A novel gene organization: intronic snoRNA gene clusters from *Oryza sativa*

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ABSTRACT

Based on the analysis of structural features and conserved elements, 27 novel snoRNA genes have been identified from rice. All of them belong to the C/D box-containing snoRNA family except for one that belongs to the H/ACA box type. The newly found genes fall into six clusters that comprise at least three snoRNA genes, and in one case as many as nine genes. Interestingly, four of the six clusters are located within the largest intron of a protein coding gene. The majority of intronic snoRNA gene clusters are simply formed by multiple copies of the same species of snoRNA gene that possess the identical functional elements. This implies a possible mechanism of duplication for the origin of repeating snoRNA coding regions in one intron. However, a few intronic snoRNA gene clusters consisting of different snoRNAs species were also observed. Polycistronic precursors from two independently transcribed clusters were demonstrated by RT-PCR and individual snoRNAs processed from the polycistronic precursors were positively determined bv reverse transcription assav. Analyses of the intergenic spacers in the clusters showed that, in addition to a very high AT content, the processing signals in rice snoRNA polycistronic transcripts might be different from those of yeast. Our results demonstrate that, in both plants and mammals, numerous snoRNAs can be produced simultaneously from an mRNA precursor of a host gene despite the different arrangements. The intronic snoRNA gene cluster is a novel gene organization, which is so far unique to plants. The conservation of intronic snoRNA gene clusters in plants was further demonstrated by the study of a similar snoRNA gene organization in the first intron of a Hsp70 gene from wild rice and Zizania caduciflora.

INTRODUCTION

Small nucleolar RNAs (snoRNAs) play an important role in ribosome biogenesis (1). Since the 1990s, a myriad of snoRNA genes have been identified and characterized in a wide range of eukaryotes, from yeast to mammals (2), and recently snoRNA homologs have also been found in Archaea (3-5). Besides RNase MRP, the vast majority of snoRNAs fall into two families, which can be distinguished on the basis of common sequence motifs and structural features (6,7). Many snoRNAs share the conserved 5'-end C box (consensus sequence UGAUGA) and 3'-end D (CUGA) boxes (1,8). The rest possess a common hairpin-hinge-hairpin-tail secondary structure in addition to the H box (AnAnnA) in the hinge region and an ACA motif 3 nt from the 3'-end of the molecule (6,9). As to their diverse functions, several snoRNAs, such as U3, MRP, U14, U8, U22, snR10 and snR30, have been shown to play key roles in rRNA processing, leading to production of mature 18S, 5.8S and 28S rRNAs in both vertebrates and yeast (10-12). The majority of C/D box snoRNAs act as guides for site-specific 2'-O-ribose methylation (7,13,14), while the H/ACA box snoRNAs are responsible for pseudouridylation of rRNA (15,16). Moreover, recent findings have indicated that some snoRNAs target snRNAs or cellular RNAs instead of rRNAs (17-20). In addition to their diverse potential functions, studying snoRNAs is of special interest because of their diverse genomic organizations and the corresponding expression modes, which vary among different organisms. In vertebrates, most snoRNAs are intron-encoded by protein coding genes (1,21). Although a few intronic snoRNAs also exist in yeast, the majority of them are independently transcribed under their own promoters; clustered snoRNA genes driven by a single promoter were reported in a couple of cases (1,22,23). In both vertebrates and yeast, an intron of a host gene encodes, at most, a single copy of a snoRNA. Examples of such an organization, in particular, are vertebrate U22 host genes (UHG), which contain a different snoRNA gene (U22 and U25-U31) in each intron, but whose spliced mRNA lacks an open reading frame (24). The presence of only a single snoRNA coding region per intron is important for both vertebrate and yeast because processing of intronic snoRNAs in these organisms is largely splicing dependent (11,25) and involves exonucleolytic

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trimming of linearized snoRNA-containing intron lariats (25,26). In contrast to yeast and vertebrates, a distinct feature of snoRNA organization in plants is the prevalence of snoRNA gene clusters (27-29) that require endonucleolytic cleavage in a splicing-independent processing pathway (30). The early study of five clusters in maize (27,28) first revealed this gene organization and its expression mode, which is further demonstrated by recent research on Arabidopsis thaliana (29,31,32). Over 50% of the snoRNAs identified from A.thaliana are organized in clusters and transcribed independently from their own promoters. In contrast, as we have previously reported, a cluster containing six snoRNAs is nested in the first intron of the rice Hsp70 gene (33; L.-H.Qu, L.Zhong and H.Zhou, unpublished results). Clustered snoRNA genes in a single intron have not been reported from yeast and mammals to date. One can therefore ask whether this is a general phenomenon in plants or merely an anomaly encountered by chance. Here, we have taken advantage of our earlier observation that an intronic snoRNA gene cluster was found in rice. With the Rice Genome Project, a large number of rice DNA sequences have now been documented. The relatively large, complex genome of rice is expected to reveal more information than that of A.thaliana. In this study, we report six novel snoRNA gene clusters from rice. Notably, four of them were found located within introns of protein-coding genes. The structural features and conservation of this gene organization in plants was analyzed and is discussed here.

MATERIALS AND METHODS

Search of the database

The *Oryza sativa* DNA database in GenBank and EMBL was searched for potential C/D box snoRNAs on the basis of structural features and functional elements in addition to comparative analyses with the known snoRNAs in *A.thaliana* and *Zea mays*. The flanking sequences of the candidate genes were carefully examined for other possible snoRNA genes. All the newly found rice snoRNA gene candidates were further studied using the PC gene 6.0 package.

RNA and DNA preparation

RNA was isolated from rice germs, which were collected and homogenized on ice in the presence of NIB (20 mM Tris–HCl pH 7.4, 10 mM MgCl₂, 40% glycerol, 20 mM β -mercaptoethanol, 0.5% Triton X-100, 0.1% bovine serum albumin, 5 vol per 1 g tissue weight). The homogenate was centrifuged at 8000 r.p.m. (Sovall, rotor SL-50T) for 10 min at 4°C. The cell debris was ground into a fine powder in liquid nitrogen. RNA was then extracted and purified as described (34). DNA was extracted from leaf tissue of *Zizania caduciflora* by the improved potassium acetate method (33). After treatment with RNase A, the DNA was further purified with glass milk or phenol/chloroform.

Detection of polycistronic transcript

Total cellular RNA was treated with DNase I before reverse transcription with primers. About 50 μ g RNA in 100 μ l of DNase I buffer was incubated (30 min, 37°C) with 10 U RQ1 RNase-free DNase I (Promega) and submitted to phenol/

chloroform extraction. The RNA was used for reverse transcription with specific primers. PCR was then carried out with the reverse primer and the corresponding forward primer, using the following program: 30 cycles of denaturation ($30 \text{ s}, 94^{\circ}$ C), annealing ($30 \text{ s}, 55^{\circ}$ C) and extension (1 min, 72° C), followed by a final extension (10 min, 72° C).

