ISSN 0026-8933, Molecular Biology, 2007, Vol. 41, No. 2, pp. 214–226. © Pleiades Publishing, Inc., 2007.
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RNA AND PROTEINS

UDC 577.241

# Small Nucleolar RNA

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Received May 2, 2006

Accepted for publication June 13, 2006

**Abstract**—The review considers small nucleolar RNAs (snoRNAs), an abundant group of non-protein-coding RNAs. In association with proteins, snoRNAs determine the two most common nucleotide modifications in rRNA and some other cell RNAs: 2'-O-methylation of ribose and pseudouridylation. In addition, snoRNAs are involved in pre-mRNA cleavage and the telomerase function. Almost all snoRNAs fall into two families, C/D and H/ACA, distinguished by conserved sequence boxes. Although the proteins of C/D and H/ACA snoRNPs have homologous regions, these snoRNPs are assembled differently. The RNA components of RNases P and MRP are also classed with snoRNAs. Another problem considered is the structure and function of small RNAs from Cajal bodies (small organelles associated with the nucleoli), which are similar to snoRNAs.

**DOI:** 10.1134/S0026893307020045

*Key words*: small nucleolar RNA, rRNA, RNA 2'-O-methylation, RNA pseudouridylation, Cajal bodies, noncoding RNA

## INTRODUCTION

Small nucleolar RNAs (snoRNAs) were identified in mammalian cells in the late 1960s, but it was not until the mid-1990s that considerable progress in snoRNA research was made owing to new experimental approaches. More than 100 snoRNAs are known, and their number is continuously increasing. To date, snoRNAs have been found in all eukaryotes and even in archaea. The latter lack nucleoli, and, consequently, their snoRNAs are termed sno-like (sRNAs).

It is known that snoRNAs belong to noncoding RNAs, which perform a surprisingly wide range of functions in the cell; snoRNAs are located in the nucleus, and their number is taxon-specific. For instance, the snoRNA number in vertebrates is twice that in yeasts. Moreover, there are $10^4$–$10^5$ molecules of each snoRNA per cell in vertebrates and only $10^2$–$10^3$ in yeasts [1].

Almost all known snoRNAs are involved in rRNA processing. The 18S, 5.8S, and 25/28S rRNAs are synthesized in the nucleolus as parts of the 35/45S pre-rRNA. This precursor is cleaved at several sites during maturation: external and internal transcribed spacers are removed, and the three mature rRNAs are released. Modification of rRNA nucleotides starts simultaneously with transcription. With a few exceptions [2], modifications are introduced in pre-rRNA before transcription is complete [3]. Interestingly, modification is completely absent from the transcribed spacers [3]. The two most common modifications are methylation of 2'-OH of ribose and transformation of uridine into pseudouridine. The amount of modified nucleotides in mature rRNA varies with taxa: for instance, modification of each type affects about 100 sites in vertebrates and about 50 sites in yeasts [4]. The main functions of snoRNA are associated with determination of the sites of rRNA modification and with cleavage of pre-rRNA during its processing.

Although the mechanisms of modification and the structure of rRNA are highly conserved, the snoRNA genes greatly vary in organization. In addition to independently transcribed genes, some snoRNA genes are in introns of other genes, some form clusters transcribed from a common promoter, and some others cluster in introns. Interestingly, every taxon has mostly one mode of snoRNA gene organization. In vertebrates, most snoRNAs are generated from pre-mRNA introns, while a few snoRNAs are encoded by introns of non-protein-coding genes.

There are several conserved elements in snoRNA. According to these elements, most snoRNAs fall into two families. One is the C/D family (box C/D snoRNAs), which includes snoRNAs that have conserved sequences C and D. Most of these snoRNAs are involved in ribose methylation. The other is the H/ACA family (box H/ACA snoRNAs), which includes snoRNAs that have conserved H and ACA sequences. Almost all snoRNAs of this family direct pseudouridylation.

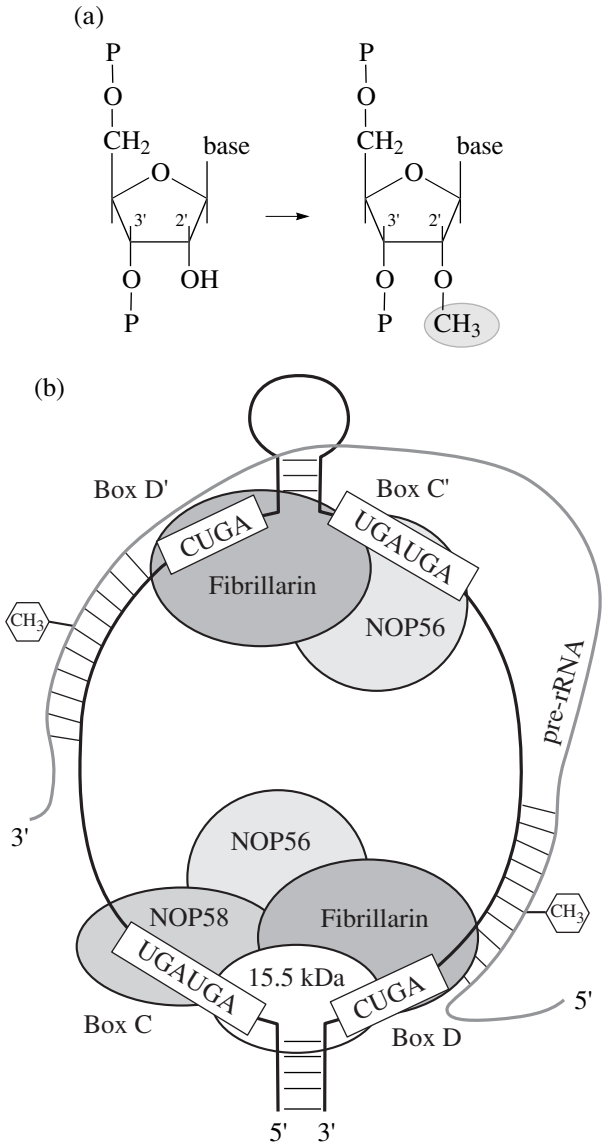


**Fig. 1.** C/D snoRNAs determine the target nucleotide of 2'-O-methylation. (a) 2'-O-methylation of ribose. (b) Structure of the snoRNA/pre-rRNA complex. The antisense elements of snoRNA interact with pre-rRNA (gray line), and the conserved snoRNA sequences (C, D, C', and D' boxes) interact with snoRNP proteins (NOP56, NOP58, fibrillarin, and the 15.5-kDa protein). The target nucleotide (hexagon) of rRNA is 4 nt away from the D and D' boxes. Fibrillarin acts as a methylase.

