The λ-Turn: A New Structural Motif in Ribosomal RNA

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Abstract. RNA structural motifs are recurrent structural elements occurring in RNA molecules. They play essential roles in consolidating RNA tertiary structures and in binding proteins. Recently, we identified a new type of RNA structural motif, namely λ-turn, from ribosomal RNAs. This motif has a helix-internal loop-helix structure. The directions of its two helices are changed ~90° due to the existence of the internal loop. A guanine from the 3′-end of the internal loop extrudes out and forms a base triple with a G-C WC base pair from one helix of the motif. From the global perspective, the λ-turn is often capped by a helix and a tetraloop. A nucleotide between the capped helix and the tetraloop forms a consecutive base triple next to the first one with a G-C pair from the same helix that the first G-C pair resides. The λ-turn motif has a consensus sequence pattern and its 3D structure is conserved across different species. All the identified λ-turns are located on surfaces of ribosomal RNAs. Structures of ribosomes reveal direct interactions between λ-turns and ribosomal proteins. All these observations indicate that λ-turns have an important role in binding with ribosomal proteins.

Keywords: λ-Turn · RNA structural motif · Ribosomal RNA

1 Introduction

RNA structural motifs are recurrent structural elements occurring in RNA tertiary structures and they play essential roles when RNAs performing their functions in various biological processes (Moore 1999; Hendrix et al. 2005; Leontis et al. 2006; François et al. 2005). Furthermore, they are key components to consolidate RNA tertiary structures. In view of their importance, researchers endeavour to understand RNA motifs with their structures, characteristics, and functions. Currently, more than twenty types of RNA structural motifs have been identified (Woese et al. 1990; Szewczak et al. 1993; Cate et al. 1996; Chang and Tinoco 1994; Klein et al. 2001;

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Nissen et al. 2001; Wadley and Pyle 2004; Leontis and Westhof 2003). However, the steps of discovering new motifs will never stop. For instance, motifs like G-ribo and adenosine wedge, to name a few, have been recently identified. (Steinberg and Boutorine 2007; Jaeger et al. 2009; Gagnon and Steinberg 2010; Shen et al. 2013). These newly identified motifs greatly enrich our knowledge about RNA structures and their functions.

In this paper, we present a new type of RNA structural motif, namely λ-turn, the name of which is given by the shape of its characteristic strand. We successfully identified nine λ-turns from ribosomal RNAs (rRNAs). Based on observations on these nine instances, we find that the λ-turn motif has a consensus sequence pattern and its 3D structure is conserved across different species. In addition, the structure of the λ-turn has several distinct characteristics. Firstly, the λ-turn contains two helices which are arranged orthogonally. Secondly, the two helices are connected by a bulge which is composed by 2–4 nucleotides. Thirdly, a guanine located at the 3′-end of the bulge extrudes out and forms a triple base pair with the first G-C pair on a helix of the motif. All the identified λ-turns are located on surfaces of ribosomal RNAs, which implies that they may be involved in binding with other molecules, such as ribosomal proteins. The observations on four structures of ribosomes further support this assumption. In these ribosomes, λ-turns have direct interactions with ribosomal protein S17 or S11, which suggests that λ-turns play an important role when rRNAs try to bind with ribosomal proteins. Any mutation in positions of λ-turns may affect the stableness of ribosome structures.

2 Results

2.1 Definition of the λ-Turn

Similar to the kink-turn motif, the λ-turn is a helix-internal loop-helix-structure motif. It is composed of two strands containing 14–17 nucleotides (see Fig. 1(A)). The longer strand, which contains an unpaired region, is regarded as the characteristic strand. The other strand in the motif is called the complementary strand. Two strands form two segments of helices which are arranged orthogonally in the λ-turn. The first helix (Helix 1 in Fig. 1(A)), which is called the "UG-stem", is composed of two base pairs, a C-G WC pair and a U•G wobble base pair. The second helix (Helix 2 in Fig. 1(A)), which is called "AU-stem", is composed of four base pairs, two A-U (or U-A) pairs followed by two G-C pairs. Between the two helices, there is a bulge which is formed by the unpaired region of the characteristic strand. The bulge contains 2–4 nucleotides. At its 3′-end, there is always a guanine which interacts with the first G-C pair on the AU-stem and they together form a base triple (see Fig. 1(A)). The other residues in the bulge extrude out to form remote interactions with residues from other parts of the RNA to consolidate the tertiary structure of the RNA molecule.