Reverse transcription analyses and mapping of ribose methylation

Reverse transcription was carried out in a 20 μ l reaction mixture containing 10 μ g RNA, 10 ng 5'-labeled primer and 200 μ M dNTPs. After denaturation of the resulting RNA product at 65°C for 5 min, the mixture was cooled to 42°C for 10 min. Then 200 U MMLV reverse transcriptase (Promega) were added and the mixture was further incubated at 42°C for 60 min. The reaction products were examined by electrophoresis on 10% acrylamide–8 M urea gels.

Ribose-methylated nucleotides of rice rRNA were determined by primer extension at low dNTP concentrations as described previously (29). In brief, two reactions of reverse transcription were performed in parallel using 5 μ g total RNA and a dNTP concentration of either 4 or 500 μ M. The rice 25S rDNA were amplified by PCR with the primer pair 25SF/ 25SR, then cloned into the *SmaI* site of plasmid pTZ18. An rDNA sequence ladder was prepared with the same primer used for rRNA methylation mapping and run in parallel with the reverse transcription reaction as a molecular weight marker.

PCR, cloning and sequencing

DNA extracted from Z.caduciflora was amplified with primers Hsp2 and Hsn, which are designed according to the sequence of the rice Hsp70 gene. Hsp2 is located in the boundary region covering 10 nt of exon 1 and 10 nt of intron 1 of the Hsp70 gene while Hsn is complementary to a 20 nt tract of exon 2 near the 3'-end of the intron. The 1972 bp PCR product was cloned into the SmaI site of the pTZ19 plasmid after electrophoretic purification and the recombinant plasmid was named pTZJB. Two fragments were obtained after treating pTZJB with BamHI. The large one, ~3681 bp in length, is self-ligated and formed pTZJB1. The small fragment, ~1167 bp in length, was subcloned into the BamHI site of pTZ19. The resulting positive clones identified by enzyme digestion were named pTZJB2. pTZJB1 was then sequenced using the universal sequencing primer and pTZJB2 with both universal sequencing primer and reverse sequencing primer in a 377 sequencer.

Oligonucleotides

Oligonucleotides were synthesized and purified by Sangon Co. (Shanghai, China). The following oligonucleotides were used in reverse transcription of RNA: Pz102, 5'-ATAGAG-CTAATACAATTTGAGGCCA-3'; Pz104, 5'-CAAATG-CCTCGATTGTCCCCATG-3'; Pz105, 5'-GCATTCAGA-ATGAGTAGGAGGAG-3'; Pz106, 5'-GAAGTATGAGTG-CTTCATTGTAG-3'; Pz107, 5'-TCAGCGGAAAAATCGG-CATACAA-3'; Pz109, 5'-GGATTCAGATGCAAAGA-TGTGTA-3'. All the above oligonucleotides are complementary to the 3'-end of the corresponding snoRNA genes. The RT–PCR experiments were performed with the following primers: C2101F, 5'-GCAGATGAGGAGGCACAAG-ATT-3'; C2102R, 5'-ATAGAGCTAATACAATTTGAGG-

CCA-3'; C2102F, 5'-ATTAGCTCTATCTGATCATCTTCC-5'-CATCATGGCGGCCAATCAAACC-3': 3': C2103R. C6107F, 5'-TGGTAATATTCAAGCTCAACAGAC-3'; C6110R, 5'-CAGAAAGAAAAGCCTTCTCATTC-3'; C6110F, 5'-AGGATGAAACCTTTTATAACAATCT-3'; C6113R, 5'-CATCAGGCCCAAACTATCACA-3'. The following oligonucleotides were used for amplification of rice 25S rDNA: 25SF, 5'-TATAGGGGCGAAAGACTAATCG-3'; 25SR, 5'-ATCTCAGTGGATCGTGGCAGCAA-3'. Rice rRNA ribosemethylated nucleotides were assayed by reverse transcription with the following primers: Ri1386R, 5'-GGCTCG-CGCCCCGGGTTTTG-3'; Ri2438R, 5'-GGGCTCCCACT-TATCCTACA-3'; Ri2948R, 5'-AACTAACCTGTCTCA-CGACGGTC-3'. The following oligonucleotides were used for amplification of the first intron of the Hsp70 gene from Z.caduciflora: Hsp2, 5'-ACACCGTCTTCGGTAACTACT-3'; Hsn, 5'-AAGGGCCAGAGCTTAATGTC-3'. The primers used in reverse transcription, RT-PCR and rDNA sequencing were 5'-end labeled with $[\gamma^{-32}P]ATP$ (Yahui Co.) and submitted to purification according to standard laboratory protocols as described previously (23).

RESULTS

Identification of six novel snoRNA gene clusters, including four located in introns, from *Oryza sativa*

Careful examination of the GenBank and EMBL DNA database allowed us to identify 27 novel snoRNA genes from O.sativa. The snoRNA genes fall into six clusters and were termed rice snoRNA clusters I, II, III, IV, V and VI, respectively. The sequences of each cluster are shown in Figure 1. Interestingly, four of the six clusters, i.e. clusters I, III, IV and V, were found located within the introns of protein coding genes. These host genes encode RPS20, NADH dehydrogenase, RPL30 and an unknown protein, respectively. The host genes possess one to four introns but, in all cases, snoRNA gene clusters were found in only one intron, whose size is usually several-fold larger than the others. The RPL30 gene, for example, is the host gene of cluster IV, which contains four introns of 145, 1872, 369 and 90 bp. Evidently, the second intron was unusually large, and it turned out to contain snoRNA genes. All of the introns in the host genes possess the standard boundary signals, i.e. GU at the 5'-end and AG at the 3'-end of the intron, in spite of considerable variation in their sizes (Fig. 1).

It appears that most intronic snoRNA gene clusters are simply formed by duplication of the same species of snoRNA gene, which possess identical functional elements. Cluster I consists of four copies of snoRNA Z100. Likewise, clusters III and IV, though scattered over different chromosomes and nested in different protein coding host genes, are both made up of multiple copies of snoRNA Z103, four in cluster III and three in cluster IV. However, cluster V, which contains three different snoRNA species, is far more complex.

As for intronic snoRNAs in mammals, rice intronic clustered snoRNAs were frequently found in introns of genes involved in ribosome biogenesis, i.e. ribosomal proteins or nucleolar proteins. This organization is suggested to have evolved for coordinate expression of functionally related genes. Interestingly, snoRNA cluster V is encoded in an intron

of a hypothetical protein-coding gene with a small ORF that is composed of only 25 amino acids. Whether this mRNA accumulates in the cell is not clear, although the evidence from cDNA sequences in the EST database (GenBank accession nos BI797167 and AU065122) supports transcription and splicing of the mRNA precursor from the host gene.