The RNA component of RNase MRP is also classed with snoRNAs. Some authors assign the RNA component of RNase P to this group.

There is evidence that distorted expression of snoRNA has severe consequences for the organism. For instance, deletion of the genes for two snoRNAs described recently, HBII-52 and HBII-85, plays an important, if not crucial, role in pathogenesis of the Prader–Willi syndrome [5, 6].

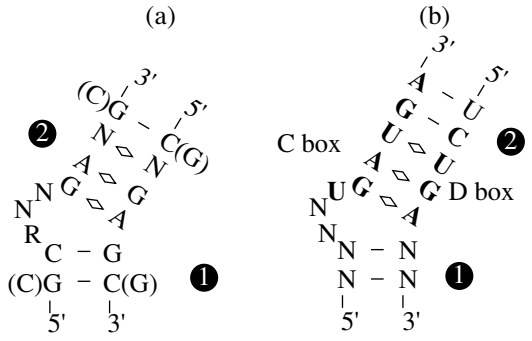


**Fig. 2.** Consensus secondary structures of (a) the K-turn motif and (b) its variant occurring in C/D snoRNA. Modified from [15].

## C/D FAMILY OF snoRNAs

Several RNAs of this family (U3, U8, U14, and U22 [7]) are necessary for pre-rRNA cleavage: they interact with several complementary pre-rRNA regions, acting as RNA chaperones [8]. A lack of these snoRNAs is lethal for the cell. The general mechanism of pre-rRNA processing is conserved, but its details differ among different taxa. For instance the U8 snoRNA, which is involved in the generation of the 18S rRNA, was found in vertebrates but not in plants and yeasts [9]. The other C/D snoRNAs are involved in 2'-O-methylation of ribose (Fig. 1a): snoRNA determines the target ribose moiety, and snoRNA-associated proteins methylate it. Most of these guide snoRNAs play a role in rRNA modification.

C/D snoRNAs are about 70 nt in size and contain two conserved sequences, boxes C (UGAUGA) and D (CUGA). The boxes are in the 5'- and 3'-terminal regions of snoRNA and are usually brought together owing to a hairpin formed by the snoRNA ends (Fig. 1b). The structure including boxes C and D and the terminal hairpin was termed the C/D motif [10]. Recent studies revealed that the C/D motif is more intricate, consisting of two short double-stranded regions and a loop between them. The resulting element includes a so-called kink-turn (K-turn), which was identified recently [11]. This structural motif is common in RNA, occurring in rRNAs, the U4 snoRNA, several mRNAs, and the RNase MRP RNA. The consensus sequence of the K-turn motif is 15 nt (Fig. 2a). The first double-stranded region usually consists of two GC pairs; the other starts with two noncanonical pairs (usually GA) and includes four complementary pairs. The loop between the two double-stranded regions is always asymmetrical, often containing three nonpaired nucleotides on one side and none on the other. The two terminal nucleotides of the loop are involved in stacking with the corresponding double-stranded regions, while the central nucleotide is exposed to the solvent. The two double-stranded regions are at an angle of 120° to each other.

The motif often has variations (Fig. 2b), e.g., nucleotide substitutions in the double-stranded regions without altering the complementary interactions. The number of nucleotides in the internal loop also varies: there are 2–4 nt on one side and 1 or 0 nt on the other [11].

The C/D motif provides a binding site for C/D-snoRNP proteins. The motif is necessary and sufficient for the formation, stability, and nucleolar localization of snoRNP [10], as well as for hypermethylation of the cap in snoRNAs encoded by independent genes and synthesized by RNA polymerase II [12].

In addition, all C/D snoRNAs contain boxes C' and D' in the central region. Their nucleotide sequences are degenerate; the most conserved nucleotides are $G_5$ and $A_6$ of C' and $G_3$, and $A_4$ of D'. The distance between C' and D' is usually 3–9 nt; in the case of a greater distance, the boxes are close owing to an intermolecular hairpin [13] (Fig. 1b). The order of the conserved boxes in snoRNA is always 5'-C–D'–C'–D-3'. The region 5' of D and/or D' contains so-called antisense elements, which are 10–21 nt in size, are fully complementary to the corresponding rRNA regions and capable of interacting with these regions (Fig. 1b). Modification affects the nucleotide that is contained in the resulting RNA/RNA helix and is 5 nt away from the D and/or D' box, which corresponds to half-turn of the helix [14]. The C and D and/or C' and D' boxes bind with a complex of proteins responsible for methylation. Two antisense elements contained in snoRNA determine two nucleotides subject to 2'-O-methylation, but most snoRNAs harbor only one antisense element.

Recent studies revealed two snoRNAs that each contain two C/D and two C'/D' motifs, as if two snoRNA molecules were linked. Despite their complex structure, these snoRNAs each determine only two methylation sites [16]. The same authors described another snoRNA that contains not only the usual C, D', C', and D boxes along with an antisense element, but also an additional upstream fragment with the C' and D' boxes and an antisense element. Thus, the C/D motif and an antisense element probably serve as an elementary unit, which can be employed in more intricate structures.

2'-O-Methylated nucleotides occur not only in rRNA, but also in small nuclear RNAs (snRNAs) involved in splicing (U1, U2, U4–U6). To date, snoRNAs that have regions complementary to snRNA sequences and determine their 2'-O-methylation have been found in all eukaryotes examined [17, 18]. The targets of several snoRNAs identified recently are as yet unknown [19–22].

As expected, snoRNAs are expressed in all tissues, because every cell contains ribosomes, whose maturation requires snoRNAs. However, some snoRNAs are tissue-specific; e.g., there are snoRNAs that are synthesized predominantly or exclusively in the brain. It is these snoRNAs that have unknown targets. Probably, their targets are mRNAs containing modified nucleotides. A search for such targets is rather difficult: databases have many mRNAs with regions complementary to the antisense elements of snoRNAs, but such findings are statistically nonsignificant because of the small size of such regions and the large size of the databases. Recent studies provided convincing evidence that snoRNA does direct 2'-O-methylation of the serotonin receptor mRNA, which is expressed in the brain [6, 23].