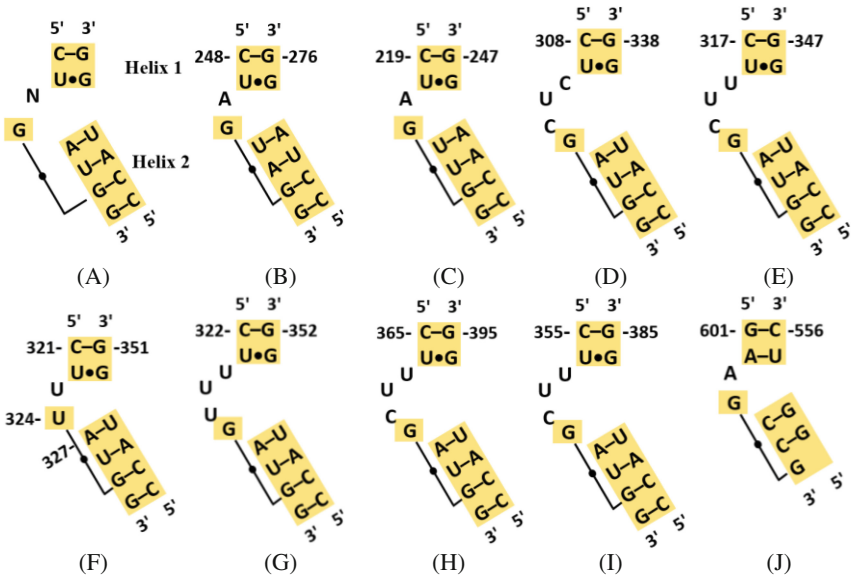


Fig. 1. Diagrams of secondary structure of λ-turns found in ribosomal RNAs. Solid lines represent Watson-Crick (WC) base pairings and dots represent non-WC base pairings. Yellow shading indicates the consensus sequence pattern. (A) The consensus sequence pattern of λ-turn. N represents one or three nucleotides of any type; (B) A:C248-G255/C271-G276 (PDB:2AW7); (C) A:C219-G226/C242-G247 (PDB:3BBN); (D) A:C308-G317/C333-G338 (PDB:2XZM); (E) 6:C317-G326/C342-G347 (PDB:3U5F); (F) A:C321-G330/C346-G351 (PDB:3IZ7); (G) 2:C322-G331/C347-G352 (PDB:3J3C); (H) 2:C365-G374/C390-G395 (PDB:3J3D); (I) i:C355-G364/C380-G385 (PDB:4KZX); (J) 3:G601-G607/G553-C556 (PDB:1S1I) (Color figure online).

2.2 Identification of λ-Turns

We have searched 550 RNA complexes (their PDB ID have been listed in Table S1 in the supplementary data) and identified nine instances of the λ-turn from nine rRNAs, which include two 16S rRNAs (PDB:2AW7, PDB:3BBN), six 18S rRNAs (PDB:2XZM, PDB:3U5F, PDB:3IZ7, PDB:3J3C, PDB:3J3D, PDB:4KZX), and a 5.8S/25S rRNA (PDB:1S1I). Since 16S and 18S rRNAs are homologous gene products, λ-turns mostly reside in 16S/18S rRNAs. The sequences of nine λ-turns have been listed in Table S2 in the supplementary data. It needs to be stressed that, we didn't find λ-turn-like structures from non-ribosomal RNAs.

2.3 Consensus Pattern of λ-Turns

The diagrams of secondary structure of nine identified λ-turns have been shown in Fig. 1(B)–(J). These diagrams clearly reveal that the λ-turn is composed of two strands which form two segments of helices and a bulge connecting the two helices. The first helix (Helix 1) contains a WC base pair (C-G) and a wobble base pair (U•G). It can be

observed that eight λ-turns from 16S/18S rRNAs share the same components of Helix 1 (see Fig. 1(B)–(I)). It indicates that, in the evolution of 16S/18S rRNAs, no substitution takes place in Helix 1. However, in the λ-turn from 5.8S/25S rRNA, the C-G pair is replaced by a G-C pair and the U•G pair is replaced by an A-U pair (see Fig. 1(J)). The difference is possibly because that 16S/18S rRNAs are not homologous with 5.8S/25S rRNAs.

