (1989) Laser temperature-jump, spectroscopic and thermodynamic study of salt effects on duplex formation by dGCATGC. *Biochemistry* **28**, 4283–4291.

14. Xia, T., McDowell, J.A. & Turner, D.H. (1997) Thermodynamics of nonsymetric tandem mismatches adjacent to G–C base pairs in RNA. *Biochemistry* **36**, 12486–12497.

15. Dougherty, J.P., Rizzo, C.J. & Breslow, R. (1992) Oligodeoxynucleotides that contain 2′,5′ linkages: Synthesis and hybridization properties. *J. Am. Chem. Soc.* **114**, 6254–6255.

16. Hashimoto, H. & Switzer, C. (1992) Self-association of 2′,5′-linked deoxynucleotides: meta-DNA. *J. Am. Chem. Soc.* **114**, 6255–6256.

17. Robinson, H., Jung, K.-E., Switzer, C. & Wang, A.H.-J. (1995) DNA with 2′-5′ phosphodiester bonds forms a duplex structure in the A-type conformation. *J. Am. Chem. Soc.* **117**, 837–838.

18. Freier, S.M., Kierzek, R., Jaeger, J.A., Sugimoto, N., Caruthers, M.H., Neilson, T. & Turner, D.H. (1986) Improved free energy parameters for predictions of RNA duplex stability. *Proc. Natl. Acad. Sci. U.S.A.* **83**, 9373–9377.

19. Sugimoto, N., Kierzek, R. & Turner, D.H. (1987) Sequence dependence for the energetics of dangling ends and terminal base pairs in ribonucleic acid. *Biochemistry* **26**, 4554–4559.

20. Quigley, G.J. & Rich, A., (1976) Structural domains of transfer RNA molecules. *Science* **194**, 791–802.

21. *Protein Data Bank* (http://www.pdb.bnl/gov), Nucleic Acid Data Base (http://ndbserver.ritgers.edu).

22. Burkard, M.E., Kierzek, R. & Turner, D.H. (1999) Thermodynamics of unpaired terminal nucleotides on short RNA helixes correlates with stacking at helix terminin larger RNAs. *J. Mol. Biol.* **290**, 967–982.

23. Sprinzl, M., Horn, C., Brown, M., Ioudovitch, A. & Steinberg, S. (1998) Compilation of tRNA sequences and sequences of tRNA genes. *Nucleic Acids Res.* **26**, 148–153.

24. Mathews, D.H., Sabina, J., Zucker, M. & Turner, D.H. (1999) Expanded sequence dependence of thermodynamic parameters improves prediction of RNA secondary structure. *J. Mol. Biol.* **288**, 911–940.