C/D snoRNAs were found in Archaea [24], a taxon evolutionarily distant from eukaryotes, but not in bacteria. Archaeal sRNAs (sno-like) have the same structure and perform the same functions as eukaryotic snoRNAs. As was found recently, archaeal C/D snoRNAs occur not only in a linear, but also in a circular form [25]. Both forms were detected in complexes with protein components of snoRNP. The biological role of such an organization is still obscure.

## H/ACA FAMILY OF snoRNAs

Several snoRNAs of this family are involved in pre-mRNA cleavage, acting as chaperones as is the case with C/D snoRNAs (U17/E1/snR30, E2, E3 [26, 27], yeast snR10 [7]). Almost all other snoRNAs of the family direct rRNA pseudouridylation [28]. Most snoRNAs of the family are 100–150 nt in size. All H/ACA snoRNAs have a common secondary structure known as hairpin–hinge–hairpin–tail. The structure includes two hairpins and two single-stranded regions: one is between the hairpins, and the other is at the 3' end (Fig. 3a) [28]. The single-stranded regions located at the bases of the hairpins have two conserved sequences. One is the H box at the base of the first hairpin; its sequence is highly degenerate and has consensus ANANNA. The other, known as the ACA box, has consensus ACA and is at the base of the second hairpin, 3 nt away from the 3' end of snoRNA (Fig. 3a). The two boxes are necessary for the correct folding of the snoRNA ends and the formation of snoRNP [29, 30]. The nuclear location of H/ACA snoRNAs requires the two boxes and the hairpin between them [31]. Both the boxes and the hairpins are essential for efficient pseudouridylation [29].

One (in a few cases, two) hairpin harbors two 3- to 10-nt regions complementary to rRNA. Upon complementary interactions with pre-rRNA, the modification target and the 3'-adjacent nucleotide are brought into the resulting single-stranded "window" and thereby become accessible for the enzyme driving pseudouridylation [28] (Fig. 3a). During the reaction, the uracil residue rotates around the $N_3$–$C_6$ axis by 180°,

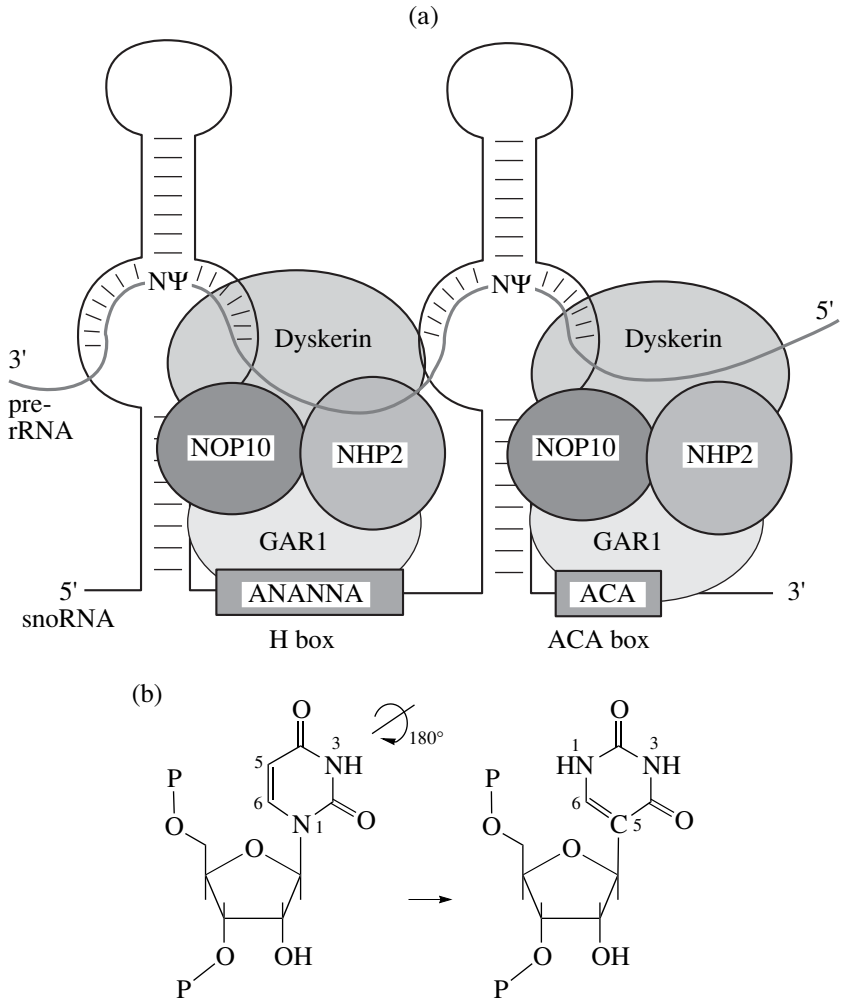


**Fig. 3.** H/ACA snoRNAs guide pseudouridylation. (a) Structure of the snoRNA/pre-rRNA complex. The antisense elements contained in the two snoRNA hairpins interact with pre-rRNA (gray line), and the conserved sequences of snoRNA (H and ACA boxes) interact with snoRNP proteins (NOP10, NHP2, Gar1, and dyskerin, acting as a pseudouridine synthase). The target nucleotide is designated as Ψ. (b) Generation of pseudouridine from uridine.

the $C_{1'}$–$N_1$ bond breaks, and a new $C_{1'}$–$C_5$ bond is formed (Fig. 3b). The distance from the target nucleotide to the H and/or ACA box is conserved and is 14–15 nt. Thus, some H/ACA snoRNAs guide pseudouridylation of one site in rRNA, while some others determine two targets.

As the C/D family, the H/ACA family includes snoRNAs that direct pseudouridylation and those whose targets are unknown [19, 22, 32]. It is probable that mRNAs are such targets.

Known H/ACA snoRNAs are fewer then C/D snoRNAs. It is rather difficult to identify H/ACA snoRNAs in databases, because their characteristic sequences are too short and degenerate. Construction of cDNA libraries of small RNAs is now the main approach to identification of H/ACA snoRNAs. It should be noted that considerable progress has recently been made in the field [32].