The second helix (Helix 2) contains four WC base pairs: two A-U (or U-A) pairs and two G-C pairs. In 16S rRNAs, Helix 2 starts with a U-A pair, followed by an A-U (Fig. 1(B)) or a U-A pair (Fig. 1(C)). As to cases of 18S rRNAs, Helix 2 always starts with an A-U pair, followed by a U-A pair (see Fig. 1(D)–(I)). The bases in the position of two G-C pairs remain the same across all λ-turns from 16S and 18S rRNAs. It indicates that, in the evolution from prokaryotes to eukaryotes, the substitution takes place in the position of two A-U/U-A base pairs in Helix 2. But it doesn't happen in the position of the following two G-C pairs. However, such pattern does not exist in the instance from 5.8S/25S rRNA, where A-U and U-A pairs are substituted by two C-G pairs (see Fig. 1(J)). In addition, the following two G-C pairs are replaced by a single guanine. It can be seen that the base-pair composition of Helix 2 in 5.8S/25S is quite different from those in 16S/18S rRNAs.

Between two helices, there is a bulge connecting them. The bulge contains two or four nucleotides (see Fig. 1(B)–(J)). Compared with the conserveness of two helices, the components of the bulge vary a lot across nine λ-turns, which suggests that substitutions occurring in the bulge are more frequent than those occurring in helices. The content of the bulge can be AG, CUCG, UUCG, UU, and UUUG. The nucleotide at the 3′-end of the bulge is often a guanine except the instance in PDB:3IZ7, where the guanine is replaced by a uracil. The base of the guanine has a remote interaction with bases of the first G-C pair in Helix 2. They together form a base triple which helps to consolidate the structure of the λ-turn. Three bases in the base triple are coplanar and their interaction diagrams have been shown in Fig. 2. There are two ways of interactions occurring in the base triple. The first one is a cis-WC-WC (cWW) interaction between the G-C WC pair in Helix 2, which connects WC edges of the guanine and the cytosine. The second one is a trans-Hoogsteen-WC (tHW) interaction, which connects the Hoogsteen edge of the guanine (or the cytosine) from the G-C pair in Helix 2 and the WC edge of the inserted guanine (or uracil) (see Fig. 2(A)–(C)). Therefore, the base triple in the λ-turn can be represented as a combination of cWW and tHW (cWW/tHW) interactions.

Since G/U-GC base triples appear in all nine λ-turns, they seem indispensable for λ-turns' formation. We also notice that, in all the base triples the G-C WC pair remains the same. Therefore, we reach to the conclusion that the first G-C pair in Helix 2 has an important role in forming the base triple and in consolidating the λ-turn's structure. At the same time, we are wondering what makes the first G-C pair in Helix 2 non-substituted. To answer this question, we looked into the RNA Base Triples Database (Petrov et al. 2013) and investigated the family of cWW/tHW, the type of which G/U-GC base triples in λ-turns belong to. In the cWW/tHW family, the total number of RNA fragments discovered by FR3D (Sarver et al. 2008) is 80, among which there are 25 G-GC triples. In other families involving cWW interactions (such as cWW/cHW, cWW/cHS), G-GC and U-GC triples also account for a large proportion.

