Nucleolar RNPs: from genes to functional snoRNAs in plants

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Abstract

The snoRNAs (small nucleolar RNAs) and related scaRNAs (small RNAs in the Cajal bodies) represent a major class of nuclear RNAs that guide 2′-*O*-ribose methylation and pseudouridylation of rRNAs, snRNAs (small nuclear RNAs) and other RNA targets. *In vivo*, all snoRNAs associate with a set of four highly conserved nucleolar proteins, forming the functional snoRNPs (small nucleolar ribonucleoproteins). The core structure of these mature snoRNPs has now been well described in eukaryotes, but less is known of their biogenesis. Recent data in animals and yeast reveal that assembly of the snoRNPs is a complex process that implicates several auxiliary proteins and transient protein–protein interactions. This new level of snoRNP regulation is now beginning to be unravelled in animals and yeast, but remains unexplored in plants. In the present paper, we review recent data from genomic and functional analysis allowing the identification and study of factors controlling the biogenesis of plant snoRNPs and their impact on plant development.

Functional diversity of snoRNAs (small nucleolar RNAs), scaRNAs (small RNAs in the Cajal bodies) and others . . .

The snoRNAs represent an abundant class of ~50–300 nucleotide *trans*-acting RNAs in all eukaryotes. They are absent from bacteria, but are present in archaea, where they are named sRNAs (for small RNAs), demonstrating an ancient origin of snoRNAs. Most snoRNAs fall into two classes: the C/D snoRNAs that direct 2′-*O*-ribose methylation and H/ACA snoRNAs that direct pseudouridylation (for reviews, see [1–3]). The C/D snoRNAs have conserved C and D motifs flanked respectively by 5′ and 3′ terminal inverted repeats. These elements fold into a kink-turn RNA motif which is specifically recognized by the 15.5K snoRNP (small nucleolar ribonucleoprotein) core protein component (see below). Adjacent to the D or D′ internal box there is a 10–21 nt guide element complementary to the RNA target sequence. The H/ACA snoRNAs have a 3′ terminal ACA motif and one or two hairpin structures separated by a short hinge region which contains an H/ANANNA box. A pseudouridylation guide sequence complementary to the RNA target is located in a bulge present in each hairpin. The major targets of snoRNAs are the rRNAs which have more than 100 methylated residues in plants and vertebrates. These modifications occur on the functional centres of the ribosomes and are important for their translational capacity [4,5].

Other classes of RNAs functionally and structurally related to snoRNAs are the scaRNAs which are located in the Cajal bodies and direct modifications of snRNAs (small nuclear RNAs) [6,7]. The scaRNAs are less abundant than snoRNAs, but the modifications of the snRNAs are essential for splicing [8].

In addition to the rRNA and snRNA targets, snoRNAs and related scaRNAs have also been shown to direct modification of tRNAs in archaea and a brain-specific mRNA in vertebrates. Moreover, many 'orphan' snoRNAs, for which no target could be predicted, have been identified in all species, suggesting that they have additional functions. In addition, some essential snoRNAs, like C/D snoRNAs U3 or U14, which are phylogenetically conserved from yeast to humans and plants, act as RNA chaperones to direct specific cleavages of the ribosomal RNA precursor [1–3].

Finally, the snoRNA structure has been co-opted to other roles. One example is hTR (human telomerase RNA), the subunit of the telomerase RNP (ribonucleoprotein), which has an H/ACA snoRNA fold recognized by dyskerin/NAP57 (see below) and which is important for telomere maintenance [9].

More recently, it has been found in humans and other species including plants that some snoRNAs can be processed into small RNAs with a miRNA (microRNA)-like function [10,11]. Interestingly, in *Arabidopsis*, these snoRNA-derived small RNAs were found associated with AGO7, which is implicated in gene-silencing pathways [11].

Thus the functional picture of snoRNAs and scaRNAs has been greatly expanded, revealing a multiplicity of targets and functions, although many still remain to be elucidated.

