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LETTER TO THE EDITOR

An extended structural signature for the tRNA anticodon loop

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ABSTRACT

Anticodon hairpins are structural motifs with contradictory functions. The recognition by aminoacyl synthetases implies extended interactions with the anticodon base triplet and thus, usually, an unfolding of the anticodon loop. The recognition by the ribosome and cognate interaction with a mRNA codon implies, on the other hand, the formation of a mini-helix with a canonical anticodon hairpin structure as observed by crystallography and NMR. To be able to understand the various properties of this motif, a precise description of its structural conservation is required. Here, on the basis of phylogenetic, structural, and molecular dynamics data, we discuss a conserved interaction established between the ribose of the U33 and the base at position 35, either a purine or a pyrimidine. This interaction involves the hydrogen bonding donor or acceptor potential of the hydroxyl group of U33 and has to be integrated in an extended definition of the anticodon hairpin. The extended structural signature provides also an explanation for the role played by pseudouridines at position 35.

Keywords: anticodon; hydrogen bond; hydroxyl group; pseudouridine; RNA motif; tRNA

INTRODUCTION

As inferred from several biochemical studies, a canonical three-dimensional structure of the anticodon hairpin is supposed to be essential for the binding of tRNA molecules to the ribosomal binding sites (Schnitzler & von Ahsen, 1997; von Ahsen et al., 1997; Ashraf et al., 1999a, 1999b; Cate et al., 1999). Up to now, the anticodon structure is defined by the presence of an array of conserved and semiconserved nucleotides. Among them, a uridine is recurrently observed at position 33; the base at position 32 is generally a pyrimidine (Y); the bases at positions 37 and 38 are essentially purines (R); the three bases at the anticodon positions 34, 35, and 36 display a nearly equal proportion of the four nucleotides; positions 34 and 37 accept a large number of modified nucleotides; a very limited number of modified nucleotides are observed at positions 35 and 36 (Grosjean et al., 1982; Auffinger & Westhof, 1998b); uridines when present at position 39 are mainly modified into pseudouridines (Auffinger & Westhof, 1998a; Yarian et al., 1999). These sequence conservations are mandatory, in the vast majority of the cases, for the formation of functional canonical anticodon hairpin structures that include the first motif that has been characterized in RNA molecules, namely the U-turn motif (Quigley & Rich, 1976). The tertiary structure of the anticodon loop U-turn is usually defined by the formation of a (U33)N3-H…OR-P(36) hydrogen bond, a stacking interaction between the aromatic cycle of U33 and the OR atom of residue 35, and a sharp reversal of the phosphodiester backbone following U33 (Quigley & Rich, 1976). Additionally, on top of the U-turn motif involving residues 33–36, it has been shown, on the basis of crystallographic and phylogenetic data, that the conservation of a set of non-Watson–Crick isosteric base pairs at position 32•38 is essential for the formation of a canonical hairpin structure (Auffinger & Westhof, 1999). All these tertiary interactions are part of the signature of the tRNA anticodon loop. In addition, from the yeast $tRNA^{Phe}$ structure, it has been inferred that a (U33)O2′-H…N7(A35) hydrogen bond is formed (Quigley & Rich, 1976). Indeed, such a hydrogen bond can be formed when a purine is present at position 35. Yet, the type of interaction that occurs when a purine at position 35 is replaced by a pyrimidine has given rise to a long-standing debate (Quigley & Rich, 1976; von

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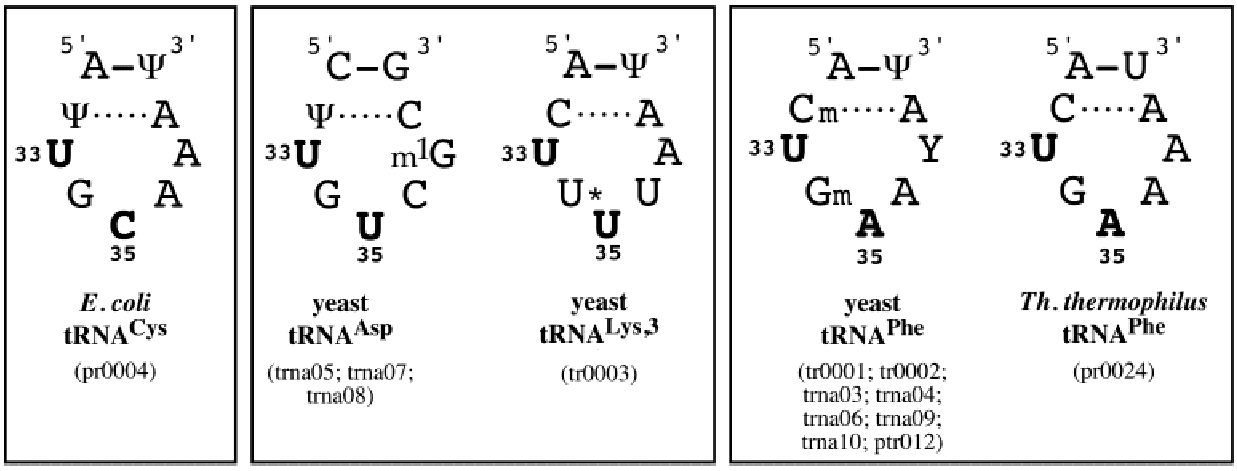

FIGURE 1. Secondary structure of tRNA anticodon hairpins for which coordinates are deposited in the Nucleic Acid Database (NDB) (Berman et al., 1992). Nucleotides 33 and 35 are shown in bold. The NDB codes are noted under each secondary structure.

Ahsen et al., 1997; Ashraf et al., 1999a) in which the (U33)ribose…base(35) interaction was defined as "non ubiquitous" (Dix et al., 1986) or "non-essential" (Ashraf et al., 1999a).