## SMALL RNAs FROM CAJAL BODIES

Recent studies revealed unusual C/D and H/ACA RNAs, which are localized in so-called Cajal bodies (CBs). CBs, or coiled bodies, are spherical or oval structures of 0.3–0.5 μm in diameter that occur in the nucleus and consist of coiled fibrils formed mostly of p80-coilin [33]. CBs were found in vertebrates, invertebrates, and plants [34]. Yeast CB-like structures are known as nucleolar bodies [35]. The CB number depends on the cell type and species, ranging from 1 to 100 (usually, 2–5) [36]. CBs are often associated with the nucleolus, occurring within the nucleus in yeasts [35]. CBs contain all three RNA polymerases, basal transcription factors, snRNAs, and snoRNAs, but not mRNA and rRNA [37]. Hence, the main role of CBs is presumably associated with maturation and assembly of snRNP, snoRNP, and transcription complexes, which function, then, in other regions of the nucleus [33].

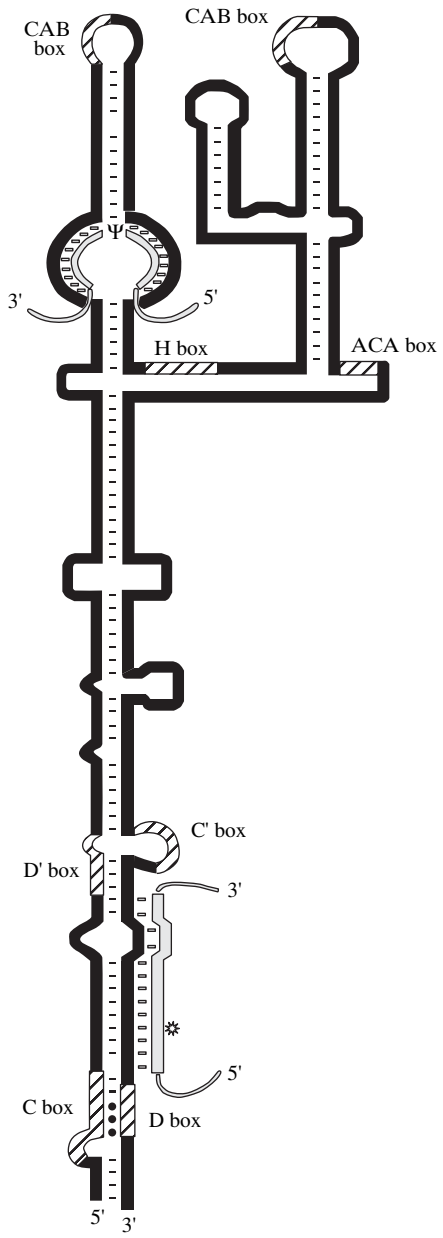


**Fig. 4.** Structure of chimeric scaRNA. The secondary structure of the U85 scaRNA is shown as in [15, 39]. The antisense elements of U85 interact complementarily with the U5 snRNA (gray). The 2'-O-methylated nucleotide is asterisked. The conserved elements of U85 are crosshatched.

According to their location the new small RNAs were termed small CB-specific RNAs (scaRNAs) [18]. Less than 20 scaRNAs are known now, but their number will probably increase. Two scaRNAs belong to the C/D family, one belongs to the H/ACA family, and one other harbors two H and two ACA boxes, actually representing two H/ACA snoRNAs joined together [38]. Four other scaRNAs are 250–350 nt in size and have an unusual structure, containing the characteristic sequences of both C/D and H/ACA families (Fig. 4) [18, 39]. Each of these scaRNAs is capable of guiding both 2'-O-methylation and pseudouridylation and is bound with both H/ACA-RNP and C/D-RNP proteins. All scaRNAs are involved in modification of snRNAs (U1, U2, U4, and U5) that have high contents of 2'-O-methylated ribose and pseudouridine (13 and 21 nucleotides, respectively) [34]. For instance, the U85 scaRNA directs $C_{45}$ methylation and $U_{46}$ pseudouridylation of the U5 snRNA [39]. Interestingly, two unrelated scaRNAs often guide the same snRNA modification, suggesting that the mechanism of snRNA modification is more error-proof than the mechanism of rRNA modification.

A search for the elements responsible for the CT localization of scaRNA, degenerate tetranucleotide ugAG, termed CAB (Cajal body) box was found in the loops of the two hairpins forming the H/ACA-RNA secondary structure (Fig. 4). The first two nucleotides of the CAB box display low conservation, while the other two nucleotides are highly conserved. The two hairpin CAB boxes act cooperatively to determine the CB localization of scaRNA [40]. It is known that C/D snoRNAs [41] and, possibly, H/ACA snoRNAs [42] pass through CBs on their way to the nucleolus during maturation. Hence, the CAB box most likely holds scaRNAs in CBs, rather than targeting them to CBs. It should be noted that the C/D motif does not ensure the CB localization of RNA [40], and it is unclear how C/D scaRNAs, possessing only the C/D motif and lacking the CAB box, are held in CBs. Apparently, other, CAB-unrelated, mechanisms also act to hold small RNAs in CBs.

Interestingly, vertebrate telomerase RNA was found to contain a motif of two hairpins and two single-stranded regions with the H and ACA boxes, as is characteristic of H/ACA snoRNA [43] (Fig. 5). The motif is at the 3' end of telomerase RNA and, in human RNA, includes 240 of the total 451 nt. The H/ACA motif of telomerase RNA is bound with all core proteins of H/ACA RNP [44] and is essential for telomerase RNA stability and the telomerase function in vivo [43]. In addition, the H/ACA motif is necessary for the nuclear localization of telomerase RNA, which is retained in the cytoplasm in its absence [45].

