

JNK1 plays an important part in this process provides an intriguing new clue about the events that underlie this complex intracellular signaling process.

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How a single protein complex accommodates many different H/ACA RNAs

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More than 100 mammalian H/ACA RNAs form an equal number of ribonucleoproteins (RNPs) by associating with the same four core proteins. The function of these H/ACA RNPs is essential for biogenesis of the ribosome, splicing of precursor mRNAs (pre-mRNAs), maintenance of telomeres and probably for additional cellular processes. Recent crystal structures of archaeal H/ACA protein complexes show how the same four proteins accommodate > 100 distinct but related H/ACA RNAs

and reveal that a spatial mutation cluster underlies dyskeratosis congenita, a syndrome of bone marrow failure.

Introduction

Most mammalian H/ACA ribonucleoproteins (RNPs) engage in the isomerization of uridines to pseudouridines (termed 'pseudouridylation') in ribosomal and spliceosomal small nuclear RNAs. Although the function of most pseudouridines is unknown, some are essential for optimal translation and for pre-mRNA splicing [1]. Perhaps the

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most intriguing species of H/ACA RNPs are defined by the small nucleolar RNA U17 (also known as E1, or snR30 in yeast), telomerase RNA (which ends in an H/ACA RNA structure), and a growing number of orphan H/ACA RNAs (which lack complementarity to any stable RNAs) [2]. U17 is the only essential H/ACA RNA and is required for processing pre-ribosomal RNA; telomerase RNA is required for replication of chromosome ends; and the orphan H/ACA RNAs have, by definition, unknown functions but are potentially involved in important processes similar to those of U17 and telomerase RNA [3] (Figure 1).

Recently, three groups have solved the crystal structures of archaeal H/ACA RNP core complexes consisting of two or three of the core proteins [4–6] (Figure 1). These structures are providing the first molecular details of both protein–protein interactions in the H/ACA core complex and the pseudouridylation itself.

H/ACA RNPs

H/ACA RNAs constitute one of the two principal classes of small nucleolar and Cajal body RNAs (Figure 1); the other class is the C/D RNAs. Comprising an average of 130–140 nucleotides, H/ACA RNAs conform to a consensus 5′-hairpin-hinge-hairpin-tail-3′ secondary structure, in which the characteristic ACA trinucleotide is exactly three residues from the 3′ end (Figure 1). H/ACA RNAs identify the ~130 known mammalian pseudouridylation sites by base-pairing to a few nucleotides flanking the target uridines. Complementarity to the substrate RNAs lies in the upper half of a bulge (pseudouridylation pocket)

of one or both of the hairpins, placing the unpaired target uridine at the bottom of a helix [7,8]. Pseudouridylation is catalyzed by the pseudouridylyase NAP57 (also known as dyskerin, or Cbf5 in yeast and archaea), which is one of the four H/ACA core proteins [9]. The small basic core proteins, GAR1, NOP10 and NHP2 (L7Ae in archaea), round out H/ACA RNPs. Except for GAR1, the core proteins are essential for the structural integrity of H/ACA RNPs [3].

The structures

The structure of the archaeal pseudouridylyase Cbf5 has been solved in complex with Nop10 alone [5,6] and with Nop10 and Gar1 [4]. On the basis of structural and sequence homology, pseudouridylyases are classified into five families called RluA, RsuA, TruA, TruB and TruD [10]. Crystal structures of bacterial and/or archaeal representatives of each family have been solved.

Cbf5 belongs to the TruB family of pseudouridylyases, which are specified by the *Escherichia coli* enzyme responsible for modifying uridine 55 in all elongator tRNAs. Unlike Cbf5, however, all other pseudouridylyases (including TruB) function as independent enzymes that recognize and isomerize uridines without assistance from other proteins or RNAs. The crystal structures of archaeal Cbf5 therefore provide the first descriptions of a pseudouridylyase that functions in the context of a RNP and that depends on a guide RNA for recognition of the target site.