Here, by analyzing available tRNA crystal structures (Fig. 1 and Table 1), relevant biochemical data, and results from molecular dynamics simulations (Auffinger et al., 1996, 1999; Auffinger & Westhof, 1996), we present evidence indicating that, to maintain the canonical structure of the anticodon loop independent of the type of the base present at position 35 (either a purine or a pyrimidine), the hydrogen bond acceptor and donor potential of the (U33)O2′-H group is alternatively used. In other words, a "universal" interaction linking the sugar of residue 33 to the base of residue 35 is part of the anticodon loop signature.

TABLE 1. Description of the crystal structures of complexed and uncomplexed tRNA molecules referenced in the Nucleic Acid Database (Berman et al., 1992) before November 1, 2000.

NDB code[a]	tRNA type	Resolution (Å)	Distance (Å)[b]	Angle (°)[b]
With A35			(A35)N7…O2′(U33)	(A35)N7…H-O2′(U33)
tr0001	yeast $tRNA^{Phe}$	1.9	2.4	167
tr0002	yeast $tRNA^{Phe}$	2.0	2.2	138
trna03	yeast $tRNA^{Phe}$	3.0	2.9	88
trna04	yeast $tRNA^{Phe}$	2.7	3.5	80
trna06	yeast $tRNA^{Phe}$	3.0	2.4	114
trna09	yeast $tRNA^{Phe}$	3.0	2.8	92
trna10	yeast $tRNA^{Phe}$	3.0	2.8	93
pr0024	*T. Thermo.* $tRNA^{Phe}$/RS	3.3	2.2	120
ptr012	yeast $tRNA^{Phe}$/EF-TU	2.7	3.2	96
		2.8 (0.4)[c]	2.7 (0.5)[c]	110 (28)[c]
With U35			(U35)C5…O2′(U33)	(U35)C5-H…O2′(U33)
trna05	yeast $tRNA^{Asp}$	3.0	3.4	101
trna07	yeast $tRNA^{Asp}$	3.0	4.4	106
trna08	yeast $tRNA^{Asp}$	3.0	3.3	104
tr0003	yeast $tRNA^{Lys,3}$	3.3	3.0	94
		3.1 (0.2)[c]	3.5 (0.6)[c]	101 (5)[c]
With C35			(C35)C5…O2′(U33)	(C35)C5-H…O2′(U33)
pr0004	*E. coli* $tRNA^{Cys}$/EF-TU	2.6	3.1	86

[a]Crystal structure references are as follows: tr0001: Shi & Moore, 2000; tr0002: Jovine et al., 2000; tr0003: Bénas et al., 2000; trna03: Brown et al., 1985; trna04: Sussman et al., 1978; trna05: Comarmond et al., 1986; trna06: Westhof & Sundaralingam, 1986; trna07, trna08, trna09: Westhof et al., 1988; trna10: Hingerty et al., 1978; pr0004: Nissen et al., 1999; pr0024: Goldgur et al., 1997; ptr012: Nissen et al., 1995.

[b]Criteria generally accepted for defining regular and C-H…O hydrogen bonds are: d(D…A) < 3.5 Å and Θ(D-H…A) $> 120°$, and d(C…A) < 4.0 Å and Θ(C-H…A) $> 90°$, respectively (Jeffrey & Saenger, 1991; Wahl & Sundaralingam, 1997).

[c]Values in parentheses represent standard deviations from mean values.

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RESULTS AND DISCUSSION

Case 1: A purine (R) at position 35

In tRNAs, purines represent roughly a little more than 50% of the nucleotides present at position 35 (Auffinger & Westhof, 1998b) as expected from the genetic code and deduced from the 550 tRNAs and 3,704 tDNA gene sequences itemized in the tRNA database (Sprinzl et al., 1998). From the nine available crystallographic structures of yeast $tRNA^{Phe}$ (including the structures extracted from the yeast $tRNA^{Phe}$/EF-TU and the *Thermus thermophilus* $tRNA^{Phe}$/RS complexes where the anticodon loop structure is not altered, ptr012 and pr0024, respectively, see Table 1), it has been inferred that the 2′-hydroxyl group of U33 forms a hydrogen bond with the N7 atom of A35 (Fig. 2). This assumes that the hydroxyl (U33)O2′-H group points toward the (A35)N7 atom (Quigley & Rich, 1976; Westhof et al., 1988), which seems reasonable given the average (U33)O2′…N7(A35) distance of ≈2.7 Å and the average (U33)O2′-H…N7(A35) angle of ≈110° (Table 1). In this orientation, the (U33)O2′-H group is located in the "*Base*" conformational domain (Fig. 3) where the hydrogen points usually toward the O2 or N3 atom of the attached base (Auffinger & Westhof, 1997). With a guanine at position 35, the (U33)O2′-H…N7(R35) would certainly be formed as well and would be part of a bifurcated hydrogen bond involving the (G35)O6 atom (Fig. 2).