The motif was recently found to contain the CAB box, characteristic of scaRNA. It is owing to this box that telomerase RNA occurs in CBs in HeLa cells [47, 48], although a minor fraction (about 10%) is detectable in the nucleolus [43, 45]. Note that CBs contain telomerase RNA in tumor cells and lack it in normal cells [48]. A possible cause is that functional reverse transcriptase occurs only in immortalized cells, while telomerase RNA is ubiquitous. Hence, the CB location of telomerase RNA in HeLa cells indicates that telomerase is assembled in CBs or accumulates there in an inactive state. This assumption is supported by the fact that reverse transcriptase is located in the

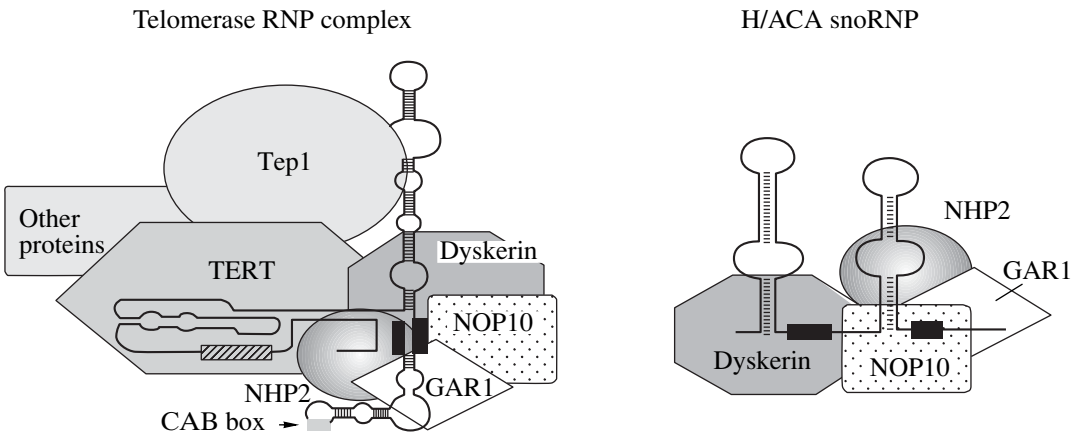


**Fig. 5.** Structure of the telomerase RNP complex. The telomerase RNA domain shown on the right (vertically) contains the H and ACA boxes (black bars), has a secondary structure characteristic of H/ACA snoRNA, and is bound with the H/ACA-snoRNP proteins. The sequence serving as a template in telomere synthesis is crosshatched. The structure of H/ACA snoRNP is shown for comparison. Modified from [46].

nucleolus, which is usually tightly associated with CBs [49].

## RNase P AND RNase MRP: RNA-CONTAINING ENDONUCLEASES

### RNase P

This RNase was found in all living cellular organisms: eukaryotes, bacteria, and archaea. Eukaryotic RNase P occurs in the nucleoplasm and, in a minor amount, in the nucleolus. In addition, RNase P activity is detectable in mitochondria and chloroplasts. RNase P is involved in removing the 5'-terminal leader from pre-tRNA: the enzyme hydrolyzes the phosphodiester bond, releasing 5'-P of mature tRNA and 3'-OH of the leader. Bacterial RNase P cleaves the precursors of several other RNAs as well, playing a role, in particular, in generation of the 4.5S rRNA [50]. Eukaryotic RNase P may also have additional substrates, although direct evidence is as yet lacking. For instance, in addition to processing pre-tRNA, nuclear RNase P probably cleaves pre-rRNA at the second internal transcribed spacer: yeast cells with mutant RNase P accumulate 3'-extended 5.8S rRNA [51].

The RNA component of RNase P has several regions conserved among all taxa. The secondary structure of the RNA core is also conserved, while the structure of the peripheral regions greatly varies. In eukaryotes, the RNA component of RNase P is synthesized by RNA polymerase III. Its processing probably follows RNase P assembly, and its details differ in different taxa. For instance, the 5'- and 3'-terminal regions are removed from the primary transcript only in yeasts, decreasing the transcript size from 486 to 369 nt. RNase P is most likely assembled in the nucleolus: the precursor and the mature form of yeast RNase P are localized in the dense fibrillar component, where assembly takes place. Then, RNase P becomes detectable in small nucleoplasmic granules, where pre-tRNA is processed with its participation [50, 52]. It should be noted, however, that some pre-tRNAs occur initially in the nucleolus, together with RNase P. Hence, pre-tRNA processing with RNase P may start in the nucleolus. In addition, it is thought that RNase P plays a role in rRNA processing in the nucleolus [50].

Bacterial RNase P has the simplest structure, consisting of 350- to 400-nt RNA and one small protein (119 amino acid residues). Its RNA component possibly has catalytic functions. In contrast, eukaryotic RNase P is an intricate RNP complex, including about ten proteins; its RNA component lacks catalytic activity. It is unclear whether activity is lacking because the proteins fold the RNA properly or take up the catalytic function. An intermediate situation is observed in archaea: their RNase P consists of several subunits, but these are fewer than in eukaryotes. The subunits are all homologous to their eukaryotic counterparts. Interestingly, none of the protein subunits of eukaryotic or archaeal RNase P is homologous to the protein component of bacterial RNase P. Thus, to generate functionally identical enzymes, eukaryotes and archaea, on the one hand, and bacteria, on the other hand, utilize similar RNAs and fundamentally different protein components [50].

All RNase P subunits are vital for yeast cells [50]. It is surprising in view of this that RNase P subunit composition varies with taxa. For instance, RNase P of some taxa lacks one or several subunits contained in the human and yeast enzymes. The subunit composition of archaeal RNase P is also polymorphic: the enzymes from different species have four, two, or only one subunit. This observation is as yet unexplained

[53]. Interestingly, the only subunit present in all archaea is encoded by a gene belonging to a ribosomal protein operon, as in bacteria. It seems that RNase P synthesis is coordinated with synthesis of ribosomal proteins, testifying again to the role of RNase P in ribosome biogenesis [53].

**Mitochondrial RNase P** cleaves pre-tRNA, as its nuclear counterpart does. The enzyme was found in all eukaryotes and studied in the most detail in yeasts. The RNA component of yeast mitochondrial RNase P is encoded by the mitochondrial genome and is similar to bacterial RNase P RNA. As in bacteria, RNase P has one protein subunit, which is encoded by the nuclear genome and lacks similarity to the bacterial subunit. Moreover, homologs of the bacterial gene for the RNase P protein were not found in the mitochondrial and chloroplast genomes [50].

**Chloroplast RNase P** has not been adequately studied. The enzyme possibly lacks RNA, although the chloroplast genome harbors nucleotide sequences homologous to the RNase P RNA [50].