Clusters II and VI lie in a spacer between two protein coding genes and appear to be independently transcribed. Cluster II consists of genes encoding three different species of snoRNA, including Z103, which is also found in two other intronic clusters (clusters III and IV), as stated above. Cluster VI is particularly large and contains as many as nine genes corresponding to eight different snoRNA species. The clusters may be transcribed from an upstream promoter and multiple snoRNAs are released by processing from polycistronic precursors. Transcription of the clusters as polycistronic precursors was determined by RT–PCR with specific primer pairs, as shown in Figure 2.

Predicted structure and function of the novel snoRNA genes in each cluster

Among the 27 snoRNA genes in the clusters, 26 encode C/D box-containing snoRNAs. They clearly exhibit the hallmark structures of C/D box antisense snoRNAs, which include the presence of two conserved motifs, the 5'-end C box (5'-UGAUGA-3') and the 3'-end D box (5'-CUGA-3'), immediately flanked by a 4–10 bp inverse repeat. These form the 5'-3'terminal stem structure in which the C and D boxes are in close proximity, an important feature for the stability and accumulation of snoRNAs as well as for snoRNA binding to proteins (26,35,36). Two other conserved motifs, the C' and D' boxes, can be found in the central region of most snoRNA genes. In particular, 18 snoRNA genes have one or two regions of complementarity to rRNAs, 10-14 nt long, which have been shown to function in guiding 2'-O-ribose methylation of rRNA (37). However, the remaining eight snoRNA genes of Z103, which are distributed over different chromosomes in both intronic and non-intronic clusters, do not show any complementarity to rRNAs, implying that the function of Z103 may differ from those of other snoRNAs. For example, it may interact with RNAs other than rRNAs. Based on the known relationships between antisense snoRNA structure and function (7,13,14,38,39), proposed 2'-O-ribose methylation sites in rice rRNAs, complementary to the new guide RNAs, are shown in Figure 3.

Based on the complementarity to rRNA and the corresponding target sites of methylation, a comparison with known snoRNA genes from various organisms revealed that 15 of the 26 C/D box snoRNA genes showed structural similarity to yeast or human snoRNAs (Table 1). This may imply that their functions have been conserved during the course of evolution. However, some distinct structural features of plant snoRNAs were also observed. For example, Z112 and its maize counterpart show high homology to each other, but both seem hardly related to their human counterpart other than the complementarity to rRNA. Rice Z112, with extra plantspecific regions, is more than twice the length of its vertebrate counterpart. The four novel snoRNAs, Z101, Z102, Z104a and Z104b, with dual antisense elements, display a mosaic structure as compared with their counterparts in yeast and mammals. The two methylation sites predicted by each of