Case 2: A pyrimidine (Y) at position 35

With a pyrimidine at position 35, the hydrogen bonding pattern is slightly less obvious. In this case, the (Y35)C5-H group replaces the (R35)N7 acceptor atom. Thus, no hydrogen-bond utilizing the donor potential of

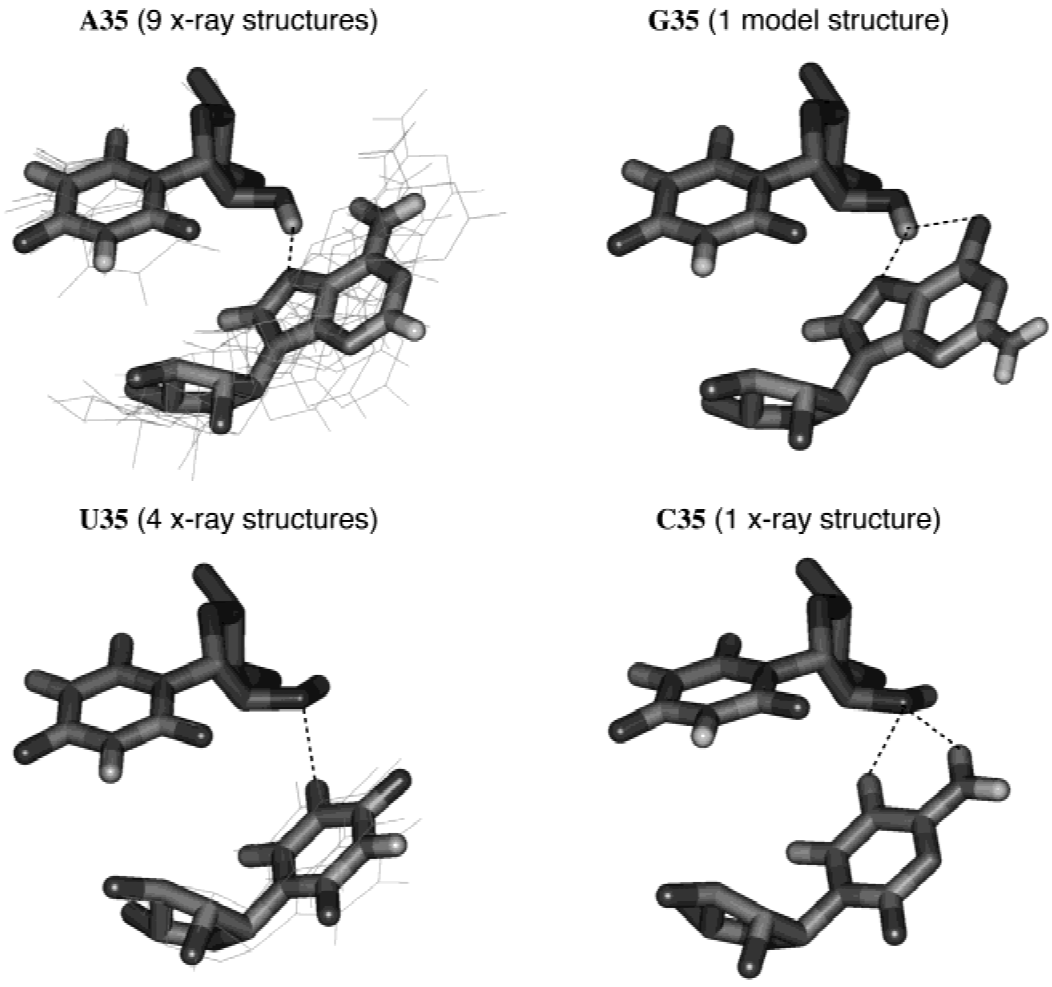

FIGURE 2. Hydrogen bond interaction between the ribose of U33 and the base 35 inferred from crystal structure analysis (see Table 1). Top left: Average structure calculated from nine yeast $tRNA^{Phe}$ crystal structures drawn with thin lines. Top right: Model structure of a U33-G35 interaction showing a bifurcated hydrogen bond. Bottom left: Average structure calculated from four tRNA crystal structures drawn with thin lines. Bottom right: View of a U33-C35 interaction extracted from the crystal structure of *E. coli* $tRNA^{Cys}$. The hydrogen atoms have been added by hand on the basis of structural considerations for tRNA(A35) and MD simulations for tRNA(U35) (see Fig. 3) and have been extrapolated for tRNA(G35) and tRNA(C35).

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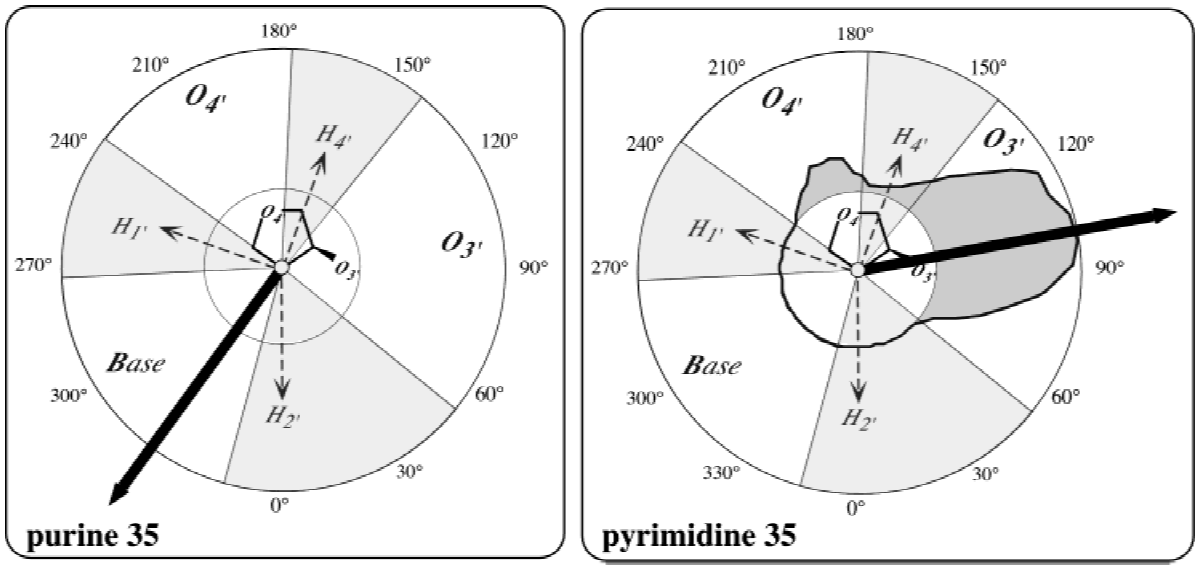