Thus, RNase P is ubiquitous in cellular organisms. Of all RNPs, such a ubiquity is characteristic of only ribosomes and signal recognition particles (SRPs). It is still an open question as to why catalysis of such a simple reaction as breakage of one phosphodiester bond requires the intricate universal machine, RNase P, while a single protein could suffice [53].

## RNase MRP

In contrast to bacteria and archaea, eukaryotes have RNase MRP, another RNP endonuclease, similar to RNase P. RNase MRP occurs mostly in the nucleolus and is present in mitochondria, where it cleaves the bond between the RNA primer and nascent DNA, owing its name to this function (MRP, mitochondrial RNA processing) [54]. In the nucleolus, RNase MRP cleaves pre-rRNA at A3 of the first internal transcribed spacer, releasing the mature 5.8 rRNA [50]. Although yeast cells lacking the 5.8S rRNA are viable, all components of RNase MRP are essential. Apparently, RNase MRP plays another important role, unidentified as yet. It is probable that RNase MRP is involved in controlling the cell cycle, in particular, mitosis [51].

The RNA component of RNase MRP is synthesized by RNA polymerase III in the nucleoplasm. The central region of the MRP RNA is necessary for its mitochondrial import, while the 5'-terminal region is responsible for the nucleolar localization [51]. In addition to RNA, RNase MRP includes several proteins, many of which are present in RNase P. For instance, yeast RNases P and MRP share eight out of nine proteins [50]. The nucleotide sequences of the RNA components of RNases P and MRP are not conserved, but these RNAs are similar in secondary structure.

Thus, eukaryotes differ from other taxa in having two similar RNP endonucleases. Notwithstanding their structural similarity, these enzymes differ in function.

## NOMENCLATURE OF snoRNAs

There is still no unified nomenclature of snoRNAs. This is partly explained by the fact that homologs found in representatives of different large taxa, e.g., in humans and yeasts, have considerable similarity only within characteristic sequences and antisense elements; i.e., their homology is relative.

Yeast snoRNAs were initially designated according to their positions in gel after two-dimensional PAGE (snR1, snR2, etc.) or to their size (snR128, snR190, etc.) [1]. The latter system was rejected, while the former is still in use.

The names of most vertebrate snoRNAs follow the snRNA nomenclature, consisting of U with the corresponding ordinal number. This form has been chosen in view of the high U content in snRNA. Although snoRNAs are not all enriched in U, new snoRNAs are usually (but not always) termed according to this convenient standard system [1]. Homologs found in other organisms are given the same names. In the case of large taxa, the name starts with two letters corresponding to the source organism. For instance, an *Arabidopsis thaliana* homolog of the vertebrate U29 snoRNA is known as AtU29. At the same time, homologs are often termed differently. The number of organisms found to have new snoRNAs and homologs of known snoRNAs is continuously increasing, which gives an opportunity for developing new nomenclatures, and this opportunity is not missed. As a result, snoRNA names are highly variable.

In early 2006, the HUGO Gene Nomenclature Committee proposed a unified nomenclature for human snoRNAs. According to this nomenclature, C/D snoRNAs are termed SNORDn, where n is the ordinal number of snoRNA. The existing snoRNA number is preserved when possible. For instance, the U87 snoRNA [55] is designated as SNORD87. Likewise, the nomenclature suggests the name SNORAn for H/ACA snoRNAs and SCARNAn for scaRNAs. When similar systems are accepted for other taxa, the snoRNA nomenclature will assume the missing plainness.

## RNP FORMATION

In vivo, snoRNAs are always associated with proteins to form snoRNP. Some snoRNPs are associated with maturing rRNAs (70–90S complexes), some others are free (10–15S complexes) [1].

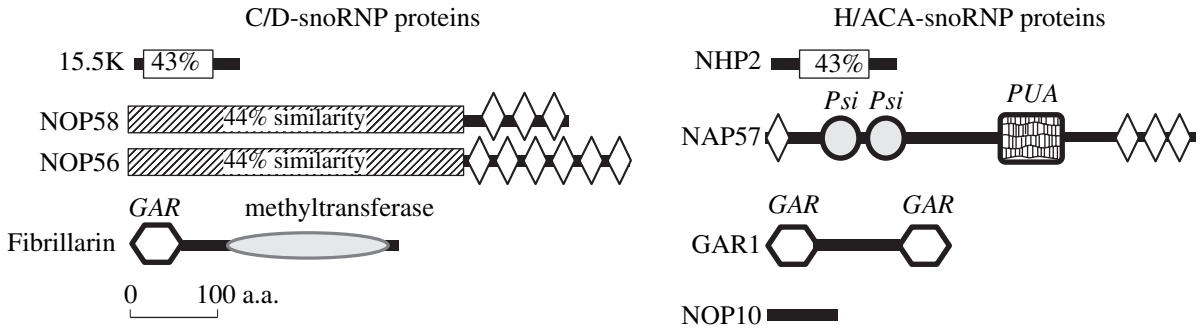


**Fig. 6.** Schemes of the amino acid sequences of human C/D- and H/ACA-snoRNP proteins. Pairwise sequence similarity (%) is indicated for NOP56 and NOP58 and for NHP2 and the 15.5-kDa protein. Indicated are Lys-rich regions (rhombi); the domain characteristic of methyltransferases (oval); the Gly/Arg-rich (GAR) domain (hexagon); two Ser clusters (Psi, circles), occurring in most pseudouridine synthases; and the PUA domain (rectangle), common for pseudouridine synthases and some transglycosylases. The bar under the dyskerin (NAP57) sequence shows the scale. Modified from [44].

## C/D snoRNP Proteins

Mature C/D snoRNAs form a stable complex with four conserved proteins, which are all essential for cell growth in yeasts [56]. These are 15.5-kDa protein/Snu13p, NOP56/Nop56p, NOP58/Nop58p, and fibrillarin/Nop1p (Fig. 1b; hereafter, the names of vertebrate and yeast proteins are given with a solidus).

All these proteins bind to the C/D motif, stabilizing snoRNA and protecting its ends from exonucleases [57]. The proteins are all necessary for the nucleolar localization of C/D snoRNAs, but the effects observed in the absence of a particular protein slightly differ: a lack of Nop1p or Sno13p distorts the nucleolar targeting of snoRNAs in yeasts to a greater extent than a lack of Nop56p or Nop58p does [35].

