Available online at www.sciencedirect.com
ScienceDirect
Current Opinion in Structural Biology

Unveiling substrate RNA binding to H/ACA RNPs: one side fits all

Hong Li[1,2]

The H/ACA RNP pseudouridylases function on a large number of extraordinarily complex RNA substrates including pre-ribosomal and small nuclear RNAs. Recent structural data show that H/ACA RNPs capture their RNA substrates via a simple one-sided attachment model. However, the precise placement of each RNA substrate into the active site of the catalytic subunit relies on the essential functions of the RNP proteins. The specific roles of each H/ACA RNP protein are being elucidated by a combination of structural and biochemical studies.

Addresses
[1] Department of Chemistry and Biochemistry, Florida State University, Tallahassee, FL 32306, United States
[2] Institute of Molecular Biophysics, Florida State University, Tallahassee, FL 32306, United States

Corresponding author: Li, Hong (hongli@sb.fsu.edu)

Current Opinion in Structural Biology 2008, **18**:78–85

This review comes from a themed issue on
Protein-nucleic acid interactions
Edited by Wei Yang and Greg van Duyne

0959-440X/$ – see front matter

DOI 10.1016/j.sbi.2007.11.004

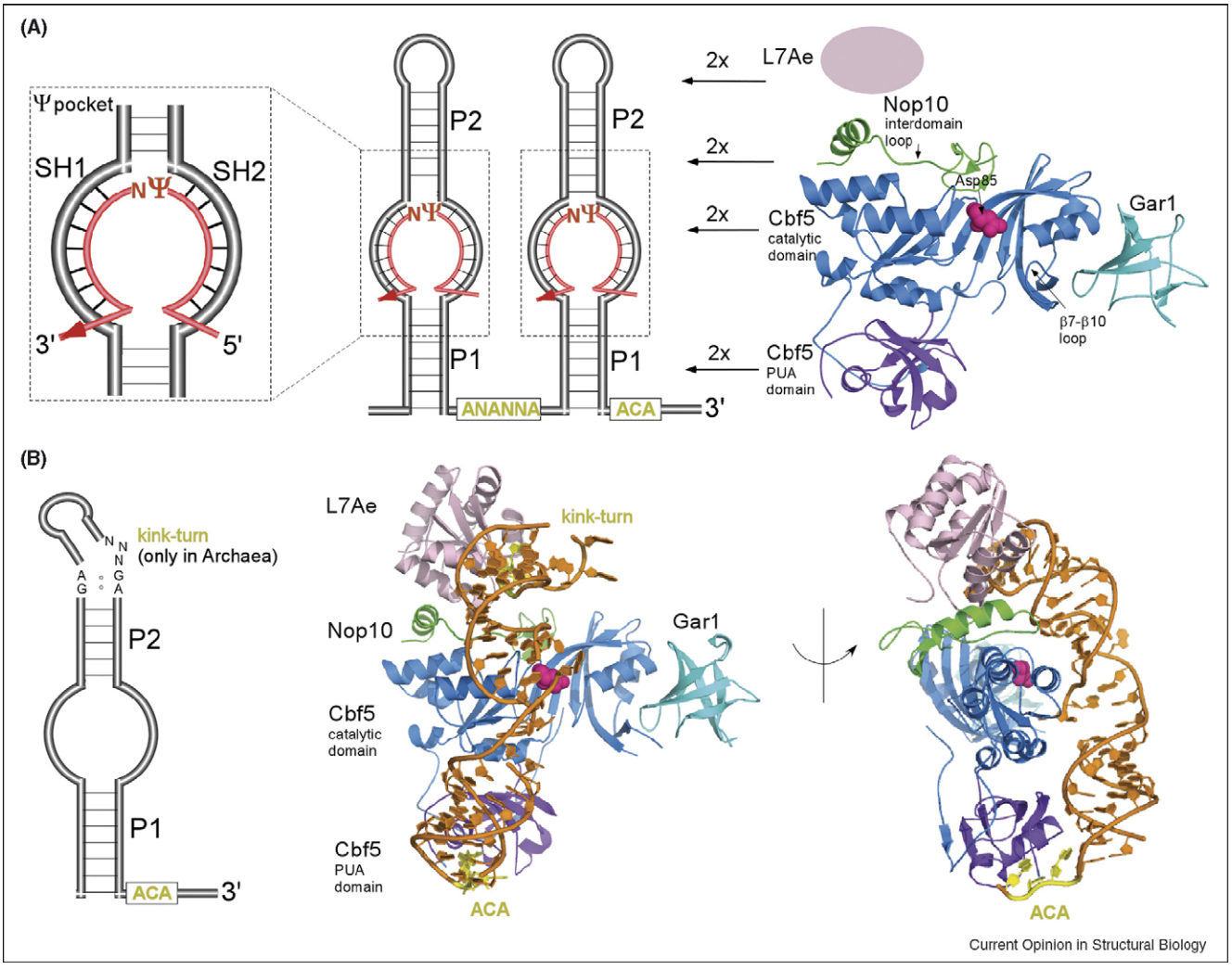
An impressive list of non-coding RNAs is now known to guide chemical reactions on other functional RNAs [1,2]. These RNAs, generally referred to as guide RNAs, secure substrate RNAs through Watson-Crick base-pairings that help to place the substrate RNA in the active site of the enzyme. The guide RNAs assemble with one or several partner proteins that are responsible for the actual catalytic processes. In guide RNA mediated enzymatic processes, an intricate network of RNA–RNA, RNA–protein, and protein–protein interactions sustains the efficiency and specificity of the enzymes. Unlike stand-alone enzymes, the presence of the guide RNA makes RNA-guided enzymes more versatile, although non-conventional, for selecting the site of chemical reactions.