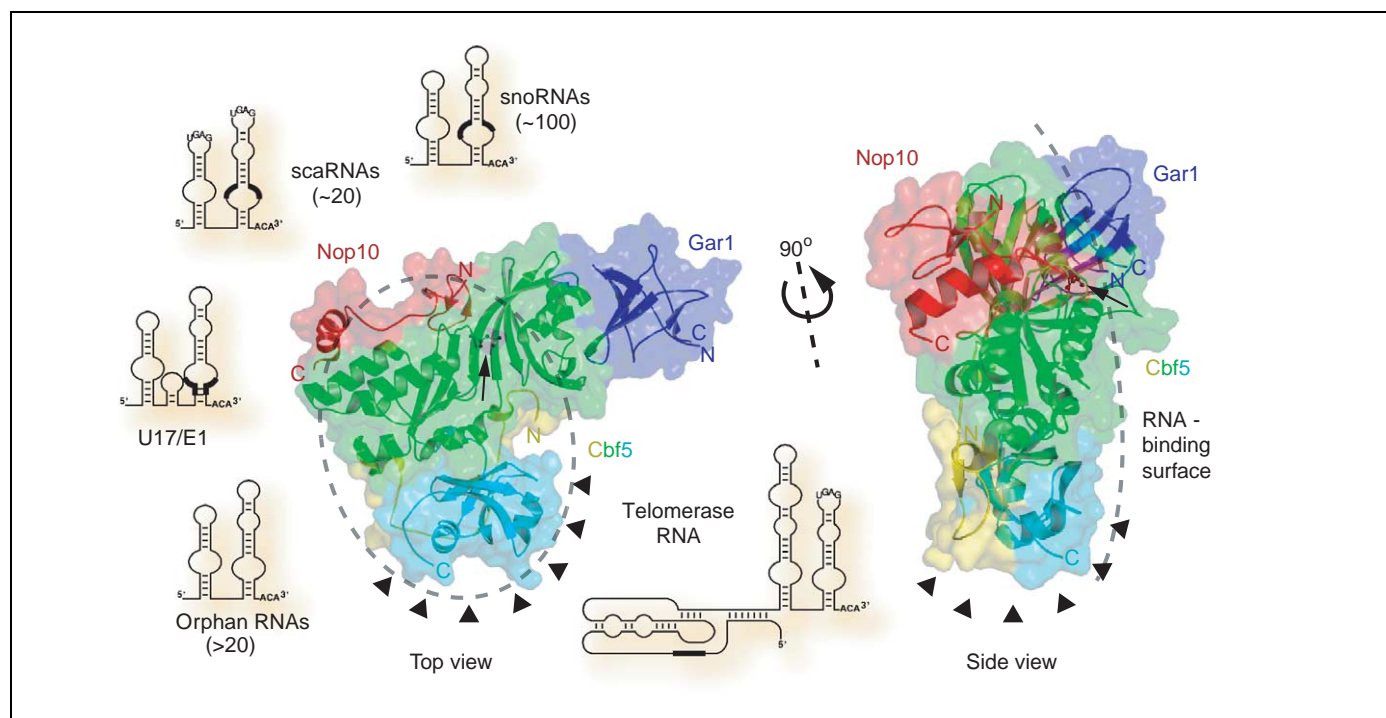


Figure 1. The archaeal Gar1–Cbf5–Nop10 complex and mammalian classes of H/ACA RNAs. Shown are a top view, with the RNA-binding surface on top, and a side view of the crystal structure of the H/ACA protein complex of Gar1 (blue), Nop10 (red) and Cbf5 with its catalytic domain (green), PUA domain (cyan) and N terminus (yellow). The α backbones are shown as ribbons through a surface rendering based on the atomic coordinates of the *Pyrococcus furiosus* proteins deposited in the Protein Data Bank (accession code 2ey4) [4] and generated with PyMOL (<http://pymol.sourceforge.net>). The N and C termini of the proteins and the catalytic aspartate (arrow, black ball-and-stick model) are indicated. The probable area and surface for binding one hairpin of any of the H/ACA RNAs shown are outlined by the broken gray oval and broken gray line, respectively. In the RNAs, the location of antisense elements in the pseudouridylation pockets and the template region of telomerase RNA are highlighted (thick black lines). Note that, relative to the protein complex, the H/ACA RNAs are not drawn to scale and are randomly arranged around the protein structures. Arrowheads indicate the location of the dyskeratosis congenita mutation cluster in the Cbf5 PUA domain and N terminus (based on a model of human NAP57). Abbreviations: scaRNAs, small Cajal body RNAs; snoRNAs, small nucleolar RNAs.

The pseudouridylase (Cbf5)

Although the structures are derived from three different archaea, *Methanococcus jannaschii* [5], *Pyrococcus furiosus* [4] (Figure 1) and *Pyrococcus abyssi* [6], they are in good agreement. Cbf5 contains a catalytic domain that superimposes closely on those of TruB [11] and the four other members of the pseudouridylase family (Figure 1, green). The matching positions of a universally conserved aspartate residue (Figure 1, arrow) and a few key residues in the catalytic center suggest that all pseudouridylases, RNA-guided or not, share a conserved mode of catalysis. In comparison to Cbf5, the catalytic domains of TruB and other pseudouridylases contain additional segments that are important for binding the substrate RNA. Apparently, Cbf5 can function without these appendages because it associates with other proteins and interacts indirectly with its substrates via its H/ACA RNA.