The 15.5-kDa protein/Snu13p belongs to the RNA-binding protein family, which also includes several ribosomal proteins and NHP2, associated with H/ACA snoRNAs. Snu13p was found not only in C/D snoRNP but also in the spliceosomal complex formed by the U4/U6-U5 snRNAs. Snu13p is bound to the K-turn motif of U4 in this complex, as in the complex with C/D snoRNA [11, 58].

Two other C/D-snoRNP proteins, NOP56/Nop56p and NOP58/Nop58p, are homologous to each other [59] (Fig. 6) and similar to the 61-kDa protein/Prp31p, a component of the U4-U6/U5 snRNP complex [58].

It is unclear whether the structural similarity of C/D snoRNP and snRNP is functionally significant. Possibly, Sno13p moves from the spliceosome to a newly formed snoRNP complex after host-gene mRNA splicing. It is of interest in this respect that the distance from the end of the snoRNA gene to the branching point is critical for snoRNA formation [60].

Methylation is apparently performed by fibrillarin/Nop1p, although direct evidence of this has not been obtained for animals or yeasts [56]. Fibrillarin possesses a domain binding S-adenosyl methionine, a methyl group donor. Moreover, fibrillarin is similar in amino acid sequence and ternary structure to known methyltransferases. Finally, methyltransferase activity was observed for the archaeal homolog of fibrillarin, suggesting the same activity for eukaryotic fibrillarin [61].

## Assembly of C/D snoRNP

Assembly of C/D snoRNP can start after the C/D motif is formed during the folding of the snoRNA precursor. The C/D motif binds with the 15.5-kDa protein/Snu13p, which seem to directly interact with the loop of the K-turn motif [58]. It is essential for the binding of this protein that the two double-stranded regions of the C/D motif are intact, while their nucleotide sequence is unimportant [62]. The binding of the 15.5-kDa protein/Snu13p probably changes the conformation of snoRNA to allow its binding with the other snoRNP proteins [62]. Thus, assembly of C/D snoRNP is a hierarchic process that proceeds through several steps.

The vertebrate 15.5-kDa protein binds to intron-encoded snoRNA during splicing, at the so-called complex C1 stage, when pre-mRNA is bound only with the U2, U5, and U6 snRNAs. Spliceosomal proteins probably recruit the 15.5-kDa protein or remove some factors hindering its binding. The 15.5-kDa protein is bound and snoRNP assembled only when the snoRNA gene is at an optimal distance (about 70 nt) from the branching point. A possible cause is that snoRNP proteins compete with spliceosomal proteins for the binding sites when the distance is too short (less than 50 nt), while the spliceosome cannot recruit the 15.5-kDa protein when the distance is too great (more than 200 nt) [63].

Interestingly, some snoRNAs are far away (about 300 nt) from the branching point, but their processing is still normal [60]. It was found that the nucleotide

sequences flanking the snoRNA genes form extended double-stranded regions (12 bp or more). The snoRNA precursor stabilized by such a structure can bind the 15.5-kDa protein and, apparently, the other C/D-snoRNP core proteins even in the absence of splicing, although snoRNA is not formed in this case. Thus, an extended double-stranded region formed by the snoRNA precursor compensates for the nonoptimal snoRNA position in an intron [63].

Not only the integrity of the double-stranded regions of the C/D motif but also the nucleotide sequence of helix 2, including the C and D boxes (Fig. 2b), are absolutely essential for the binding of the other three C/D-snoRNP proteins to snoRNA. Any changes in helix 2 sequence almost completely abolish the binding of these snoRNP proteins to snoRNA even when the complementary interactions are preserved. Thus, the C and D boxes, which form helix 2, are important for the binding of the snoRNP core proteins.

The details of the association of these proteins with snoRNA are still far from being completely understood. It is known only that fibrillarin and NP58 independently bind to the D and C boxes, respectively [64]. The association of fibrillarin with the D box agrees with its putative methyltransferase activity, because it is the distance from the D box that determines the methylation target site. NOP56 is not essential for snoRNP stability and does not bind to snoRNA until fibrillarin is bound [65]. Direct interactions of NOP56 with the C/D motif were not detected [64]. Apparently, NOP56 binds to the C/D motif via protein–protein interactions (Fig. 1b).

It should be noted that C/D snoRNAs contain not only the C and D boxes but also the C' and D' boxes, which are in the central region of the molecule and are often close together owing to hairpin formation. It seems logical to assume that C/D snoRNA forms two copies of the C/D motif and binds two sets of proteins, displaying a module structure. This assumption is supported by the fact that many snoRNAs have two antisense elements or their only antisense element is close to the D' box. As expected, the putative C'/D' motif proved to guide methylation [13]. However, the C'/D' motif is conserved to a far lesser extent as compared with the C/D motif: the C' and D' boxes and their flanking sequences are usually degenerate and incapable of forming the K-turn motif, which is necessary for the binding of the 15.5-kDa protein/Snu13p and subsequent snoRNP assembly. It was observed, indeed, that the 15.5-kDa protein interacts with the C/D, but not with the C'/D' motif [66]. Likewise, NOP58 binding to the C'/D' motif was not detected [64]. Only NOP56 and fibrillarin are capable of binding to the C'/D' motif, the former interacting with C' and the latter, with C' and D' [64]. Thus, C/D snoRNP complexes are structurally asymmetrical (Fig. 1b).

Interestingly, even when the internal snoRNA regions form a fully functional C'/D' motif, it cannot bind with the 15.5-kDa protein [66]. A possible cause is that fibrillarin and NOP56 interact with the same nucleotides of the C'/D' motif as the 15.5-kDa protein does, hindering its access to the binding site [15]. Since only fibrillarin is directly involved in methylation, it is possible that methylation does not require a stable binding of all core proteins to the C'/D' motif. Fibrillarin binds both to the C/D and C'/D' motifs, suggesting that C/D snoRNP contains two fibrillarin molecules [64].

Thus, different requirements are apparently imposed upon the C/D and C'/D' motifs, since the latter only guides methylation, while the former additionally ensures snoRNP stability and nucleolar localization. It is probably this difference in function that determines the asymmetrical structure of C/D snoRNP.

A strikingly different situation is characteristic of archaea, whose C/D and C'/D' motifs both bind the complete sets of the C/D-snoRNP proteins. It should be noted that the C' and D' boxes are almost never degenerate in archaea [15].