Cluster I		Cluster II	
ggagccaagg acaagagctt gaaggtgaag ggccccgtgc ggatgcccac caaggtgctc	60	ttgatttgat ttgttctcgc tttcaaatct gtttgctttt ttttgggctg ttttgtcgat	60
cacatcacca ccaggaagtc tccttgtgga gaaggtgaca (70nt) ttgtcatgtt	180	gggattttgt ttgggcttga ggatgatgga t	
gatgatgatc		GATGCAGAT GAGGAGGCAC AAGATTTAAT	Z101
TECTEACEAG ATEATETTAA ATACTAGTTE ATCACTEAGA TCTTEATCTT	Z100a	GGGTAATTTG CGTCTGAGGC ATCTCTGTGA TGTCACACCC TTTTTCACCT TGGAGATCTG	180
TGATGTCTAA AAAAATCGAG CTTTTTAAAA CTGATGCTTA GCA		ATCCATC	
tatcatc atcatcttca	300	tac aaatettetg eettetttt titteetega itaaetiita iittettita	240
attgtgttgt ttgttaggag ttagcattgc cttgattaaa gcattttaga tgagagctga	360	aaaagttttc ctctattagc ccttgttctt ttagtctttc gaagaaaaga aaaaacgctg	300
gttgttagtt gt		gggatt	
AGGCCGAG CATGTGATGT TACAAGGAGA TGCTACTGAG ATGTTATATC	Z100b	GATG ATAITGATGAT CAAATTTGAT TCCCTATTGG TTTGATTGGC CGCCATGATG	Z102
GTTGAAGTCT CTAAAACCGA GCTTTTTAAA ACTGATGCTT GGCCT		есстсалатт статтаесте татетелтса те	
tgtaa tagcaagtgt	480	ttcctcca cattttctt gtttcatttt	420
agigiggaig gittlaiggi tailgcaigg (130nt) titaaigigg tiaitgitci	660	itecelectig galetitetg etgetitette (190ht) aatttattig gegetgetta	660
at	7100		71026
ACAAAATTOOL COUTTETTAAA ACTON TO COOCTATO	21000	TATTTCTCCCT TTCACAACCA ACCCACTATT CTCATTCTCC A	21030
ACAAAAICGA GEIIIIIAAA ACIGAIGGII GOCCIAIC			
	780		780
cadataciga continggg itaatgacit agtigotici tagtacitot tigtaccatg	840	citotatiti gagoaagoag iggitgitio ciagagtatg alggatgiat igatatgito	840
tattictara cat	900		
	71004		
CTAAAAATCG AGCTTTTTAA AACTGAITECT CAGC	21000		
cttgaa gtaataatgt gaaccccttg	1020		
(190nt) cag <i>etaccaa cacctegeat ceptttgaga tecepteca caaaageete</i>	1260		
	1200		
attgatettg teagetetge agatgttgte aageagatea eeteaateae eattgageea	1320		
attgatettg teagetetge agatgttgte aageagatea eeteaateae eattgageea	1320		
attgatettg teagetetge agatgttgte aageagatea eeteaateae eattgageea	1320		
attgatettg teagetetge agatgttgte aageagatea eeteaateae eattgageea Cluster III	1320		
attgatettg teagetetge agatgttgte aageagatea eeteaateae eattgageea Cluster III teageeggeeggeggeggeggeggeggeggeggggggggg	60		
attgatottg toagototgo agatgttgto aagoagatoa cotoaatoao cattgagooa Cluster III toagooggoo ggoggoggog cagooggotg gggggaggog goggoggoa ggaggooggo ggoggtgggo aggoggogg coggoogtg gttogttgc (3450nt) ogttiggttt	60 3560		
attgatettg teagetetge agatgttgte aageagatea eeteaateae eattgageea Cluster III teageeggee ggeggeggeg eageeggegg gggggggg	60 3560		
attgatettg teagetetge agatgttgte aageagatea eeteaateae eattgageea Cluster III teageoggee ggeggeggeg eageeggetg ggggggggge geggeggge ggggggge gggggggg	60 3560 Z103a		
Attgatettg teagetetge agatgttgte aageagatea eeteaateae eattgageea Cluster III teageoggee ggeggeggeg eageeggetg gggggaggegge ggggggge ggggggggg ggeggtggge aggegggegg eeggeggetg gttegtttge (3450nt) egtttggttt teagattggt tttaegeggt tgeaaatetg atgeagt TTT GAAGTGATGA TECTTTECCE AAATTATEGA CTATGATTGT ACTGTEGEGAT TTTTECAAGE TETTTECTTE GEAGAAGEET	60 3560 Z103a 3680	Cluster IV	
Attgatettg teagetetge agatgttgte aageagatea eeteaateae eattgageea Cluster III teageoggee ggeggoggeg eageeggegg gggggggggg	60 3560 Z103a 3680	Cluster IV caacaagotg cagotogtga tgaagagogg caagtacaog otoggotaca agacogtoot	60
attgatettg teagetetge agatgttgte aageagatea eeteeateae eattgageea Cluster III teageoggee ggeggegge eageeggegge gggggggggg	60 3560 Z103a 3680 3740	Cluster IV caacaagctg cagotogtga tgaagagcgg caagtacaog otoggotaca agacogtoot caggaccoto aggaactoca aggglaagat (370nt) ttattgootg tatgttgtat	60 480
Cluster III toagcoggoo ggoggoggog cagcogogtg ggggggggg goggogggga ggaggcoggo ggoggtggg aggogggog cagcogogtg gtgggggggg goggoggga ggaggcoggo ggoggtggg aggogggog cagcogodtg gttegttige (3450nt) egttiggtt teagaitggt titaegeggt tgeaaatetg atgeagt TTT GAACTEATEA TECTTCCCC AAATTATCGA CTATGATTET ACTETECGAT TITTEGAAGC TECTTCTCCC GAGAAGGCT TCTAAGCCAG TAGTTCTGAT CAAA cegeta cattigatti geatetgatt tggttiegtt teletgggag tteatecaet agategtt etgagtatt catecgegg gitetgatti	60 3560 Z103a 3680 3740 3800	Cluster IV caacaagctg cagctcgtga tgaagagcgg caagtacacg ctcggctaca agaccgtcct caggaccctc aggaactcca agggtaagat (370nt) ttattgcctg tatgttgtat igctgigtgc tgttgtctac attttagctt tggatatgtc catacg	60 480
Cluster III toggcoggco ggcggcggog cagcogcgtg ggggggggg gcggcgggca ggaggccggc ggcggtgggc aggcgggcg cagcogcgtg gttogtitgc (3450nt) cgttiggttt tcagattggt titacgcggt tgcaaatctg atgcagt TTT GAACTGATGA TCCTTTCCCC AAATTATCGA CTATGATTGT ACTGTGCGAT TTTTGCAAGC TCTTCTCTC GGAGAAGGCT TCTAAGCCAG TAGTTCTGAT CAAA ccgcta cattigattt gcatctgatt tggtticgtt tctctgggag ttcatccact agatctgtt ctgagttatt catccgcggt gitctgattt tcgctgttg tittggtiga gattatat aatctt	60 3560 Z103a 3680 3740 3800	Cluster IV caacaagotg cagotogtga tgaagagogg caagtacaog otoggotaca agacogtoot caggacooto aggaactoca agggtaagat (370nt) ttattgootg tatgttgtat tgotgtgtgo tgttgtotac attttagott tggatatgto cataog GAGG ATATGATTA	60 480 Z103e
Cluster III toggocggoc ggoggoggog oggocggotg gggggggggg	60 3560 Z103a 3680 3740 3800 Z103b	Cluster IV caacaagctg cagctogtga tgaagagcgg caagtacaog otoggotaca agacogtoot caggaaccotc aggaactoca agggtaagat (370nt) ttattgootg tatgtigtat tgotgigtgc tgitgtotac attitagott tggatatgtc catacg CAGG ATA[TGATGA]T CCTTTCCCCA GATGATCGAC AATGAACATA CCGTGCGATT GTTTTCTCTT AGGAGATAAT	60 480 Z103e 600
Cluster III tcagccggcc ggcggcggcg cagccgcgtg gggggaggcg gcggcgggca ggaggccggc ggcggtgggc aggcgggcg cagccgcgtg gtcgttgc (3450nt) cgtttggttt tcagattggt tttacgcggt gcaaatctg atgcagt TTT GAACTGATCA TCCTTCCCC AAATTATCGA CTATGATTGT ACTGTGCGAT TTTTGCAAGC TCTTCTCTC GGAGAAGGCT TCTAAGCCAG TAGTTCTAACTGTGCGAT TTTTGCAAGC TCTTCTCTC GGAGAAGGCT TCTAAGCCAG TAGTTCTAACTGTGCGAT CATH ccgcta catttgattt gcatctgatt tggtttcgtt tctctgggag ttcatccact agatctgttt ctgagttatt catccgcggt gttctgattt tcgctgttg ttttggttga gatattatt aatctt GATG GAACTGAGCA TCCTTCCCC AGCTGATCGA CTATGATTAT ACTGTGCGCA AATGTAATCC CTCTTCAGAG GATTCAAGCC	1320 60 3560 Z103a 3680 3740 3800 Z103b 3920	Cluster IV caacaagctg cagctogtga tgaagagcgg caagtacacg otoggotaca agacogtoot caggacoctc aggaactoca agggtaagat (370nt) ttattgootg tatgtigtat igotgigtge tgitgtotac attitagott iggataigte catacg CAGG ATA CCTTTCCCCA GATGATCGAC AATGAACATA CCGTGCGATT GTTTTCTCTT AGGAGATAAT CAAGCCAGAA ATCTGATCCT C	60 480 Z103e 600