In addition to its catalytic domain, Cbf5 contains a C-terminal pseudouridylase archaeosine tRNA-guanine transglycosylase (PUA) domain [12] (Figure 1, cyan), which is larger and is enveloped by an N-terminal extension (Figure 1, yellow) in comparison to that of the eubacterial TruB enzyme. In archaeosine transglycosylase (archaeosine is a guanine derivative specific to archaeal tRNAs), this domain is important for recognizing the CCA terminal residues of tRNA (the CCA residues are essential for tRNAs to be charged with their amino acids). Interestingly, the PUA domain in Cbf5 is similarly perched for potential recognition of the defining ACA trinucleotide at the 3' end of H/ACA RNAs.

The bracket (Nop10)

Nop10, a protein of 60 amino acids, lines the oblong catalytic domain of Cbf5 with a random coil central segment that separates its own N-terminal Zn²⁺-binding and C-terminal α -helical domains (Figure 1, red). Although Nop10 seems to be intrinsically disordered on its own [13], it becomes structured on binding to Cbf5. This tight interaction with Cbf5 might be important to freeze the catalytic domain in the most favorable position for catalysis and to extend the positive surface potential of Cbf5 for binding the H/ACA and substrate RNAs [4–6]. In fact, Nop10, as well as its C-terminal α -helix alone, promotes binding of substrate RNA in gel-shift assays and is sufficient for reconstituting RNA-guided pseudouridylase activity in the context of Cbf5 and L7Ae [6].

Nop10 also might be involved in docking of L7Ae to the complex, as modeled by Rashid *et al.* [4] and demonstrated for its mammalian counterpart NHP2 [14]. By mutating one of the four Zn²⁺-coordinating cysteine residues, which are conserved in archaeal but not eukaryal Nop10, Manival *et al.* [6] have shown that Zn²⁺ binding is not required for RNP assembly and activity. Thus, Zn²⁺ seems to be important for the structural integrity of specifically archaeal Nop10 (which must withstand challenging temperatures).

The outsider (Gar1)

The crystal structure of Gar1 in the Gar1–Cbf5–Nop10 complex is the first structure of any Gar1 homolog to be

solved and it shows that Gar1 belongs to the superfamily of reductase, isomerase and elongation factor folds [4] (Figure 1, blue). Gar1 binds to one end of the Cbf5 catalytic domain without contacting Nop10. This observation is in good agreement with biochemical data demonstrating that archaeal, yeast and mammalian Gar1 interact independently with Cbf5 homologs [14–17].

Although Gar1 has been 'tied' to the catalytic core by crosslinking in mammalian H/ACA RNPs [14], it is situated too far from the active-site aspartate to make contact with this residue. The missing one-fourth of archaeal Gar1 in the structure and/or the defining (glycine/arginine)-rich N and C termini of eukaryal GAR1 might account for this discrepancy. Interestingly, the location of Gar1 in the complex might prove to be identical to that of Naf1 in eukaryal H/ACA RNPs. Naf1, which is required for the biogenesis of H/ACA RNPs, shares homology with the domain of Gar1 that contacts Cbf5 [18].

How do H/ACA and substrate RNAs bind this protein complex?

The structure of the related TruB in association with its substrate tRNA provided the basis for identifying the probable RNA-binding surface in the complex [4,5]. Specifically, together with Nop10, the PUA and catalytic domains of Cbf5 form a platform with a positively charged surface potential that is ideal for binding RNA (Figure 1, broken gray oval and line). Indeed, mutation of two conserved basic and surface-exposed amino acids in the catalytic domain abolishes binding between archaeal H/ACA RNA and the Cbf5–Nop10 dimer [5]. Moreover, the PUA domain is essential for RNP assembly and activity because (in the presence of substrate RNA) it supports both processes, even when added in *trans* [6].