FIGURE 3. Conformational preferences for the (U33)O2′-H group. Left and right: Conformational wheel outlining the three favored O3′, O4′ and "Base" domains and the three forbidden H1′, H2′, and H4′ domains (in gray) for the orientations of the 2′-OH group of a ribose in a C3′-*endo* conformation as deduced from MD simulations (Auffinger & Westhof, 1997). The C2′-O2′ axis is perpendicular to the plane of the page. Left: With a purine at position 35, the (U33)O2′-H bond (bold arrow) is located in the "base" domain with an average H2′-C2′-O2′-H dihedral angle close to 323° as deduced from structural considerations involving the position of the (R35)N7 atom. Right: With a pyrimidine at position 35, the (U33)O2′-H bond (bold arrow) is located in the O3′ domain with an average H2′-C2′-O2′-H dihedral angle close to 108° as deduced from the distribution of the orientations adopted by the (U33)O2′-H deduced from MD simulations (Auffinger & Westhof, 1997) and shown by the gray curve which has for baseline the internal circle.

the (U33)O2′-H group can be formed. Instead, from stereochemical considerations, it is possible to infer that a C-H…O interaction, namely the (U33)O2′…H-C5(Y35) contact, can be formed (Fig. 2). The average (U33)O2′…C5(U35) distance (≈3.5 Å, see Table 1) and the average (U33)O2′…H-C5(U35) angle (≈101°) are compatible with the formation of a weak hydrogen bond (Wahl & Sundaralingam, 1997; Desiraju & Steiner, 1999). In this case, the (U33)O2′-H bond points away from the C5-H group. Multiple molecular dynamics (MMD) simulations of the yeast $tRNA^{Asp}$ anticodon hairpin, with explicit consideration of the solvent, support the existence of such a C-H…O interaction (Auffinger et al., 1996; Auffinger & Westhof, 1996). From these MMD simulations, and from a MD simulation of the entire yeast $tRNA^{Asp}$ molecule in an aqueous environment (Auffinger et al., 1999), it has been observed that the (U33)O2′-H group is located in the O3′ conformational domain (Fig. 3) and, therefore, points systematically away from the (U35)C5-H bond. The average (U33)O2′…C5(C35) distance estimated from the molecular dynamics simulations is close to 3.5 Å.

Alternatively, from a recent NMR structure of the anticodon hairpin of *Escherichia coli* $tRNA^{Lys,3}$ including all the modified nucleotides (Sundaram et al., 2000), the average (U33)O2′…C5(U35) distance is calculated to be close to 5.0 (±0.1) Å. Although the (U33)O2′ and (U35)C5 atoms are facing each other, these data invalidate the existence of a (U33)O2′…H-C5(U35) interaction and support instead the presence of a hydration pocket delimited by the (U33)O2′ and the (U35)C5 atoms. Yet, given the position of the hydrophilic atoms that delineate this putative binding site, a stable water molecule at this location is not really expected and is, indeed, not observed in the crystal structures. Therefore, it seems reasonable to propose that the long (U33)O2′…C5(U35) distance may result from a local lack of NMR constraints that are particularly difficult to collect in loop regions. Another example emphasizing the difficulty of deriving precise distances from NMR experiments in loop regions is given by the strong (U33)N3-H…OR-P(U36) hydrogen bond. The average (U33)N3…OR-P(U36) distance of 3.2 (±0.4) Å derived from the NMR data is overestimated when compared to the distances of 2.8 and 2.6 Å extracted from the two recent high resolution crystal structures of yeast $tRNA^{Phe}$, tr0001 and tr0002, respectively. Thus, although NMR structural models lead to the very important conclusion that anticodon hairpins adopt folds in solution similar to those observed in tRNA crystal structures, they may, locally, lack precision for checking fine contacts.

When a cytosine is located at position 35, the (U33)O2′-H group may establish a "stronger" hydrogen bond with the (C35)NH2 group, as was proposed earlier (Quigley & Rich, 1976), rather than with the (C35)C5-H group. The recent $tRNA^{Cys}$ structure (extracted from the *E. coli* tRNA complex with EF-Tu,

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pr0004) points rather to a bifurcated interaction involving both the (C35)NH2 and the (C35)C5-H donor groups and the (U33)O2′ acceptor atom (Fig. 2).

Modified nucleotides at positions 33 and 35

As deduced from the tRNA sequences extracted from the tRNA database (Sprinzl et al., 1998), modified nucleotides are absent at position 33, and are very rare at position 35 (<1%) and 36 (<2%) (Auffinger & Westhof, 1998b). At the latter locations, modifications of the Watson–Crick sites would prevent recognition of the mRNA. Similarly, at position 35, methylation of the (R)N7 and the (Y)C5 sites would prevent the formation of the identified interaction between the base of residue 35 and the ribose of residue 33. Interestingly, the only modified base that is tolerated at position 35 is a pseudouridine (Ψ) in which the less hydrophilic C5-H group found in pyrimidines is replaced by a N1-H group (Charette & Gray, 2000). This observation constitutes a strong evidence for the formation of a (U33)O2′…H-C5(Y35) interaction because a Ψ35 allows for the formation of a (U33)O2′…H-N1(Ψ35) hydrogen bond in replacement of the weaker (U33)O2′…H-C5(U35) interaction without perturbing the loop structure (Fig. 4).