## H/ACA-snoRNP Proteins

Like C/D snoRNA, mature H/ACA snoRNA is associated with four conserved proteins [67, 68]: GAR1/Garp1, dyskerin/Cbf5p, NHP2/Nhp2p, and NOP10/Nop10p (Fig. 3a). Each of the proteins directly interacts with snoRNA [69], is essential for yeast cell growth, and, with the exception of GAR1/Gar1p, stabilizes snoRNA [44].

The central domain of GAR1/Gar1p directly binds to snoRNA. The terminal domains contain Gly/Arg repeats, determining the name of the protein [70]. These domains stabilize the snoRNA–rRNA complex and are involved in protein–protein interactions [71]. A lack of GAR1 suppresses pseudouridylation [72], but has no effect on snoRNP accumulation.

Dyskerin (NAP57)/Cbf5p probably acts as a pseudouridine synthase, although direct evidence is as yet lacking, as in the case of fibrillarin. Dyskerin harbors two motifs occurring in most pseudouridine synthases (Fig. 6) and an extended region displaying 34% homology to *E. coli* TruB pseudouridine synthase [44]. Moreover, point mutations affecting dyskerin dramatically reduce the level of rRNA pseudouridylation, and a knockout in the dyskerin gene leads to embryonic death in *Drosophila* and mice. Some mutations of the dyskerin gene cause dyskeratosis congenital, a hereditary disorder that is accompanied by poikiloderma (dystrophic changes in the skin), nail dystrophy, and several other signs [46].

The role of the two small core proteins, NHP2/Nhp2p and NOP10/Nop10p, is still incompletely understood, although the proteins are essential for the snoRNP function. NHP2 was initially identified as a chromatin-associated nonhistone protein [44]. NHP2 is homologous to the C/D-snoRNP 15.5-kDa protein and ribosomal protein L30. The NOP10 amino acid sequence is conserved among various taxa, as is the case with the other snoRNP core proteins, but NOP10 does not contain any known motif, nor does it have homologs among the other snoRNP core proteins.

Interestingly, C/D and H/ACA snoRNPs have homologous proteins and proteins with common domains (Fig. 6).

Common domains were found in dyskerin (NAP57) and C/D-snoRNP proteins NOP56 and NOP58 and in fibrillarin and GAR1. The most interesting finding is that NHP2 is similar to the C/D-snoRNP 15.5-kDa protein, which binds to the K-turn motif. The archaeal ortholog of these proteins is ribosomal protein L7Ae, which is also a core component of C/D and H/ACA snoRNPs [44]. The assumption that H/ACA snoRNAs contain the K-turn motif needs verification.

## Assembly of H/ACA snoRNP

While C/D snoRNP assembly proceeds through several steps, the H/ACA-snoRNP proteins interact with each other even in the absence of RNA [73]. For instance, GAR1 and NOP10 independently bind dyskerin, while dyskerin binds with NHP2 only when bound with NOP10. The dyskerin–NOP10–NHP2 trimer is sufficient for H/ACA snoRNA binding in both vertebrates and yeasts [44]. The H/ACA-snoRNP proteins do not interact with the snoRNA precursor during splicing, as is the case with C/D snoRNA. Their interaction takes place earlier, when the splicing sites are selected. Thus, H/ACA snoRNA processing is splicing-independent, and, accordingly, the H/ACA-snoRNA genes are not preferentially located in the vicinity of the 3'-splicing site in contrast to the C/D-snoRNA genes [74]. Electron microscopy of purified yeast H/ACA snoRNP showed that every H/ACA snoRNA is associated with two sets of proteins, suggesting that the hairpins are each bound with the four core proteins [67].

## Proteins Involved in Biogenesis of C/D and H/ACA snoRNPs

Assembly of snoRNP occurs during transcription [75]. Both assembly and transport of snoRNP involve accessory proteins, which are not associated with mature snoRNP, nor are they significant for the snoRNP function. Less than ten such proteins are known now [44]. Here we consider only the proteins studied in the most detail.

C/D snoRNAs are weakly associated with two structurally related nucleoplasmic proteins, TIP48 (also known as TIP49b, RUVBL2, p50, TAP54β, or Reptin42) and TIP49 (TIP49a, RUVBL1, p55, or TAP54) [56]. These proteins possess DNA helicase activity and play a role in many processes, including DNA repair, transcription [76], and snoRNP assembly and transport. For instance, the yeast ortholog of TIP48 is necessary for the accumulation of C/D and H/ACA snoRNPs and for the nucleolar localization of Nop1p and Gar1p, core components of snoRNP [56].

The nucleolus and CBs contain Nopp140, which shuttles between these organelles and between the nucleolus and the cytoplasm. Nopp140 interacts with C/D and H/ACA snoRNPs and, possibly, plays a role in their assembly and transport. In yeasts, a Nopp140 homolog is necessary for H/ACA snoRNA accumulation and C/D RNA retention in the nucleolus [56].

The SMN (survival of motoneurons) protein, whose mutations cause spinal muscular atrophy, interacts with the C/D- and H/ACA-snoRNP core proteins, fibrillarin, and GAR1 and is probably involved in snoRNP assembly. In addition, SMN is one of the most important proteins necessary for assembly of RNP involved in ribosome biogenesis, splicing, and transcription [56]. It should be noted that, important as it is in eukaryotes, SMN is absent from yeasts: its yeast homolog has not been found [44].

In contrast to snoRNAs guiding nucleotide modification, snoRNAs involved in rRNA processing are usually associated with greater sets of proteins. For instance, the U3 RNP, which is involved in the formation of the 18S rRNA from pre-rRNA, contains one other protein in addition to the four usual core proteins and is associated with at least seven other proteins for a certain period [56].

To conclude, snoRNAs and microRNAs are the two most abundant and well-studied groups of small noncoding RNAs. Still, the functional significance of the overwhelming majority of modifications guided by snoRNAs are as yet unclear, notwithstanding numerous studies (e.g., see [77–82]). Currently available experimental findings demonstrate that modifications of a few nucleotides in rRNA, snRNA, and mRNA are distinctly expressed phenotypically [6, 83–85], while the effect of other modifications is detectable only in the case of their cooperative interactions, stabilizing the RNA structure.

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