The high diversity of plant snoRNA genes

At present, more than 200 snoRNA genes have been reported in *Arabidopsis thaliana* and rice. Most of them

Key words: *Arabidopsis thaliana*, assembly factor, Cajal body, RNA modification, small nucleolar RNA (snoRNA).

Abbreviations used: NAF1, nuclear assembly factor 1; NUFIP, nuclear Fragile X mental retardation protein-interacting protein; Pol, polymerase; RNP, ribonucleoprotein; scaRNA, small RNA in the Cajal bodies; snoRNA, small nucleolar RNA; pre-snoRNA, snoRNA precursor; snoRNP, small nuclear RNP; snRNA, small nuclear RNA; snRNP, small nuclear RNP; TIP, TATA-box-binding protein-interacting protein.

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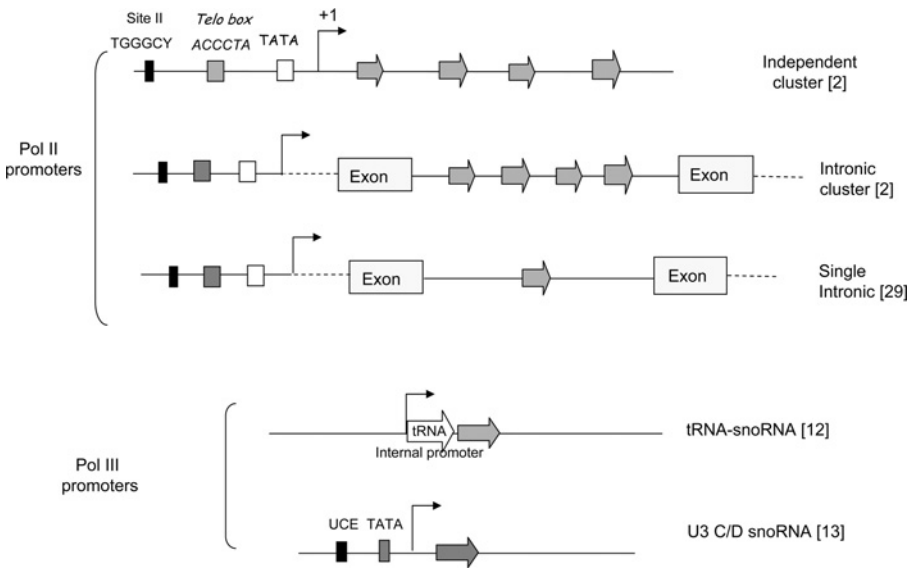
Figure 1 | **Structural organization of genes encodings snoRNAs directing modification of rRNAs in *Arabidopsis***

The scheme and the position of snoRNAs and elements are not to scale. +1 indicates transcription initiation site. Boxes indicate different promoter elements. The grey arrows indicate snoRNA. The white arrow indicates tRNA. UCE, upstream control element.



encode C/D snoRNA targeting rRNAs that were identified by bioinformatic screening of the model plant genomes (reviewed in [2]). More recently, high-throughput sequencing of small RNA fractions from *Arabidopsis* has expanded their number, particularly the number of H/ACA snoRNAs as well as predicted scaRNAs [12]. However, the localization of these potential scaRNAs in the Cajal bodies has to be confirmed by *in situ* hybridization. In addition, these studies reveal many orphan snoRNAs, suggesting additional functions and/or targets in plants [12].

The eukaryotic snoRNAs exhibit very diverse modes of genomic organization. In animals, most snoRNAs are located within introns of host genes that usually encode proteins related to ribosome biogenesis [1]. In yeast, the majority of snoRNAs are encoded by monocistronic genes transcribed from their own Pol (polymerase) II promoter. In plants, most snoRNAs are found in genomic clusters which are independent, transcribed from their own promoter or located in introns of protein-coding genes (Figure 1). Polycistronic gene clusters are not found in animals and are rarely found in yeast. In addition, plants also have a unique class of genes corresponding to dicistronic tRNA–snoRNA genes (Figure 1). These are transcribed by Pol III and produce a dicistronic precursor that is processed into a mature tRNA and a C/D snoRNA [13].