Cluster III tcagccggcc ggcggcggcg cagccgcgtg gggggaggcg gcggcgggca ggaggccggc ggcggtggg aggcgggg cagccgcgtg gtcgttgc (3450nt) cgtttggttt tcagattggt ttacgcggt ggaaatctg atgcagt TTT GAAGTGATGA TCCTTTCCCC AAATTATCGA CTATGATTGT ACTGTGCGAT TTTTGCAAGC TCTTCTCTT GGAGAAGGCT TCTAAGCCAG TAGTT <u>CTCA</u> CCGCta catttgattt ggattcgtt tggttcgtt tctctgggag ttcatccact agatcgttt ctgagttatt catccgcggt gttctgattt tcgctgttg ttttggttga gatattatt aatctt GATC GAAGTGGAG TCCTTCCCCC AGATTCCAA CTATGATTAT ACTGTGCGCA AATGTAATCC CTCTTCAGAG GATTCAAGCC AGAAATCCGA CTATGATTAT ACTGTGCGCA AATGTAATCC CTCTTCAGAG GATTCAAGCC AGAAATCCGA TCATGATTAT CCGTGCGCA AATGTAATCC CTCTTCAGAG GATTCAAGCC AGAAATCCGA	60 3560 Z103a 3680 3740 3800 Z103b 3920	Cluster IV caacaagctg cagctogtga tgaagagcgg caagtacacg otoggotaca agacogtoot caggaccotc aggaactoca aggglaagat (370nt) ttattgootg tatgtigtat tgotgigtgc tgitgtotac attitagoti tggatatgic catacg CAGG ATA CCTTTCCCCA GATGATCGAC AATGAACATA CCGTGCGATT GTTTTCTCTT AGGAGATAAT CAAGCCAGAA ATCTGATCCT C gaattitaa tgiagagtit tittitciga aagatticot	60 480 Z103e 600 660
Cluster III tcagcoggoc ggoggoggog cagcoggogg gggggggggg	60 3560 Z103a 3680 3740 3800 Z103b 3920 3980	Cluster IV caacaagctg cagctogtga tgaagagcgg caagtacacg otoggotaca agacogtoct caggacoctc aggaactoca aggglaagat (370nt) ttattgoctg tatgtigtat igcigigige tgitgtotac attitagott iggataigte catacg CAGG ATA[TGATCA]T CCTTTCCCCA GATGATCGAC AATGAACATA CCGTGCGATT GTTTTCTCTT AGGAGATAAT CAAGCCAGAA ATCTGATCCCT C gaattitaa igiagagitt ittitticiga aagatticct itatcitcaa aigagitggi (370nt) ittattiagt itiggitgaa iiggiag	60 480 Z103e 600 660
Cluster III tcagcoggcc ggcggcggcg cagccgcgtg gggggaggcg gcggcgggca ggaggccggc ggcggtggg aggcgggg cagccgcgtg gtcgttgc (3450nt) cgtttggttt tcagattggt tttacgcggt gcagacgctg gttcgtttgc (3450nt) cgtttggttt tcagattggt tttacgcggt gcagacgctg gttcgtatt tcagattggt tttacgcggt gcagacgctg gttcgtatt tcttgggag ttcatccact agatctgttt cigagitatt catccgcggt gttctgatt1 tcgctgttg tttggttga gatattatt aatctt GATC GAAGTGGAAG CTCTTCCCC AGCTGATCGA CTATGATTAT ACTGTGCGCA AATGTAATCC CTCTTCAGAG GATTCAAGCC AGAAATCTGA TCCATC tgct attctcaaca caatttcagc tttgtattt tgtttgcag tgttttttg cgcgaccctt tttigttigt ttgtttgttt ga	60 3560 Z103a 3680 3740 3800 Z103b 3920 3980	Cluster IV caacaagotg cagotogtga tgaagagogg caagtacaog otoggotaca agacogtoot caggacocto aggaactoca aggglaagat (370nt) ttattgootg tatgtigtat igotgigtgc igtigtotac attitagoti iggataigto cataog CAGG ATA CCTTTCCCCA GATGATCGAC AATGAACATA CCGTGCGATT GTTTTCTCTT AGGAGATAAT CAAGCCAGAA ATCTGATCCT C gaatttaa igiagagtti ittitticiga aagatticot ittacticaa aigagtiggt (370nt) ttiattiagt ittggitgaa tiggiag TGC	60 480 Z103e 660 Z103f
Cluster III tcagcoggcc ggoggogg cagcoggogg gggggggggg	60 3560 Z103a 3680 3740 3800 Z103b 3920 3980 Z103c	Cluster IV caacaagotg cagotogtga tgaagagogg caagtacaog otoggotaca agacogtoot caggacocto aggaactoca agguaagat (370nt) ttattgootg tatgttgtat tgotgtgtgc tgttgtotac attitagott tggatatgtc catacg GAGG ATA[TGATCA]T CCTTTCCCCA GATGATCGAC AATGAACATA CCGTGCGATT GTTTTCTCTT AGGAGATAAT CAAGCAGAA ATCTCA]TCCT C gaattitaa tgtagagttt ttittitotga aagatticot ttatottcaa atgagttggt (370nt) ttatttagt titggtgaa ttggtag TGG AGGATA[TGAC GA]TCCTTCC CAGATGATC GACGATGAGC ATAATGTGCG CTATTGTTCT	60 480 Z103e 660 Z103f 1140
Cluster III tcagcoggcc ggoggogg cagcoggogg gggggggggg	1320 60 3560 Z103a 3680 3740 3800 Z103b 3920 3980 Z103c 4100	Cluster IV caacaagotg cagctogtga tgaagagocgg caagtacaog ctoggotaca agaccgtoot caggaocoto aggaactoca agguaagat (370nt) ttattgootg tatgttgtat tgotgtgtgc tgttgtotac attttagott tggatatgtc catacg CAGG ATA[TGATCAA]T CCTTTCCCCA GATGATCGAC AATGAACATA CCGTGCGATT GTTTTCTCTT AGGAGATAAT CAAGCCAGAA ATCTGATCCAC AATGAACATA CCGTGCGATT GTTTTCTCTT AGGAGATAAT CAAGCCAGAA ATCTGATCCT C gaattttaa tgtagagttt ttttttctga aagatttcot ttatcttcaa atgagttggt (370nt) tttatttagt tttggttgaa ttggtag TGG AGGATA[TGAC GA]TCCTTCC CCAGATGATC GACGATGAGC ATAATGTGCG CTATTGTTCT CTTAAGAGAT AATCAAGCCA GAACT[ATGA]T TCTCCA	60 480 Z103e 660 Z103f 1140
Cluster III toggoggc ggoggogg cagocgogt gggggggg goggggggg ggggggggggggg	1320 60 3560 Z103a 3680 3740 3800 Z103b 3920 3980 Z103c 4100	Cluster IV caacaagotg cagotogtga tgaagagogg caagtacaog otoggotaca agacogtoot caggaocoto aggaactoca aggtaagat (370nt) ttattgootg tatgttgtat tgotgtgtgc tgttgtotac atttagott tggatatgtc cataog GAGG ATA TGATGATCAACGAA ATGTGATCGAC AATGAACATA CCGTGCGATT GTTTTCTCTT AGGAGATAAT CAAGCCAGAA ATGTGATCGAC AATGAACATA CCGTGCGATT GTTTTCTCTT AGGAGATAAT CAAGCCAGAA ATGTGATCGAC AATGAACATA CCGTGCGGATT GTTTTCTCTT AGGAGATAAT CAAGCCAGAA ATGTGATCGAC (370nt) tttatttagt tttggttgaa ttggtag TGG AGGATAATGAGC GATCATTCC CAGAGATC GACGATGAGC ATAATGTGCG CTATTGTTCT CTTAAGAGAT AATCAAGCCA GAACTAATGAT TCTCCA aatt taatgcagtc tctttigctg	60 480 Z1 0 3e 660 Z1 0 3f 1140 1200
attgatettg teagetetge agatgttgte aageagatea ecteaateae cattgageea Cluster III teageoggee geoggegeg eageeggege geoggegege geoggegegegegegegege	1320 60 3560 Z103a 3680 3740 3800 Z103b 3980 Z103c 4160	Cluster IV caacaagotg cagotogtga tgaagagogg caagtacaog otoggotaca agacogtoct caggacoto aggaactoca aggitaagat (370nt) ttattgootg tatgitgtat tgotgtgtgc tgttgtotac atttagott tggatatgtc cataog CAGG ATA CCTTTCCCCA GATGATCGAC AATGAACATA CCGTGCGATT GTTTTCTCTT AGGAGATAAT CAAGCCAGAA ATCTGATCCT C gaatttaa tgtagagttt ttttttotga aagatttoot ttatottcaa atgggtggt (370nt) tttatttagt tttggtgaa ttggtag TGG AGGATA TGATCATCC CCAGATGATCGAC GAACTAATCGAGCA ATAATGTGCG CTATTGTTCT CTTAAGAGAT AATCAAGCCA GAACTATCATC GACGATGAGC ATAATGTGCG CTATTGTTCT CTTAAGAGAT AATCAAGCCA GAACTATCATCTCC aatt taatgcagtc tctttgctg aaagatttgt tttatctgaa aatgtactga tttgtggtta atttcattgt gctgcctatt	60 480 Z1 0 3e 660 Z1 0 3f 1140 1200 1260
Cluster III tcagcoggcc ggcggcggc cagccgcgt ggggggggg gcggggggg gggggggggg	1320 60 3560 Z103a 3680 3740 3800 Z103b 3920 3980 Z103c 4100	Cluster IV caacaagotg cagotogtga tgaagagogg caagtacaog otoggotaca agacogtoot caggacoto aggaactoca aggutaagat (370nt) ttattgootg tatgitgtat tgotgtgtgc tgttgtotac atttagott tggatatgtc cataog GAGG ATATGATCGAC AATGAACATA CCGTGCGATT GTTTTCTCTT AGGAGATAAT CAAGCCAGAA ATCTGATCCT C gaatttaa tgtagagttt ttttittotga aagatticot ttatottcaa atgagttggt (370nt) tttatttagt titggitgaa tiggtag TGG AGGATATGAC CATCATCC CCAGATGATC GACGATGAGC ATAATGTGCG CTATTGTTCT CTTAAGAGAT AATCAAGCCA GAACTATGAT CACGATGAGC ATAATGTGCG CTATTGTTCT CTTAAGAGAT AATCAAGCCA GAACTATGAT TCTCCA aatt taatgcagt totttigctg aagattigt tttatctgaa aatgtactga titgitggta atticatigt gotgcotatt titcctgatt ctttagcat gitgiggtga aattitatt ggtgctgct aata	60 480 Z1 0 3e 660 660 Z1 0 3f 1140 1200 1260