On the basis of experimental data, it has been proposed that the Ψ35 modification increases the activity of yeast suppressor $tRNA^{Tyr}$(UΨA) by increasing the stability of the anticodon(Ψ)–codon(A) interaction (Johnson & Abelson, 1983). In plants, modified cytoplasmic $tRNA^{Tyr}$(GΨA) is required for an efficient recognition of the UAG or UAA codons, whereas the unmodified GUA anticodon does not recognize the same UAG or UAA codons (Zerfass & Beier, 1992). Recently, it has been proposed that Ψ35 in echinoderm mt $tRNA^{Asn}$ is involved in the decoding of the three AAC, AAU, and AAA codons, whereas the U35 variant can only decode the two AAC and AAU codons (Tomita et al., 1999). In these systems, strengthening of the anticodon(Ψ35)–codon(A) interaction is necessary and is helped presumably through the formation of a (Ψ35)N1-H…O2′(U33) hydrogen bond equivalent to a (U35)C5-H…O2′(U33) interaction.

Several biochemical studies with natural or chemically modified nucleotides at various locations of the anticodon loop were designed with the goal of assessing the existence and significance of specific tertiary interactions. It has been shown that modifications of U33 do not affect the aminoacylation ability of yeast $tRNA^{Phe}$ (Wittenberg & Uhlenbeck, 1985) for which a canonical structure of the anticodon loop is not required. Yet, numerous examples indicate that modifications of U33 (for example, U33 to m^3U, C33, dU33, Um, m^6U, or D) introduced in wild-type tRNA sequences (Uhlenbeck et al., 1982; Dix et al., 1986) and tRNA transcripts (von Ahsen et al., 1997; Ashraf et al., 1999a, 1999b) alter the translational efficiency, supporting the idea that the ability of tRNA anticodon hairpins to adopt a canonical structure involving an intact U-turn is mandatory for ribosome binding. Methylation of the N3 atom that prevents the formation of the (U33)N3-H…OR-P(A36) interaction shows especially strong effects on the translational efficiency (Uhlenbeck et al., 1982; Dix et al., 1986; von Ahsen et al., 1997). Similarly, methylation of the U33 ribose of yeast $tRNA^{Phe}$ as well as the U33 → dU33 substitution that prevents

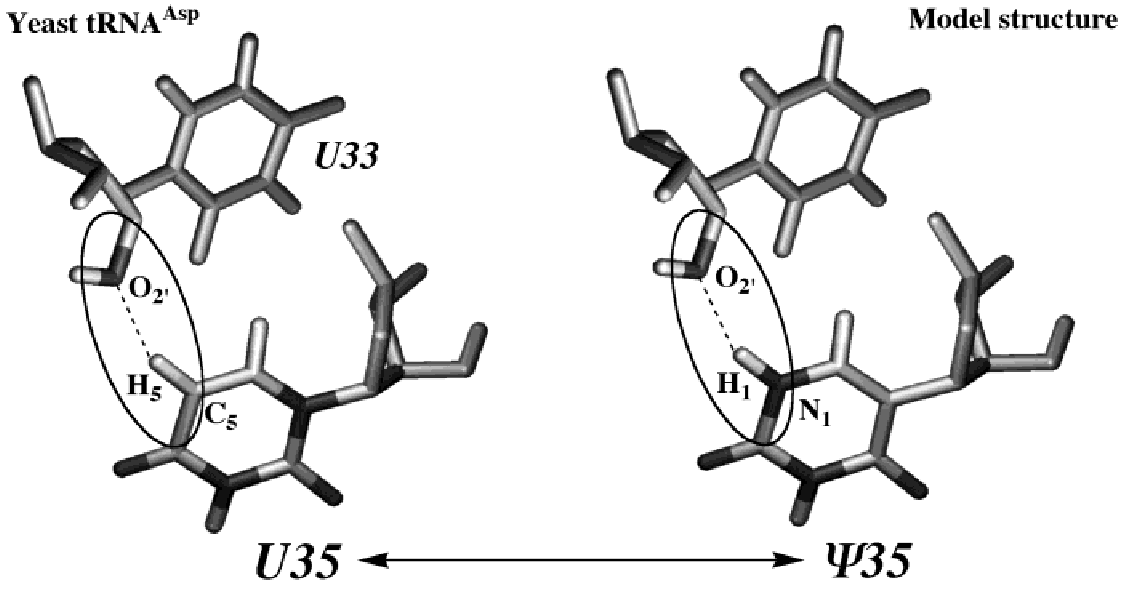

FIGURE 4. Substitution of a pyrimidine at position 35 of the anticodon loop by a pseudouridine. Left: C5-H…O2′ interaction inferred from tRNA(U35) crystal structures and MD simulations (see Figs. 2 and 3). Right: A U35 → Ψ35 substitution strengthens the anticodon loop structure by replacing a C-H…O contact by a N-H…O bond.

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the formation of the (U33)O2′-H…N7(U35) interaction alters the translational efficiency (Uhlenbeck et al., 1982; Dix et al., 1986). To a lesser extent, it has been shown that the U33 → dU33 substitution affects the translational efficiency of tRNAs containing pyrimidines at position 35 (von Ahsen et al., 1997). This confirms the fact that, in tRNA(Y35), a weaker interaction involving the (U33)O2′-H group replaces the yeast $tRNA^{Phe}$ (U33)O2′-H…N7(U35) hydrogen bond and leads to an alternative to a recent proposition suggesting that the overall uridine conformation, including a dynamic (C3′-endo ↔ C2′-endo) sugar pucker, *anti* conformation, and ability of uracil to stack between C32 and A35-phosphate is more important than specific hydrogen bonds involving the (U33)N3-H and (U33)O2′-H groups (Ashraf et al., 1999a).

Extended canonical anticodon loop structure

From the preceding, an extended anticodon hairpin signature can be proposed. This signature summarizes all the available phylogenetic and structural considerations and includes interaction as characterized above between ribose33 and base35 (Fig. 5).