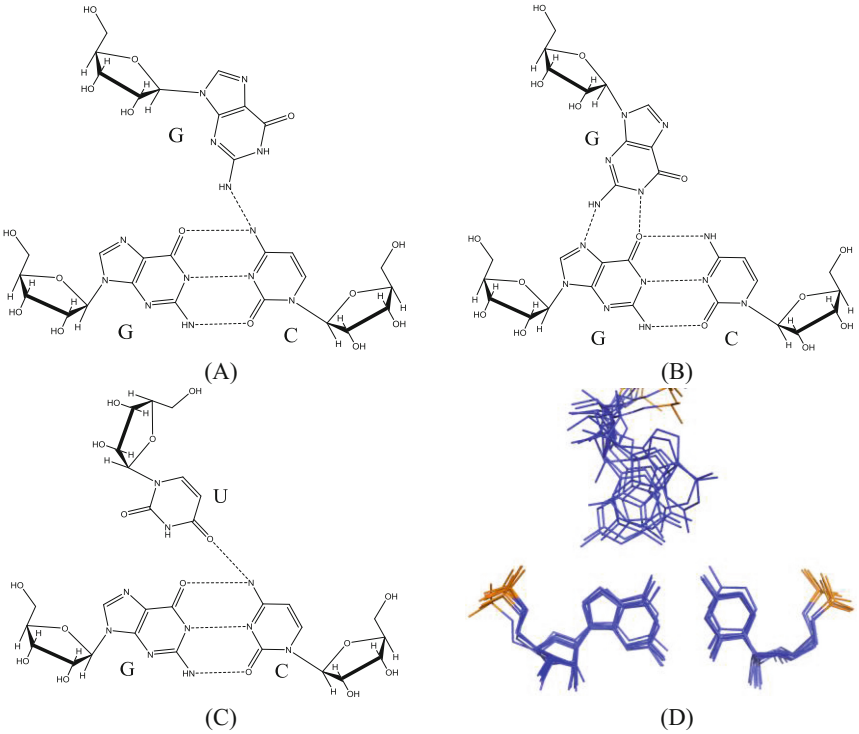


Fig. 2. Interaction diagrams of the G-GC and U-GC base triples occurring in λ-turns. (A) The G-GC base triple with interactions occurring between the inserted guanine and the cytosine from G-C WC pair; (B) the G-GC base triple with interactions occurring between the inserted guanine and the guanine from G-C WC pair; (C) the U-GC base triple with interactions occurring between the inserted uracil and the cytosine from G-C WC pair; (D) structure alignment of nine triple base pairs.

The great number of G/U-GC triples indicates that their structures are more stable than other types of base triples. It further suggests that, as long as the nucleotide at the 3′-end of the bulge is a guanine, the G-C pair in Helix 2 prefers to be non-substituted. Otherwise the stableness of the base triple will be weakened. Interestingly, in the case of PDB:3IZ7, the guanine in the bulge has been replaced by a uracil. We can't help wondering that, in this circumstance, whether the G-C pair could be substituted by an A-U pair without affecting the stableness of the base triple and the whole RNA structure[1]. However, the consequence of such substitution remains unclear.

The tertiary structure of the λ-turn has some distinct characteristics. Firstly, the directions of two helices are changed ∼90° due to the existence of the bulge (Fig. 3

[1] The replacement of G-C pair to A-U pair is possible. First, the cytosine is substituted by a uracil which consequently forms a G•U wobble pair. Then, the guanine is substituted by an adenosine and A-U pair will be formed finally. The U-AU triples are also abundant in the RNA Base Triples Database.

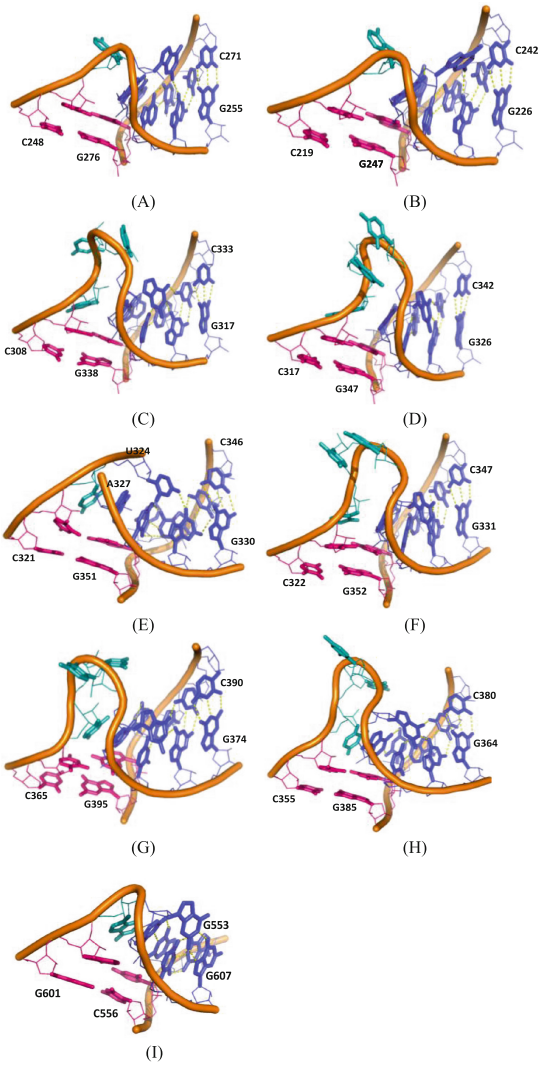
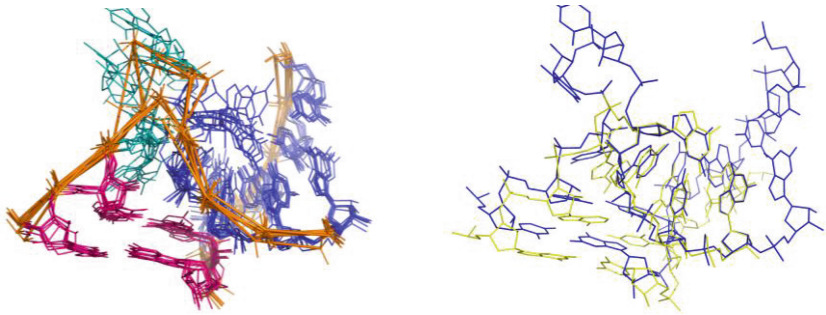