Cluster III toggocggoc ggoggogg cagoogogtg gggggggg goggoggggg gggggggg ggoggtggg aggogggog cagoogogtg ggggggggg goggogggg gggggggg ggoggtggg aggogggog cagoogogtg gtggggggg goggogggg gggggggg ggoggtggg aggoggggg cagoogogtg gttogttt togattggt titacgoggt tgoaaatotg atgoagt TTT GAACTGATGA TCCTTTCCCC AAATTATCGA CTATGATTGT ACTGTGCGAT TTTTGCAAGC TCTTCTCTC GGAGAAGGCT TCTAAGCCAG TAGTT <u>CTGAT</u> CAAA ccgcta catitgattt gcatctgatt tggtttogtt tototgggag ttoatocact agatctgttt ctgagttatt catcogogg gittegattt tcgctgttg tittggtigg gatattatt aatott GATC GAAGTGGACGA TCCTTTCCCC AGCTGATCGA CTATGATTAT ACTGTGCGCA AATGTAATCC CTCTTCAGAG GATTCAAGCC AGAAATCTGA tgct attotcaaca caatttcagc titgtattit tgtttgcag tgtttittg cgcggacctt tittgtttgt tgttgttt tg ATTGAACT GAAGATCCTT TCCCAGATG ATCGATCAG ATTATACTGT GCGCAAATGT AATCCTTTT CCGAGGATC AAGCAGAAA TCTGATCAT acagaatgca atatttaact ttacttttg tggttttgta attittttt ctgtacagtt titgctcgtc caattgttg tttg ATTGGA ACTGCAGTC	1320 60 3560 Z103a 3680 3740 3800 Z103b 3980 Z103b 4100 4160 Z103d	Cluster IV caacaagotg cagctogtga tgaagagogg caagtacaog otoggotaca agacogtoot caggacotc aggaactoca agggtaagat (370nt) ttattgootg tatgttgtat tgotgtgtgc tgttgtotac atttagott tggatatgtc cataog CAGG ATATGATCGAC CCTTTCCCCA GATGATCGAC AATGAACATA CCGTGCGATT GTTTTCTCTT AGGAGATAAT CAAGCCAGAA ATCTGATCCT C gaatttaa tgtagagttt ttttitictga aagatticot ttatottcaa atgagttggt (370nt) tttatttagt titggtgaa tiggtag TGG AGGATA ATGATCATCCC CCAGATGATC GACGATGAGC ATAATGTGCG CTATTGTTCT CTTAAGAGAT AATCAAGCCA GAACTATGAT TCTCCA aatt taatgcagtc totttigotg aaagattgt tttatctgaa aatgtactga titgtggta atticattgt gotgcotatt titoctgatt citttagota gitgtggtga aattitatt ggtgotgct aata TGCACA	60 480 Z103e 660 Z103f 1140 1200 1260 Z103g
Cluster III toggocggoc ggoggogg cagoogocgt ggggggggg goggoggggg gggggggggg	1320 60 3560 Z103a 3680 3740 3800 Z103b 3980 Z103b 4100 4160 Z103d 4280	Cluster IV caacaagotg cagotogtga tgaagagogg caagtacaog otoggotaca agacogtoot caggacootc aggaactoca agggtaagat (370nt) ttattgootg tatgitgtat tgotgtgtgc tgttgtotac attitagott tggatatgtc cataog CAGG ATATGATCGAC CCTTTCCCCA GATGATCGAC AATGAACATA CCGTGCGATT GTTTTCTCTT AGGAGATAAT CAAGCCAGAA ATCTGATCCT C gaattitaa tgtagagtit tittitictga aagatticot ttatcitcaa atgagitggt (370nt) titattagt titggitgaa tiggtag TGC AGGATATGACCATACCACAGACATA CACGATGAGC ATAATGTGCG CTATTGTTCT CTTAAGAGAT AATCAAGCCA GAACTATGAT CTCCA aatt taatgcagit cittigctg aagattigt titatcigaa aatgtactga titgiggita atticatigt gotgcotatt titcotgatt cittiagcat gitggtgga aattitatt ggtgcgctt aata TGCAGA ATATGATGAT CCTTTCCCCA GATGATCGAC AATGAACATA CTGAGCGCTA TTATTTCTCT	60 480 2103e 660 660 2103f 1140 1200 1260 2103g 1380
Cluster III toggocggoc ggoggoggog oggocgotg ggggggggg goggoggga gggggoggc ggoggtggg aggoggggg oggocgotg gtlogttige (3450nt) ogtitiggtit toggatggt titaogoggt tgoaaatotg atgoagt TTT GAACTGATCA TCCTTTCCCC AAATTATCGA CTATGATTGT ACTGTGCGAT TITTGCAAGC TCTTCTCTC GGAGAAGGCT TCTAAGCCAG TAGTT <u>CTGAT</u> CAAA cogota cattigatti gcaictgatt iggiticgit tototgggag ticatocaot agatotgit cigagitatt catcogoggt gitotgatti togotgit titiggitag gatattata aatott GATE GAACTGAGGA TCCTTTCCCC AGCTGATCGA CTATGATTAT ACTGTGCGCAA AATGTAATCC CTCTTCAGAG GATCAAGCC AGATATCGA TCCATC iggt attotcaaca caatticage titigattit tgittigcag tgittitig cgcggaccett titigtitgt tigtitgit ga ATGGAAGT GAACATCTT TCCCCAGATG ATCGACTACG ATTATACTGT GCCCAAATGT AATCCCTTT CGGAGGATC AAGCCAGAAA TCTGATCAT acagaatgca atattaact ttacittitg tggttitgta attitittit ctgtacagit titigctogic caattgitg titig ATGGAAGT GAACGACACGATC AGCCAGAAA TCTGATCAT	1320 60 3560 Z103a 3680 2103a 3980 Z103b 3980 Z103c 4100 4160 Z103d 4280	Cluster IV caacaagotg cegotogtga tgaagagocgg caagtacaog otoggotaca agaocgtoot caggacootc aggaactoca agggtaagat (370nt) ttattgootg tatgitgtat tgotgtgtgc tgttgtotac atttagott tggatatgtc catacg CAGG ATATCGATCGAC AATGAACATA CCCTGCGATT GTTTTCTCTT AGGAGATAAT CAAGCCAGAA ATCTGATCCT C gaatttaa tgtagagttt ttttittetga aagatticot ttatetteaa atgagttggt (370nt) ttattagt titggitgaa tiggtag TGC AGGATATCGAC GATCCTTCC CCAGATGATC GACGATGAGC ATAATGTGCG CTATTGTTT CTTAAGAGAT AATCAAGCCA GAACTATCGAT CACGATGAGC ATAATGTGCG CTATTGTTCT CTTAAGAGAT AATCAAGCCA GAACTATGAT TCTCCA aatt taatgcagt c tottitgetg aagattigt titatetgaa aatgtactga titgtggta atticattg getgcctatt titectgatt citttagcat gitgggtga aatttatt ggtgctgct aata TGCACA ATATGATCAT AGGAACC AAGCCAGTAT TCTCA	60 480 Z103e 660 Z103f 1140 1200 1260 Z103g 1380
Cluster III tcagcoggco ggcggcggog cagcogogtg ggggggggcg gcggcgggca ggaggccggc ggoggtggc aggcggcgg cagcogogtg ggggggggg gcgggggga ggaggcggc ggoggtggg aggcgggg cagcogogtg gtlogtitgc (3450nt) cgtitggtit tcagaitggt titacgcggt tgcaaatctg aigcagt TTT CAACTGATCA TCCTTCCCC AAATTATCGA CTATGATTGT ACTGTGCGAT TTTTGCAAGC TCTTCTCTC GGAGAAGGCT TCTAAGCCAG TAGTTCTGAT ACTGTGCGCAT TTTTGCAAGC TCTTCTCTC GGAGAAGGCT TCTAAGCCAG TAGTTCTGAT CAAA ccgcta catitgatit gcaictgati tggticgti tctctgggag ticatccact agatctgtit ctgagtiatt catccgcggt gitctgatti tcgcigtig tittggtiga gatattat aatctt GATE CAACTGCAG TCCTTTCCCC AGCTGATCGA CTATGATTAT ACTGTGCGCA AATGTAATCC CTCTTCAGAG GATCAAGCC AGAAATCTGA TCCATC igct attctcaaca caattcagc titgtattit tgtttigcag tgtttittig cgcgaccctt titigtitgt tigttigtit ga ATGGAACT GAACATCCTT TCCCCAGATG ATCGACTACG ATTATACTGT GCGCAAATGT AATCCCTTT CGGAGGATC AAGCCAGAAA TCTGATCAT acagaatgca atatttaact ttactttitg tggttitgta atttittitt ctgtacagti ttigctcgtc caattgtitg titg ATGGA ACTGCACGATC CTTTCCCCAG ATGATCGACT ATGATTATACTGT GCGCACAATGT AACCCCTTT CGGAGGATC AAGCCAGAAA TCTGATCAT III ttatatagti tiggtcgt citigtgtt tiggt cattof caccac attrong contactor c	1320 60 3560 Z103a 3680 3740 3800 Z103b 3980 Z103b 3980 Z103c 4160 2103d 4280	Cluster IV caacaagotg cagotogtga tgaagagogg caagtacaog otoggotaca agaocgtoot caggacootc aggaactoca agggtaagat (370nt) ttattgootg tatgitgtat tgotgtgtgc tgttgtotac atttagott tggatatgtc catacg CAGG ATATCGATCGAC AATGAACATA CCCTGCGATT GTTTTCTCTT AGGAGATAAT CAAGCCAGAA ATCTGATCCT C gaatttaa tgtagagttt tittitotga aagatticot ttatcitcaa atgagtiggt (370nt) ttattagt ittggitgaa tiggiag TGC AGGATATCGAC GATCCTTCC CCAGATGATC GACGATGAGC ATAATGTGCG CTATTGTTCT CTTAAGAGAT AATCAAGCCA GAACTATCGAC GACGATGAGC ATAATGTGCG CTATTGTTCT CTTAAGAGAT AATCAAGCCA GAACTATGAT TCTCCA aatt taatgcagtc tcittigctg aagattigt titatcigaa aatgtactga titgiggta atticatigt gotgcciatt titcotgatt cittiagcat gitgggtga aattitatt ggigctgct aata TGGACA ATATCATGATC AGGAACC AAGCCAGTAT TCTCCA CATGAACATA CTGAGCGCTA TTATTTCTCT TTTGAGAACC AAGCCAGTAT TCTCA aatcttat gcaggcallt ccititatti	60 480 Z1 03e 660 Z1 03f 1140 1200 1260 Z1 03g 1380 1440