1. The 31–39 pair, which is the last base pair of the anticodon stem, is of the Watson–Crick type in more than 90% of the instances. Besides the G=C and C=G pairs (48%), the A31–U39 pairs dominate (37%) and are systematically modified into A31–Ψ39 pairs (Auffinger & Westhof, 1998a; Yarian et al., 1999). The systematic modification of A31-U39 pairs into A31–Ψ39 pairs has been shown to strengthen the anticodon hairpin structure to a level probably equivalent to hairpins with C31=G39 or G31=C39 pairs by forming a water mediated base–backbone interaction (Auffinger & Westhof, 1998a).
2. Among all the tRNA sequences, 93% of the 32•38 oppositions can be assigned to two families of isosteric base pairs (Auffinger & Westhof, 1999). The first family (86%) is characterized by the formation of a bifurcated hydrogen bond between the carbonyl group of a pyrimidine at position 32 and an amino group of a base located at position 38, and comprises the C32•A38, U32•A38, U32•C38, and C32•C38 pairs. The second family (7%) implies the formation of a U32•U38 non-Watson–Crick pair. A third family (7%) comprises a set of 11 infrequent 32•38 sequences that are not isosteric to the base pairs found in families 1 or 2. The proportion of G=C or C=G pairs is close to zero.
3. A (U33)N3-H…OR-P(36) hydrogen bond is recurrently observed in tRNA crystal structures and is part of the signature of U-turns and, thus, more specifically of the anticodon hairpin signature (Quigley & Rich, 1976; Jucker & Pardi, 1995).
4. A conserved interaction that utilizes either the donor or the acceptor hydrogen bond potential of the (U33)O2′-H group links the ribose of U33 to the base at position 35. With a purine at position 35, a (U33)O2′-H…N7(R35) bond is formed (Quigley & Rich, 1976), with a pyrimidine at position 35, a (U33)O2′…H-C5(Y35) interaction is observed.
5. Last, a stacking interaction involving the aromatic cycle of U33 and the OR atom of the phosphate group of nucleotide 35 is recurrently observed in all the known tRNA structures (Quigley & Rich, 1976).

Are anticodon hairpins present in other RNAs?

U-turns are ubiquitous in RNA structures (Jucker & Pardi, 1995; Gutell et al., 2000). A recent report (Carter et al., 2000) indicates that the spur hairpin element of the 16S ribosomal subunit may be a mimic of the anticodon hairpin. A close examination of the structure adopted by residues 75–95 of the 16S rRNA constituting the spur hairpin reveals that the structure of the stem loop is different from that of a canonical anticodon hairpin as defined above (Fig. 5). In the spur element, a G79=C89 Watson–Crick pair at a position equivalent to that of the anticodon 32•38 pair restricts the length of the loop to 5 nt instead of 7. Although the 16S spur element may be able to form Watson–Crick interactions with three bases of the codon and present an overall similarity to an anticodon hairpin, its signature does not match that of anticodon stem-loops. Among all the known motifs including a U-turn, only the tRNA anticodon hairpins are known to accommodate pyrimidines at position U + 2, the position equivalent to that of the residue 35 in tRNAs (Gutell et al., 2000). Thus, it appears that the anticodon hairpins constitute a very unique and specific class of structural motifs.

CONCLUSION

To be able to recognize specific motifs in sequence databases and to construct reliable three-dimensional models, it is of great importance to integrate a maximum of phylogenetic and structural information on specific structural motifs in a comprehensive signature. The present work provides an additional piece to the anticodon hairpin signature by gathering experimental and theoretical evidence indicating that the well-characterized (U33)O2′-H N7(R35) hydrogen bond can be replaced by a (U)O2′…H-C5(Y35) interaction. It is the first described occurrence where a classical hydrogen bond is replaced by a C-H…O hydrogen bond to form a sequence independent interaction. This is made possible by a donor/acceptor swap at the level of the (U33)O2′ hydroxyl.