Fig. 3. The tertiary structures of nine identified λ-turns. UG stems are shown in magenta, AU stems are shown in blue, and the internal loops are shown in teal. (A) A:C248-G255/C271-G276 (PDB:2AW7); (B) A:C219-G226/C242-G247 (PDB:3BBN); (C) A:C308-G317/C333-G338 (PDB:2XZM); (D) 6:C317-G326/C342-G347 (PDB:3U5F); (E) A:C321-G330/C346-G351 (PDB:3IZ7); (F) 2:C322-G331/C347-G352 (PDB:3J3C); (G) 2:C365-G374/C390-G395 (PDB:3J3D); (H) i:C355-G364/C380-G385 (PDB:4KZX); (I) 3:G553-C556/G601-G607 (PDB:1S1I) (Color figure online).

(A)–(I)). Secondly, the nucleotides in the bulge extrude out and have remote interactions with other parts of RNA molecule. Despite that nine λ-turns are identified from

different species, such as E. coli and Homo sapiens, their structures are quite conserved. To verify our claim, we aligned structures of eight λ-turns from 16S/18S rRNAs based on the backbones of helices using PyMOL software and we use RMSD (Root Mean Square Distance) to measure the similarity of two λ-turns (see Fig. 4(A)). The largest RMSD is merely 2.996 Å and the average RMSD is 0.8944 Å (all RMSD values can be found in Table S3 in the supplementary data). The conserveness of structures of λ-turns



(A) (B)

Fig. 4. Structure alignment of λ-turns. (A) Structure alignment of λ-turns from 16S/18S rRNAs based on the backbones of their helices; (B) structure alignment of λ-turns from 5.8/25S rRNA (PDB:1S1I, show in yellow) and 18S rRNA (PDB:3U5F, show in blue) based on positions of G-G pair (1S1I) and G-GC triple (3U5F) (Color figure online).

implies important functions that λ-turns may have.

However, the structure of the λ-turn from 5.8S/25S rRNA varies a bit from other ones. We aligned two λ-turns from PDB:1S1I and PDB:3U5F based on positions of their G-GC (or G-G) triples (Fig. 4(B)). It can be seen that the λ-turn in 3U5F is more compact than its counterpart, although their structures are similar in general. This observation again evidences the difference between the λ-turn from 5.8S/25S rRNA and those from 16S/18S rRNAs.

From a global perspective, a λ-turn from 16S/18S rRNA is actually folded by a single strand (Fig. 5(A)–(H)). The strand forms two segments of helices and bends at the position of the λ-turn. The Helix 2 in the λ-turn is capped by another helix and then a tetraloop. In this global structure, an interesting pattern is observed: between the capped helix and the tetraloop there is always a guanine stretching inside and interacting with the second G-C pair in Helix 2 (see nucleotides coloured blue in Fig. 5). The new G-GC base triple is also formed by cWW/tHW interactions, the same way by which the first G-GC base triple in Helix 2 is formed. The new base triples can be found in all λ-turns from 16S/18S rRNAs. Due to the existence of the inserted guanine, the second G-C pair in Helix 2 prefers not to be substituted for the same reason as the first G-C pair in Helix 2. It further explains why the second G-C pair in Helix 2 remains the same across 16S/18S rRNAs.