Independent monocistronic genes are not frequent in plants. Indeed, the only clear example corresponds to U3. Remarkably, the U3 gene in plants is transcribed by Pol III, whereas in yeast and animals, the U3 gene is transcribed by Pol II [14]. Notably, U3 is also a rare example of a monocistronic snoRNA gene in animals.

The clustered organization of snoRNA genes in plants could serve to co-ordinate their expression with the factors implicated in ribosome biosynthesis. In *Arabidopsis*, *in silico* analysis reveals that most gene clusters encoding snoRNA-targeting rRNAs have a common promoter structure characterized by Telo box (ACCCTA) and site II (TGGGCY) elements associated with a TATA box (Figure 1). These Pol II promoter elements have been shown to control the transcription of genes encoding ribosomal proteins or other proteins involved in ribosome biogenesis (Figure 1) [15,16].

The processing of pre-snoRNAs (snoRNA precursors)

Mature snoRNAs are produced by processing of a pre-snoRNA that can be polycistronic, intronic or monocistronic. In animals, the major pathway for processing intronic snoRNAs depends on intron debranching followed by exonucleolytic trimming of the 5′ and 3′ free ends [17].

Processing of polycistronic pre-snoRNA has been well described in yeast [18]. A key factor in this process is Rnt1p, an RNase III endonuclease that cleaves the pre-snoRNA and liberates the individual snoRNAs with 5′ and 3′ extensions. These extensions are eliminated by Rat1p or Xrn1p 5′→3′ exonucleases and the nuclear exosome which has 3′→5′ exonuclease activity [19]. The mature snoRNA ends are protected by assembly of snoRNP core proteins [20].

In plants, most snoRNAs are processed from polycistronic precursors and this implicates an endonucleolytic cut to

release the snoRNAs [21]. Nevertheless, the endonuclease responsible for this cleavage has not been identified. AtRTL2, the closest homologue of Rnt1p in *Arabidopsis*, could be implicated, but no effect on pre-snoRNA accumulation was detected in *atrtl2* mutants [22]. This could be due to gene redundancy, because *AtRTL2* is part of a multigene family, and/or the existence of an alternative pathway independent of Rnt1-like activity.

The *Arabidopsis* homologue of the yeast exosome was recently implicated in 3′ end processing of plant snoRNAs. This was revealed by RNA profiling of *Arabidopsis* mutants depleted in two distinct core subunits of the exosome [23]. Interestingly, this depletion led to accumulation of polyadenylated snoRNAs derived from polycistronic precursors. The polyadenylation of snoRNAs had already been observed in yeast exosome mutants. This was intriguing because 3′ end transcription termination of snoRNA genes depends on the Nrd1 complex and is not associated with poly(A) polymerase-dependent polyadenylation [24]. It was shown that snoRNA polyadenylation is catalysed by a TRAMP (Trf4–Air2–Mtr4p polyadenylation) complex associated with the Nrd1 complex and this recruits the exosome, either for correct maturation of the 3′ end of the snoRNAs or to degrade aberrant snoRNAs [25,26].

Plants also have specific pathways which operate for processing dicistronic tRNA–snoRNA precursors. The study of processing of tagged tRNA–snoRNA precursor mutants both *in vitro* and *in vivo* revealed two alternative pathways specific to plants [27]. The first depends on tRNA-processing activities, including the 3′ end tRNA-processing tRNAse Z endonuclease and the *Arabidopsis* homologue of La protein that binds to the U-rich queue of Pol III transcripts [28]. The alternative pathway to process tRNA–snoRNA precursors occurring *in vivo* probably depends on the tRNA decay system that liberates the snoRNA with concomitant degradation of the tRNA from the dicistronic precursor [27].

