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Structural comparison of yeast snoRNP and spliceosomal protein Snu13p with its homologs

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Abstract

Snu13p is a bifunctional yeast protein involved in both messenger RNA splicing as well as ribosomal RNA maturation. Snu13p initiates assembly of ribonucleoprotein particles by interacting with a conserved RNA motif called kink turn. Unlike its archaeal homolog, L7Ae, Snu13p displays differential specificity for functionally distinct kink turns. Thus, the structures of Snu13p at different functional states, including those alone and bound with RNAs, are required to understand how the protein differentially interacts with kink turns. Although the structure of the human homolog of Snu13p bound with a spliceosomal RNA is known, there has not been a report of a structure of free Snu13p. This has hindered our ability to understand the structural basis for Snu13p's substrate specificity. We report a crystal structure of free Snu13p at 1.9 Å and a detailed structural comparison with its homologs. We show that free Snu13p has nearly an identical conformation as that of its human homolog bound with RNA. Interestingly, both eukaryotic proteins exhibit notable structural differences in their central β-sheets as compared to their archaeal homolog, L7Ae. The observed structural differences offer a possible explanation to the observed difference in RNA specificity between Snu13p and L7Ae.

Keywords: X-ray crystal structure; Protein–RNA complex; rRNA modification; mRNA splicing; K-turn

Yeast protein Snu13p and its human homolog were first isolated from purified U4/U6·U5 tri-snRNP particles of the spliceosome [11,8], which was found to be associated with the 5′ stem of U4 snRNA [8]. Subsequently, Watkins et al. [16] identified Snu13p to also be a box C/D small nucleolar ribonucleoprotein particle (snoRNP) by noticing the resemblance between box C/D RNA sequence and that of the 5′-stem of the U4 snRNA. They then show that Snu13p bound to several box C/D RNAs specifically, and that it could be co-purified from the U3 box C/D snoRNP. Spliceosomes are dynamic ribonucleoprotein particles responsible for removal of introns in messenger RNA [10]. Box C/D snoRNPs process and methylate precursor ribosomal RNAs [6,13,17]. Snu13p's association with distinct ribonucleoprotein particles suggests its dual functional roles in RNA processing. The common thread linking Snu13p to two different RNPs is an RNA motif recognized by Snu13p. Snu13p interacts specifically to a helix–bulge–helix, or kink-turn (K-turn) motif [5,8,16].

Despite a high degree of sequence homology, Snu13p and its eukaryotic homologs nevertheless appear to have different RNA specificity than their archaeal homolog, L7Ae, for box C/D snoRNA (or sRNAs for small RNAs in Archaea) (Fig. 1). In both Eukarya and Archaea, a majority of box C/D s(no)RNAs contain two sets of conserved sequences; box C/C′ (RUGAUGA) and box D/D′ (CUGA), where box C and D or box C′ and D′ are close in space, forming a K-turn motif. The two K-turn motifs differ in their flanking RNA structures. The terminal K-turn, formed by box C/D,

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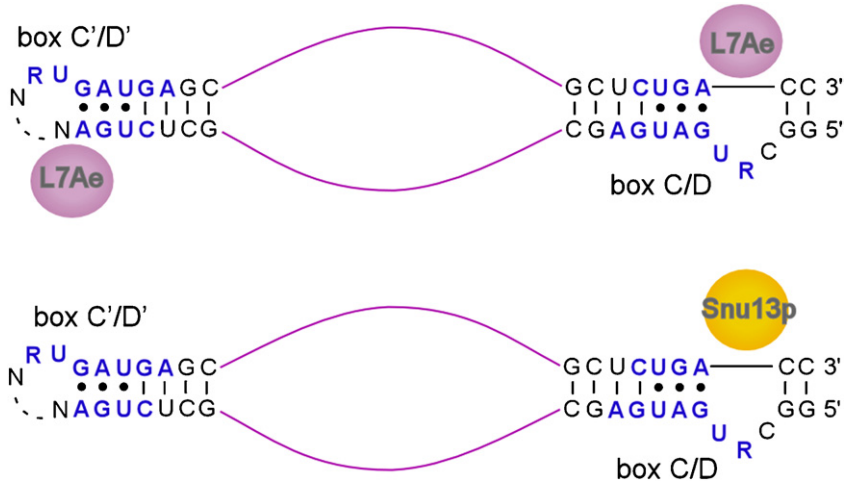