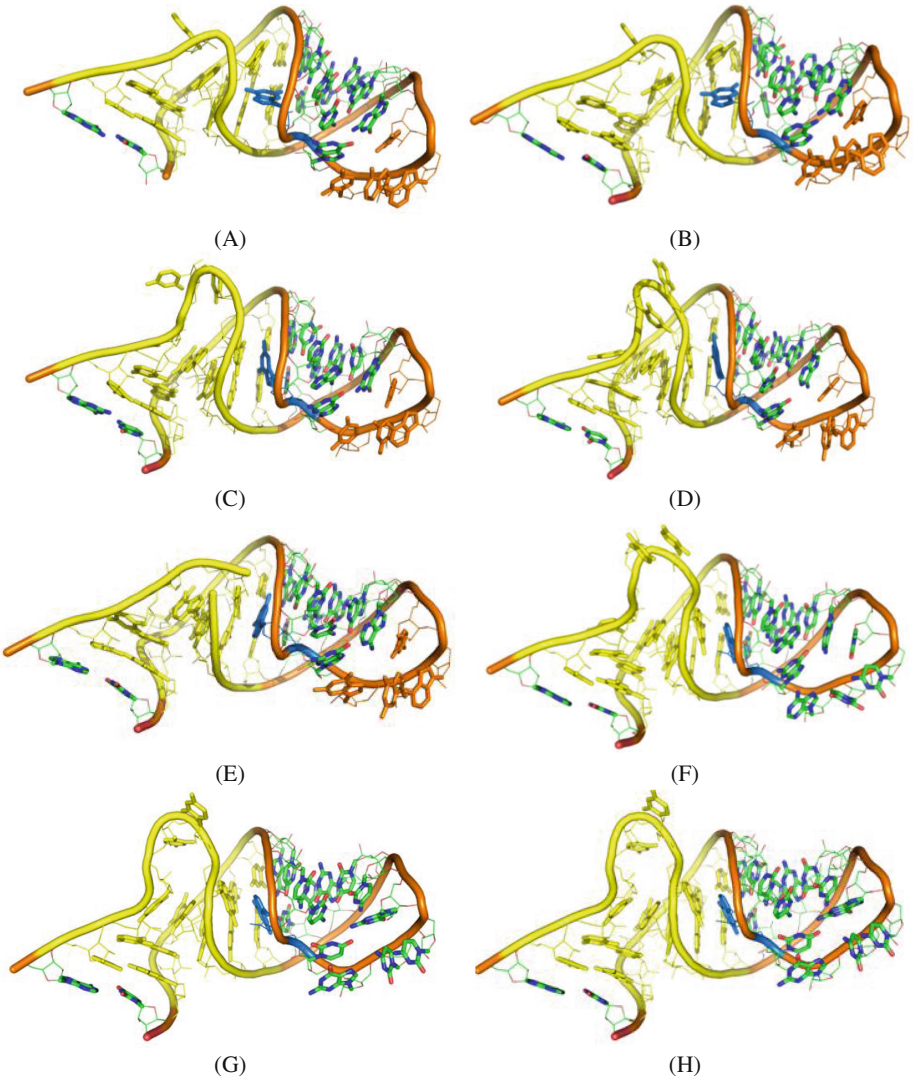


Fig. 5. Overall structures of λ-turns and their capped helices and tetraloops. λ-turns are coloured yellow and the guanines located between the capped helix and the tetraloop are coloured blue. (A) A:C248-G255/C271-G276 (PDB:2AW7); (B) A:C219-G226/C242-G247 PDB:3BBN); (C) A:C308-G317/C333-G338 (PDB:2XZM); (D) 6:C317-G326/C342-G347 (PDB:3U5F); (E) A:C321-G330/C346-G351 (PDB:3IZ7); (F) 2:C322-G331/C347-G352 (PDB:3J3C); (G) 2: C365-G374/C390-G395 (PDB:3J3D); (H) i:C355-G364/C380-G385 (PDB:4KZX) (Color figure online).