A conserved core structure of snoRNPs

In vivo, all snoRNAs form a core snoRNP with a set of four nucleolar proteins which are conserved in all eukaryotes. The C/D snoRNAs associate with fibrillarin (Nop1p in yeast) which is the RNA methylase, NOP58, NOP56 and 15.5K (yeast Snu13p) [1–3]. The 15.5K is also a component in the U4 snRNP (small nuclear RNP) spliceosome and is part of the L7Ae RNA-binding protein family, which includes ribosomal proteins L7A and L30 [29]. The H/ACA snoRNAs associate with dyskerin/NAP57 (Cbf5p in yeast), which is the pseudouridine synthase, NHP2, NOP10 and GAR1 [1–3]. NHP2 protein is also a member of the L7Ae protein family [29].

In plants, the core snoRNP proteins are highly conserved (Table 1) and have all been found in the nucleolar proteome from *Arabidopsis* [28]. Most significantly, it has been shown that AtFib2 (*Arabidopsis* fibrillarin 2) can rescue the yeast Nop1p conditional mutant, which is an essential gene [31].

Table 1 | Comparison of human core snoRNPs and snoRNPs assembly factors with the *Arabidopsis* homologues

The *Arabidopsis* core snoRNPs and NAF1 have been reported previously [30–33]. Potential *Arabidopsis* assembly factors were identified by TBLASTN (J. Rodor and M. Echeverria, unpublished work).

		human factors	potential homologs in *A.thaliana*	% identity between *A.thaliana* and human
Core proteins	C/D snoRNP	FIBRILLARIN	At5g52470	57
			At4g25630	70
		NOP58	At3g05060	37
			At5g27120	36
		NOP56	At1g56110	57
			At3g12860	57
		15,5K	At5g20160	78
			At4g12600	77
			At4g22380	77
	H/ACA snoRNP	DYSKERIN	At3g57150	58
		NOP10	At2g20410	57
		GAR1	At3g03920	52
			At5g18180	49
		NHP2	At5g08180	33
Assembly factors	C/D snoRNP	hBCD1	At1g04945	19
		hTAF9	At1g54140	33
		hPIH1/hNOP17	-	
	H/ACA snoRNP	hNAF1	At1g03530	23
		hSHQ1	-	
	C/D et H/ACA snoRNP	RVB1-TIP49	At5g22330	72
		RVB2-TIP48	At5g67630	74
			At3g49830	69
		SMN	-	
		NUFIP	At5g18440	14
		hSPAGH	At1g56440	24

identity %: 70 to 80, 60 to 70, 50 to 60, 40 to 60, 30 to 40, 20 to 30, 10 to 20, 0 to 10

The dyskerin/NAP57 plant homologue was shown to be encoded by a single gene in *Arabidopsis* [32], but *atnap57* mutants are lethal and mutants could not be characterized [33]. Interestingly, AtNAP57 has been found to be associated with the *Arabidopsis* telomerase RNP and is implicated in telomerase maintenance [34]. This suggests that the plant telomerase RNA subunit, which has not yet been identified, forms an H/ACA snoRNP particle as reported for human telomerase [9].

In addition to the four core proteins, additional proteins could be associated with snoRNPs depending on their specific targets or functions. This has clearly been shown for the conserved C/D snoRNA U3 that directs cleavage of pre-rRNA. The U3 snoRNP has been purified from yeast [35] and plants [36,36a]. In both species, it was found to be a very large snoRNP with more than ~30 proteins, in addition to the four core C/D snoRNP proteins. Interestingly, the yeast and plant U3 snoRNPs are distinguished because, in addition to common protein components, they each have specific proteins [36a].