these snoRNAs are targeted by two different individual snoRNAs in yeast and mammals (Table 1). Based on complementarity to Z102, we predict a novel site of methylation in rice rRNAs, although this snoRNA shares another antisense element with human U44. The putative target sites of Z108 and Z110 are conserved between plants and vertebrates, but not yeast, and only the cognate of Z108 has been identified from mouse (GenBank accession no. AJ278763). Three snoRNA genes, Z105, Z107 and Z109, can form a 13–14 bp long duplex with rice rRNAs at sites that do not match any known ribose-methylated nucleotide of rRNAs

aagcgaccga tccaaccacc cgcatccaca caccgcacca ccaaccatcg accgcctcgc 6620

in yeast and vertebrates. Thus, it is possible that there may be plant-specific methylation of rRNA. Recently, the counterparts of these three snoRNAs have also been reported in *A.thaliana*, but the methylation site predicted by the snoRNAs was not experimentally mapped (32). Using primer extension at low dNTP concentrations, we determined three new methylated nucleotides in rice 25S rRNA (Fig. 4), i.e. Am1364, Um2396 and Am2902, which were targeted by Z105, Z107 and Z109, respectively. However, A2925 in 25S rRNA, a methylation site predicted by Z102, was found to be unmethylated in our experiment (Fig. 4).