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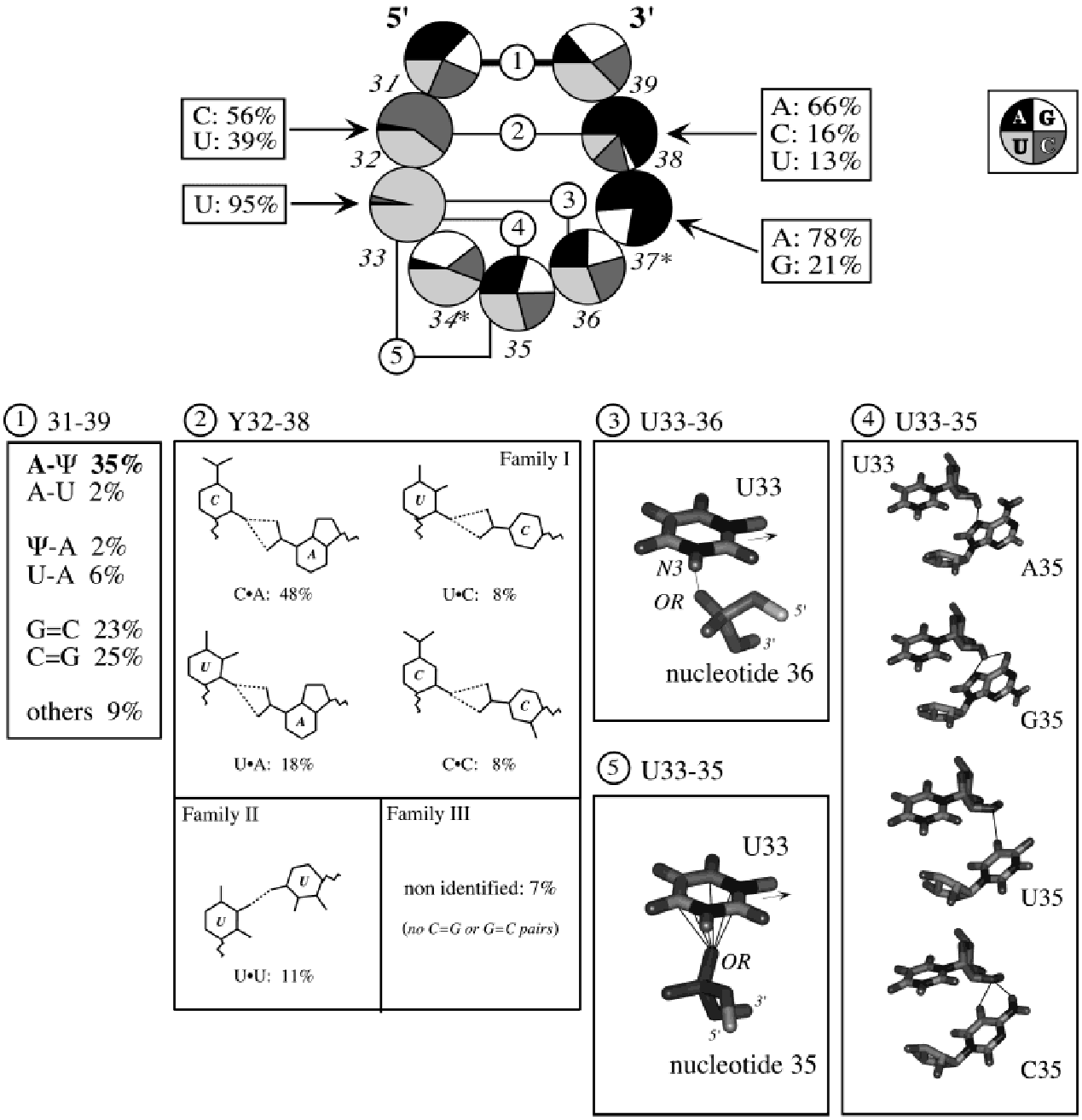

FIGURE 5. Detailed phylogenetic and structural characterization of the canonical structure of tRNA anticodon loops.

REFERENCES

Ashraf SS, Ansari G, Guenther R, Sochacka E, Malkiewicz A, Agris PF. 1999a. The uridine in "U-turn": Contributions to tRNA-ribosomal binding. *RNA 5*:503–511.

Ashraf SS, Guenther R, Agris PF. 1999b. Orientation of the tRNA anticodon in the ribosomal P-site: Quantitative footprinting with U33-modified anticodon stem and loop domains. *RNA 5*:1191–1199.

Auffinger P, Louise-May S, Westhof E. 1996. Molecular dynamics simulations of the anticodon hairpin of $tRNA^{Asp}$: Structuring effects of C-H…O hydrogen bonds and of long-range hydration forces. *J Am Chem Soc 118*:1181–1189.

Auffinger P, Louise-May S, Westhof E. 1999. Molecular dynamics simulations of the solvated yeast $tRNA^{Asp}$. *Biophys J 76*:50–64.

Auffinger P, Westhof E. 1996. H-bond stability in the $tRNA^{Asp}$ anticodon hairpin: 3 ns of multiple molecular dynamics simulations. *Biophys J 71*:940–954.

Auffinger P, Westhof E. 1997. Rules governing the orientation of the 2′-hydroxyl group in RNA. *J Mol Biol 274*:54–63.

Auffinger P, Westhof E. 1998a. Effects of pseudouridylation on tRNA hydration and dynamics: A theoretical approach. In: Grosjean H, Benne R, eds. *Modification and editing of RNA*. Washington, DC: American Society for Microbiology. pp 103–112.

Auffinger P, Westhof E. 1998b. Location and distribution of modified nucleotides in tRNA. In: Grosjean H, Benne R, eds. *Modification and editing of RNA*. Washington, DC: American Society for Microbiology. pp 569–576.

Auffinger P, Westhof E. 1999. Singly and bifurcated hydrogen-bonded base-pairs in tRNA anticodon hairpins and ribozymes. *J Mol Biol 292*:467–483.

https://doi.org/10.1017/S1355838201002382 Published online by Cambridge University Press

Bénas P, Bec G, Keith G, Marquet R, Ehresmann C, Ehresmann B, Dumas P. 2000. The crystal structure of HIV reverse transcription primer tRNA(Lys,3) shows a canonical anticodon loop. *RNA 6*:1347–1355.

Berman HM, Olson WK, Beveridge DL, Westbrook J, Gelbin A, Demeny T, Hsieh SH, Srinivasan AR. 1992. The nucleic acid database: A comprehensive relational database of three-dimensional structures of nucleic acids. *Biophys J 63*:751–759.

Brown RS, Dewan JC, Klug A. 1985. Crystallographic and biochemical investigation of the lead(II)-catalyzed hydrolysis of yeast phenylalanine tRNA. *Biochemistry 24*:4785–4801.

Carter AP, Clemons WM, Brodersen DE, Morgan-Warren RJ, Wimberly BT, Ramakrishnan V. 2000. Functional insights from the structure of the 30S ribosomal subunit and its interactions with antibiotics. *Nature 407*:340–348.

Cate JH, Yusupov MM, Yusupova GZ, Earnest TN, Noller HF. 1999. X-ray crystal structures of 70S ribosome functional complexes. *Science 285*:2095–2104.

Charette M, Gray MW. 2000. Pseudouridine in RNA: What, where, how, and why. *IUBMB Life 49*:341–351.