By using the coordinates of the TruB–tRNA complex and a previously solved complex of L7Ae and guide RNA, Rashid *et al.* [4] have modeled a fully assembled H/ACA RNP hybridized to a substrate RNA. The three-junction helix, formed by base-pairing between the substrate RNA and the pseudouridylation pocket, and by the upper stem of the guide RNA, folds into an extended structure that places the target uridine next to the catalytic aspartate. The tip of the H/ACA RNA hairpin extends beyond Nop10, where it binds L7Ae, whereas the 3' ACA and both ends of the substrate RNA point beyond the PUA domain. Therefore, consistent with all studies, H/ACA RNPs seem to be bipartite – that is, one side protein, one side RNA. This separation between RNA and protein in H/ACA RNPs contrasts with the situation in other RNPs; for example, in the large ribosomal subunit, proteins adorn the surface all over the RNA core and send extensions deep into the interior [19]. For the H/ACA RNPs, this pasting of RNAs to one side of the core protein complex seems to be exquisitely suited to accommodate the ~100 different but related H/ACA RNAs and their various substrate RNAs. It will be interesting to see whether C/D RNPs – the other main class of small

Box 1. Dyskeratosis congenita

Phenotype

Dyskeratosis congenita is a rare syndrome of bone marrow failure that is inherited in one of the three following modes (listed in descending order of frequency and severity): X-linked, autosomal recessive and autosomal dominant. Individuals affected with dyskeratosis congenita are mostly identified in their first decade of life by the triad of nail dystrophy, abnormal skin pigmentation and mucosal leucoplakia, but they often die in their third decade owing to bone marrow failure. Affected individuals are also predisposed to malignancies of rapidly dividing tissues and other complications [20].

Molecular pathogenesis

X-linked dyskeratosis congenita is caused by mutations in NAP57, the pseudouridylyase of H/ACA ribonucleoproteins (RNPs) [21]. The autosomal dominant form of dyskeratosis congenita is caused by mutations in telomerase RNA and reverse transcriptase [22,23], whereas the gene or genes responsible for the autosomal recessive form remain to be identified. Impaired maintenance of telomeres could explain both X-linked and autosomal dominant dyskeratosis congenita because telomerase RNA also forms an H/ACA RNP. Such a mechanism is supported by the observation of shortened telomeres in individuals with dyskeratosis congenita. Do mutations in NAP57, therefore, specifically affect its interaction with telomerase RNA but not with the other 100-plus H/ACA RNAs? The answer seems to be no because the dyskeratosis congenita phenotype can be reproduced by mutations in NAP57 that, in the absence of telomere defects, impair ribosome biogenesis (and possibly precursor mRNA splicing) through reduced pseudouridylation of RNA [24,25].

nucleolar RNPs – adopt a similar mechanism for binding their many C/D RNAs.

The mutation cluster

A most interesting result of these structural analyses is the observation that the N terminus of Cbf5 wraps around its C-terminal PUA domain, thereby generating a single hotspot for mutations identified in the Cbf5 ortholog NAP57 of individuals affected with dyskeratosis congenita [4–6] (Figure 1, arrowheads; Box 1). This hotspot was dramatically highlighted by modeling the structure of NAP57 (residues 35–359 out of 514) based on the structure of archaeal Cbf5 and by mapping the dyskeratosis congenita mutations onto the resultant model [4].

This tight spatial clustering of mutations that are distant from each other in the linear sequence suggests that most dyskeratosis congenita mutations affect the same function of NAP57. The location of the mutations in and near the PUA domain indicates that they might have an effect on H/ACA RNA binding. Alternatively – and more intriguingly – the exposed surface defined by the mutation hotspot is strategically situated at one end of the complex for interaction with a non-core component of the H/ACA RNP – for example, a telomerase-specific protein or an H/ACA RNP assembly factor. If so, interference with such an interaction would explain the specific effect of dyskeratosis congenita mutations on only a few select H/ACA RNPs.

Concluding remarks

As outlined here, this exciting flurry of archaeal H/ACA protein structures has yielded considerable insight into

many aspects of H/ACA RNP biology. However, several important questions remain. In terms of human H/ACA RNPs, how will the missing one-third and one-half of NAP57 and GAR1, respectively, fit into the RNP and how will they affect the overall structure? Given the often conserved nature of the dyskeratosis congenita missense mutations, what is their molecular impact? The answers will be known only once the structure of the human H/ACA RNP core has been solved.

Ultimately, the crystal structure of the whole RNP, including the H/ACA RNA and substrate RNA, will be required. Meanwhile, the solution structure of one of the H/ACA RNA hairpins might aid in modeling the complete RNP [13]. The full RNA protein complex might also provide insight into how the base-pairing between the guide and substrate RNAs is released after pseudouridylation. As such, the structure of mammalian holo-H/ACA RNPs will answer both fundamental and clinically relevant questions.

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