2.4 Locations of λ-Turns and Protein Recognition

Nine identified λ-turns are all located on surfaces of rRNAs (see Figure S1 in the supplementary data), which indicates that λ-turns may have significant functions, such as binding with ribosomal proteins. The structures of ribosomes provide insights of possible functions of λ-turns. There are four complexes (PDB:2XZM, PDB:2AW7, PDB:3BBN, and PDB:4KZX) which contain both rRNA and ribosomal proteins. The λ-turns in these four complexes all have direct interactions with ribosomal proteins, three of which are S17 ribosomal proteins (in PDB:2XZM, PDB:2AW7, and PDB:3BBN) and one of which is S11 ribosomal protein (in PDB:4KZX) (see Fig. 6). Protein S17 in human body is involved in various biological processes, such as translation (Vladimirov et al. 1996) and translational initiation (Cmejla et al. 2007), etc. Studies indicate that a shortage of protein S17 may result in the disease of anemia because of the increasing self-destruction of blood-forming cells in the bone marrow (Cmejla et al. 2007). Protein S11 in human is also involved in biological processes like translational initiation and termination. The mutation in the location of λ-turns may cause a deficiency of protein S11/S17 binding for rRNAs, which hinders the process of translation. Consequently a similar syndrome of anemia may be introduced.

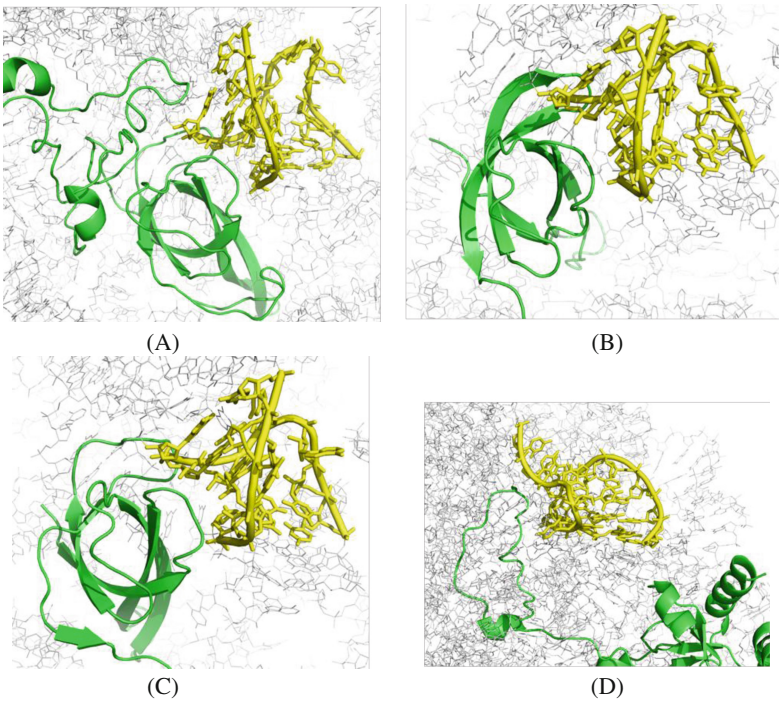


Fig. 6. Interaction diagrams between λ-turns and ribosomal proteins. λ-turns are coloured yellow and proteins are represented by green ribbons. (A) The λ-turn and protein S17 in PDB:2XZM; (B) the λ-turn and protein S17 in PDB:2AW7; (C) the λ-turn and protein S17 in PDB:3BBN; (D) the λ-turn and protein S11 in PDB:4KZX (Color figure online).

3 Discussion

In this paper, we describe a new type of RNA structural motif, namely λ-turn, which is only identified from ribosomal RNAs. The λ-turn is a helix-internal loop-helix-structure motif. It bends at the position of the internal loop, which leads to a $\sim 90°$ change in the direction of its two helices. The region of the internal loop contains unpaired nucleotides which have remote interactions with nucleotides from other parts of the RNA molecule. At the 3′-end of the internal loop, there is a guanine which extrudes out and forms a base triple with the first G-C pair in Helix 2. From the global perspective, the λ-turn is capped by another helix followed by a tetraloop. Between the capped helix and the tetraloop, there is a guanine stretching inside and forming a base triple with the second G-C pair from Helix 2. The two base triples help to consolidate the structure of the λ-turn.

All the identified λ-turns are located on surfaces of rRNAs. In structures of ribosomes, it can be seen that λ-turns have direct interactions with ribosomal proteins. Since proteins S11 and S17 are involved in the translational initiation and termination, we speculate that λ-turns may be involved the process of translation and mutations in λ-turns may lead to the disease of anemia.

Supplementary Data
Supplementary data are available at: http://sse.tongji.edu.cn/yingshen/LTurn/supp.docx.

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