A major class of non-coding RNAs in eukarya and archaea is the box H/ACA small nucleolar or small RNA (snoRNA or sRNA), which guides site-specific isomerization of uridine to pseudouridine (Ψ) in precursor ribosomal RNA (rRNA) and spliceosomal RNAs [3–5]. Several unique members of this class also function in ribosomal rRNA processing in eukarya [6] and telomerase maturation in vertebrates [7]. As such, H/ACA RNA-mediated functions are essential to ribosome and spliceosome biogenesis [8–10] and to chromatin maintenance [7]. In addition to the collective effects of uridine isomerization on RNA stability and local environment [11–13], the process of modification itself is believed to have chaperone effects on the complex process of ribosome folding and assembly [14]. In H/ACA RNA-mediated ribosome biogenesis, H/ACA RNAs act on pre-rRNA following its transcription but dissociate before its folding and assembly with ribosomal proteins [15]. Thus, H/ACA RNAs must attune their functional cycles to the rate of ribosome production that in turn, sustains the needs of cell growth.

Each H/ACA RNA contains either one or two (or less frequently, three) hairpins interrupted by internal loops (Figure 1A). Common to these hairpin repeats is the presence of an H-box (ANANNA) or ACA-box (ACA) at the 3′ tail (Figure 1A). In archaeal H/ACA RNAs, a kink-turn (K-turn) motif is also present at the base of the apical loop (Figure 1A). *In vitro* reconstitution of purified archaeal proteins and transcribed H/ACA RNAs demonstrates that a single hairpin unit of the H/ACA RNA is sufficient for both RNP assembly and pseudouridylation activities [24•,25•]. The biochemical studies with the reconstituted RNPs further show that the internal loop, the 3′ ACA tail, and the K-turn motif of the hairpin unit are all required for the *in vitro* functions. The internal loop sequences are complementary to the modification sites while the ACA tail and the K-turn motif are required for the association of H/ACA RNA with partner proteins [24•,25•]. Unlike other known guide RNAs, H/ACA RNAs bind RNA substrates through base pairing between both strands of their internal loop and the substrate RNA, leading to a unique three-way junction constrained by a fourth helix underneath (Figure 1A).

All members of the H/ACA RNAs function with a set of conserved proteins that contain the catalytic subunit for the isomerization reaction itself. Using archaeal naming conventions, these proteins are Cbf5 (Nap57 or dyskerin in mammals), L7Ae (Nhp2 in eukaryotes/mammals), Nop10, and Gar1, all of which are crucial to the pseudouridylation process. Cbf5 has sequence homology to TruB, a stand-alone bacterial pseudouridylase responsible for isomerization of U55 in tRNA [16]. L7Ae and Nhp2 belong to the family of K-turn binding proteins, but interestingly, only archaeal H/ACA RNAs are known to contain the K-turn motif. This is in accord with the fact that L7Ae is also known to be associated with other K-turn-containing RNAs in the ribosome [17] and the box C/D small ribonucleoprotein particles [18]. Much less

Figure 1



(A) Secondary structures of H/ACA RNA and substrate RNA complex and the arrangement of the four proteins. Inset illustrates detailed features of the pseudouridine pocket (Ψ pocket) and its interaction with the substrate RNA. 'K-turn' stands for kink-turn RNA motif (see reference [17]). 'N' denotes an RNA nucleotide of any sequence and 'Ψ' denotes the uridine targeted for modification. Two sets of the Cbf5-Nop10-Gar1 complex (reference [28•], PDB ID: 2EY4) and L7Ae are believed to be bound to the two hairpin units of the H/ACA RNA. **(B)** The tertiary structure of a single hairpin unit H/ACA RNA (gray and orange) bound with all four proteins in the absence of the substrate RNA (reference [36••], PDB ID: 2HVY). Note the linear RNA–protein interface bypassing the catalytic aspartate (magenta spheres).

is known about Nop10 and Gar1 based on sequence homology alone. Intense efforts are devoted to searching for the structural basis for how H/ACA RNPs function, especially for the roles of accessory proteins. Previous structural studies already revealed the mode of H/ACA RNP assembly in the absence of substrate RNA. More recently, new insights into the roles of H/ACA RNP proteins in the placement of substrate RNA are obtained. This short review summarizes these structural findings with emphasis on the topic of substrate RNA loading and the functional roles of the H/ACA RNP components in this process. For other extensive reviews on H/ACA RNPs, readers are referred to those on biological function and biogenesis [1,19], on assembly [20•,21–23], and on telomerase function [7].

The tripartite protein core

In archaea, Cbf5, Nop10, and Gar1 are known to form a stable complex independent of RNA while L7Ae associates stably only with the guide RNA [24•,25•]. Shortly after their biochemical identification and characterization, crystal structures of the archaeal Cbf5-Nop10 binary [26•,27•] and the Cbf5-Nop10-Gar1 ternary [28•] complexes were obtained (Figure 1A). The structures of these Cbf5-Nop10 and Cbf5-Nop10-Gar1 complexes, together with two previously determined structures of