Fig. 1. Current model of interactions of Snu13p and its homologs with box C/D RNA. The conserved box C/C′ and box D/D′ sequences are coloured in blue. Loops connecting the two sets of box C/D sequences represent the methylation guide regions of the box C/D RNA. Snu13p and its eukaryotic homologs are believed to have weak affinity for box C′/D′ motif while L7Ae does not discriminate between the two motifs. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this paper.)

is flanked by two stems. The internal K-turn is formed by box C′/D′, is frequently bordered by a stem and an internal loop of variable nucleotide length (Fig. 1). Snu13p and its eukaryotic homologs bind specifically to the terminal K-turn motif, but appear to discriminate against the internal K-turn motif [9,12,14]. In contrast, L7Ae displays similar binding affinities for both the terminal and the internal K-turn motifs [9,14].

Crystal structures of several Snu13p homologs and their complexes with K-turn RNAs have been reported. These include that of the human homolog of Snu13p, 15.5 kDa protein bound with the 5′-stem of U4 snRNA [15], L7Ae from *Archaeglobus fulgidus* (AF L7Ae) bound with a box C/D RNA [7], L7Ae from *Methanococcus jannaschii* (MJ L7Ae) bound to a K-turn connected to an internal loop [4], and free L7Ae from *Pyrococcus abyssi* (PA L7AL) [2]. Structural comparison among the protein or protein-K-turn RNA complexes revealed small differences among the proteins but some notable differences among the bound K-turn RNAs, particularly between the U4 snRNA and the box C/D RNA [7]. The structural rigidity displayed by the protein likely helps to ensure a high specificity of binding. In contrast, the K-turn RNA exhibits structural flexibility even in its bound state. Accordingly, a recent fluorescence resonance energy transfer (FRET) study of a K-turn RNA detected two distinct conformations in solution [3]. Therefore, it would appear that Snu13p/L7Ae binds to a K-turn RNA by first exploring the RNA's "adaptability" before reaching the final stable protein–RNA complex. Along with the idea of RNA adaptability, or "induced fit," these early studies suggest a structural mechanism that may explain the differential K-turn specificity exhibited between the eukaryotic and archaeal proteins. It can be hypothesized that the terminal K-turn and the internal K-turn on a box C/D RNA have different levels of "adaptability" caused by different flanking RNA structures. While both K-turns are able to adapt to the binding surface of the archaeal L7Ae protein, only the terminal K-turn can adapt to the binding surface of Snu13p and its eukaryotic homologs. This hypothesis predicts that Snu13p and its eukaryotic homolog have a more rigid fold than L7Ae.

To gain a complete understanding of the molecular basis for the observed RNA specificity of Snu13p and its eukaryotic homologs, we determined a high resolution crystal structure of free Snu13p. Currently, there are no structures of any other eukaryotic Snu13p homologs in the absence of bound RNA, and compared the crystal structure of free Snu13p with its human homolog 15.5 kDa protein bound with the 5′-stem of U4 snRNA, and found that the crystal structure of Snu13p is nearly identical to that of 15.5 kDa protein. Interestingly, we also identified several systematic structural differences between eukaryotic and archaeal proteins that may account for the observed difference in their RNA specificity.

Materials and methods

Protein expression and purification. Snu13 gene was cloned into pET11b vector with *Nhe*I and *Bam*HI as restriction sites for expression in *Escherichia coli* BL21(DE3) cells. Cells expressing Snu13p were grown, harvested, and lysed according to standard protocols. Snu13p was purified by Ni–NTA affinity chromatography followed by gel filtration on a Superdex 75 column. The purified protein was concentrated to 70 mg/ml in a storage buffer containing 20 mM Tris–HCl, pH 7.5, 1 M NaCl, 5% glycerol, 0.1 mM phenylmethylsulfonyl fluoride (PMSF), and 5 mM β-mercaptoethanol and stored at −80 °C.

Crystallization and data collection. Snu13p was screened for crystallization by the hanging drop vapor diffusion method. The drops