Assembly of snoRNPs

Recent genetic and biochemical studies in yeast and animals have revealed that assembly of C/D and H/ACA snoRNP implicates several auxiliary proteins and transient protein–protein interactions with the core snoRNP proteins. The emerging view is that the assembly of functional snoRNPs is tightly coupled to transcription of the snoRNA genes by Pol II, the processing of pre-snoRNA and splicing of their host

mRNA precursors [37–39]. Assembly of C/D snoRNPs is initiated by the binding of 15.5K protein to the kink–turn fold of C/D boxes. The 15.5K is the only core snoRNP protein that can directly interact with the snoRNA. This is followed by the recruitment of the three other core C/D snoRNP proteins, a process assisted by other factors [40,41]. In HeLa cells, NUFIP (nuclear Fragile X mental retardation protein-interacting protein; Rsa1 in yeast) was identified as a central protein directing this process. NUFIP interacts directly with the 15.5K protein and serves as a bridge to recruit the other core proteins [29]. It has been proposed that NUFIP is also implicated in the assembly of U4 snRNP that contains the 15.5K protein and other nuclear RNP-containing proteins from the L7Ae family, such as NHP2 from H/ACA snoRNPs [29].

In the case of H/ACA, snoRNP assembly is directed by a different set of proteins, including NAF1 (nuclear assembly factor 1), a key assembly factor both in vertebrates and yeast (Table 1). NAF1 interacts with dyskerin/NAP57/Cbf5p and subsequently recruits the other H/ACA core proteins. Most importantly, NAF1 binds to the CTD (C-terminal domain) of Pol II, and the assembly of H/ACA snoRNPs is tightly coupled to transcription [37,38,42].

In plants, the importance of snoRNP assembly was shown by impaired accumulation *in vivo* of the C/D snoRNA mutated in the C box, which is essential for the assembly process [27]. Homologues of yeast or animal assembly factors can be predicted by BLAST searches, but, except for the RNA helicases TIP48 (where TIP is TATA-box-binding protein-interacting protein) and TIP49, they are highly divergent (Table 1). In the cases of NUFIP or NAF1, the homology is restricted to a short sequence motif. For others, such as hSHQ1 or NOP17, plant homologues cannot even be identified by BLAST searches on the *Arabidopsis* genomic sequences. Overall, this suggests that snoRNP assembly implicates different or additional interactions which are specific to plant systems. This was addressed recently by the characterization of *atnufip* insertional mutants in *Arabidopsis* (J. Rodor, E. Jobet and M. Echeverria, unpublished work). This study has confirmed that, in spite of its high divergence, AtNUFIP is a functional homologue of hNUFIP and specifically directs assembly of C/D snoRNPs. Most importantly, *atnufip* mutants had strong defects in different steps of plant development, but were viable. This is an important result that should allow future studies on the impact of snoRNP biogenesis and RNA methylation on plant development.

The plant homologue of NAF1, predicted to direct H/ACA snoRNP assembly, has also been identified in *Arabidopsis thaliana*. This was based on a two-hybrid screen with AtNAP57 bait and this is a strong argument for its implication in the assembly of the plant H/ACA snoRNP [33]. Considering the central role of this factor, as well as its high divergence compared with yeast and human NAF1, it would be very interesting to pursue functional characterization of NAF1 to assess its importance in H/ACA snoRNPs and its impact on plant development.

Broader prospects for snoRNP biogenesis in plants

The work discussed in the present paper highlights a new layer of snoRNP regulation that had not been addressed until now in plants. The fact that conserved core snoRNP components interact with highly divergent assembly factors suggests that the plant factors do have functional and/or mechanistic specificities that are not reflected by their homology with animal factors. Moreover, in plants, *Arabidopsis* and rice offer the unique opportunity to address the impact of these factors on development, a domain which is virtually unexplored in animals. Studies on these events in the future should confirm the importance of snoRNP regulation for plant development, but also the adaptation of plants to abiotic stresses, as illustrated by the strong response of core snoRNP proteins to sugar stress [43].

Finally, these studies should also reveal new aspects of the biogenesis of other important RNPs in plants, as some of the regulatory proteins that control snoRNP biogenesis are also likely to be important for the biogenesis of other RNPs ([29], and J. Rodor, E. Jobet and M. Echeverria, unpublished work).

Funding

J.R. and I.L. were respectively supported by an 'Allocation de Recherche' and a 'Bourse de Cotutelle' from the French Government.

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Received 4 September 2009
doi:10.1042/BST0380672