Comarmond MB, Giegé R, Thierry JC, Moras D. 1986. Three-dimensional structure of yeast $tRNA^{Asp}$. I. Structure determination. *Acta Cryst B 42*:272–280.

Desiraju G, Steiner T. 1999. *The weak hydrogen bond.* New York: Oxford University Press.

Dix DB, Wittenberg WL, Uhlenbeck OC, Thompson RC. 1986. Effect of replacing uridine 33 in yeast $tRNA^{Phe}$ on the reaction with ribosomes. *J Biol Chem 261*:10112–10118.

Goldgur Y, Mosyak L, Reshetnikova L, Ankilova V, Lavrik O, Khodyreva S, Safro M. 1997. The crystal structure of the phenylalanyl-tRNA synthetase from *Thermus thermophilus* complexes with cognate $tRNA^{Phe}$. *Structure 5*:59–68.

Grosjean H, Cedergreen RJ, McKay W. 1982. Structure in tRNA data. *Biochimie 64*:387–397.

Gutell RR, Cannone JJ, Konings D, Gautheret D. 2000. Predicting U-turns in ribosomal RNA with comparative sequence analysis. *J Mol Biol 300*:791–803.

Hingerty B, Brown RS, Jack A. 1978. Further refinement of the structure of yeast $tRNA^{Phe}$. *J Mol Biol 124*:523–534.

Jeffrey GA, Saenger W. 1991. *Hydrogen bonding in biological structures.* Berlin: Springer-Verlag.

Johnson PF, Abelson J. 1983. The yeast $tRNA^{Tyr}$ gene intron is essential for correct modification of its tRNA product. *Nature 302*:681–687.

Jovine L, Djordjevic S, Rhodes D. 2000. The crystal structure of yeast phenylalanine tRNA at 2.0 Å resolution: Cleavage by Mg(2+) in 15-year old crystals. *J Mol Biol 301*:401–414.

Jucker FM, Pardi A. 1995. GNRA tetraloops make a U-turn. *RNA 1*:219–222.

Nissen P, Kjeldgaard M, Thirup S, Polekhina G, Reshetnikova L, Clark BFC, Nyborg J. 1995. Crystal structure of the ternary complex of Phe-$tRNA^{Phe}$, EF-Tu and a GTP analog. *Science 270*:1464–1472.

Nissen P, Thirup S, Kjeldgaard M, Nyborg J. 1999. The crystal structure of Cys-$tRNA^{Cys}$-EF-Tu-GDPNP reveals general and specific features in the ternary complex and in tRNA. *Structure 7*:143–154.

Quigley GJ, Rich A. 1976. Structural domains of transfer RNA molecules. *Science 194*:796–806.

Schnitzler W, von Ahsen U. 1997. Identification of specific Rp-phosphate oxygens in the tRNA anticodon loop required for ribosomal P-site binding. *Proc Natl Acad Sci USA 94*:12823–12828.

Shi H, Moore PB. 2000. The crystal structure of yeast phenylalanine tRNA at 1.93 Å resolution: A classic structure revisited. *RNA 6*:1091–1105.

Sprinzl M, Horn C, Brown M, Loudovitch A, Steinberg S. 1998. Compilation of tRNA sequences and sequences of tRNA genes. *Nucleic Acids Res 26*:148–153.

Sundaram M, Durant PC, Davis DR. 2000. Hypermodified nucleosides in the anticodon of $tRNA^{Lys}$ stabilize a canonical U-turn structure. *Biochemistry 39*:12575–12584.

Sussman JL, Holbrook SR, Warrant RW, Church GM, Kim S-H. 1978. Crystal structure of yeast phenylalanine transfer RNA. I. Crystallographic refinement. *J Mol Biol 123*:607–630.

Tomita K, Ueda T, Watanabe K. 1999. The presence of pseudouridine in the anticodon alters the genetic code: A possible mechanism for assignment of the AAA lysine codon as asparagine in echinoderm mitochondria. *Nucleic Acids Res 27*:1683–1689.

Uhlenbeck OC, Lowary PT, Wittenberg WL. 1982. Role of the constant uridine in binding of yeast $tRNA^{Phe}$ anticodon arm to 30S ribosomes. *Nucleic Acids Res 10*:3341–3352.

von Ahsen U, Green R, Schroeder R, Noller HF. 1997. Identification of 2′-hydroxyl groups required for interaction of a tRNA anticodon stem-loop region with the ribosome. *RNA 3*:49–56.

Wahl CM, Sundaralingam M. 1997. C-H…O hydrogen bonding in biology. *Trends Biochem Sci 22*:97–102.

Westhof E, Dumas P, Moras D. 1988. Restrained refinement of two crystalline forms of yeast aspartic acid and phenylalanine transfer RNA crystals. *Acta Cryst A44*:112–123.

Westhof E, Sundaralingam M. 1986. Restrained refinement of the monoclinic form of yeast phenylalanine transfer RNA. Temperature factors and dynamics, coordinated waters, and base-pair propeller twist angles. *Biochemistry 25*:4868–4878.

Wittenberg WL, Uhlenbeck OC. 1985. Specific replacement of functional groups of uridine-33 in yeast phenylalanine transfer ribonucleic acid. *Biochemistry 24*:2705–2712.

Yarian CS, Basti MM, Cain RJ, Ansari G, Guenther RH, Sochacka E, Czerwinska G, Malkiewicz A, Agris PF. 1999. Structural and functional roles of the N1- and N3-protons of Ψ at tRNA's position 39. *Nucleic Acids Res 27*:3543–3549.

Zerfass K, Beier H. 1992. Pseudouridine in the anticodon GΨA of plant cytoplasmic $tRNA^{Tyr}$ is required for UAG and UAA suppression in the TMV-specific context. *Nucleic Acids Res 20*:5911–5918.

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