tgcccacctc ttcggaagtc agaaattgag tactacgcta tgttggccaa ggtcaccgtc 2040

Cluster	V
Cluster	•

cgcctccaca aatccgccgc cgccgacgtc gagagagaag ggacagagag cgagtgcttc	60
$gccgtccccg\ ccgccgcgat\ gagggccaag\ {\tt gttcgttacc}\ ($ 190 nt) gttgttggtt	300
tattttgtgg cttgcg	
ATTC TCCGTGATGA CGAACACAGA TGACGAGTCC GATCTAATCC	Z104a
ATTCCATTAA ACCATGGGGA CAATCGAGGC ATTTGTCTGA GAGAAT	
tgca ttgcgtgtcg	420
cgcttgcgcg atttaacttt cttcttctgc tactactgct atatagctgt aaaaatgttt	480
ctttcgctag caatcttcgt gtgtactata ggagtaggtg ttctaaattg ttatatttct	540
ggatgcg	
GTT CTCCTTGATG AACACACAGA TGACGAGTCC GATCTAATCC ATTCCATTAA	Z104b
ACCATGGGGA CAATCGAGGC ATTTGTCTGA GAGAAC	
attt tatttatttt cttgccgatt	660
ttcctttgtt ctgtatgtga ttcttcttta tgatactgta	
TTGGCATGAT_GACGAAACCT	Z105
TACGTTTATE TGATCTATTE GATGATAAAT TECTECTAET CATTETGAAT GECAA	
atcct	780
gtacatttgt ttctttcttt tcatatgtac gttagtcagt acgttagtgt tctgcttgct	840
aaatcatgaa catgattcag tcaaggetet atgetgtatg actaceacaa aattaategt	900
ttta	
TEGAAC ATCATGAAGA ACCATTEGTC CETETTTCTE ATCATCTCET GATGAAACTA	Z106
CAATGAAGCA CTCATACTTC TGATTCCA	
aa acttttatcc tcatgagata gattacttct	1020
atccacacga (90nt) taatctgcag <i>tggaagaaga agagaatgag gaggctcaag</i>	1160
aggaagcgcc gaaagatgag gcagagatcc aagtagtcgt tatcagtagt gtggatgtct	1220

Cluster vi						
tgttttatta	tgcaagcgat	gcgatGGCAG				
			TGACGACCTG	GTAATATTCA	AGCTCAACAG	Z107
ACCAAATCAC	AGGTCTTTCT	CTCTGGATCT	ACTCCTCAGG	GATTGATTTG	TATGCCGATT	120
TTTCCGCTGA	ACCGAGCCAT	CTGA				
		TTTTGC	Tettttttet	(70nt)	tctttgcact	240
GGAGGAGTCT	ATTGCGATGA	GGATATTAAG	CTCCCTCT	TGAAGGCATT	GAGGACACTC	Z108
GCTGGCAGAA	TCGAACCAAT	TAACAAAGCC	ACCACTGAGT	AACTTGAAAT	CCTCC	
					ttcaa	360
tttgttctgc	tatgttttt	ctgttgtttt	tcttagatct	gt		
		mmo mio ve via		TTGGATTG	TGAGGAGGTA	Z109
ATTGATGCAT	AATCCAACAC	TTGATCAGAG	GACGGCTTCT	GTCGTCCATG	TGACGTTACA	480
CATCITIGCA	ICIGAAICCA	A				
		tcacagaac	agatettate	gtttatggtt	ttatgtggat	540
IIIIIac	TOLOGIT	CHARGETAT	ALCONTROL	COLO LOOT TO	TODOLOLOTO	7110
AUI	ATCAGAGGAT	LOCOTTTTOT	TTOTO ITO IT	GGACACCIT	IGACACAGIG	2110
CIAIAACACI	GIGAAIGAGA	AGGUITTICI	TICIGATGAT	GUI	*****	(())
	ttooototta	agtattatat		ttaaaga		000
+	liaaaiciig	cgigiiciai	aattittici	cerrectata	letaegatti	720
AACCOT ATC	ATCATTACCA	TATTOTTOCA	ATTACCCTCA		TOACCACAT	7111
TETETERCAT	CCTCACCCTT	TATIOTIOUA	ATTACCOTCA	GATTATCAAA	TUACUACATT	2111
TUTETUUCAT	Geronouerr	tataatccan	tootottato	atatotoooa	atotagattt	840
tettcateta	ttatoaotto	caate	taatattgta	Sigiataaag	atotagatti	040
ligitourgiu		TAGTO	CCAGTGAGGA	TTCTATGACC	ATAGGAAGTG	Z112
CCGTATGACA	CTTTAAACAG	CAGCTGCTTT	TCTCATTTGC	AAGTGTCCAT	CCTTCGACCA	960
TAACCTTTGT	GGTTTGGTCT	GGGATGGAGT	ACCCATTTGC	AATTGGTTAA	TTCAGCTGTG	1020
TGTGTGATTT	TAAACGGGTC	TCTTGACGGA	TTA			
			tgcgaag	ctgtctcata	ataatataga	1080
atttgtatct	gttattttgt	gat		0	0	
		AGTTTGG	GCCTGATGCC	ACGTCACCTG	TAGCTACGGC	Z113
TTTGACCGGT	GATGCTTTGA	TTAGGCCAAA	ACAGAGTTGG	TTTCCTACGT	TTCACATGTT	1200
GATAGGAACC	TGGGACTGCA	TCTCCATCGG	TCTACAGAGC	CCGTATCTGT	CATTACATT	1260
gatttatttg	aattaacaac	cttttttgtt	ttgcttttat	tttttggtc	ccttgattat	1320
ATGCCAATGA	TGATAAATTT	AAGGCTTGTT	TCTCATAACA	TTCGCAGTTG	CCGCCTAAGA	Z114a
GCTTTCGCCC	TGCCAGGCTT	GAGAGCTAAT	GCTGTTAATT	CCTTCCTTGG	ATGTCTGACC	1440
CAT						
tttcttc	atgcaagttt	atttgttgtt	cttctgtaaa	ttggtgatc		
				T	GCATGGCAAA	Z114b
TGATGCTAAA	AGCAAGGCTT	GTTTCTCATA	ACATTCGCAG	TTGCCGCCTA	AGAGCTTTCG	1560
CCTTGCCAGG	TTTGAGAGCT	AATGCTGCTA	ATTCCTTCCT	TGGATGTCTG	ATGCAATGCA	1620
acaccaactg	tattctcttt	aaattattgt	tttgccactt	ctataattaa	tgtttactga	1680
L						

Figure 1. (Previous page and above) The sequences of the snoRNA gene clusters from rice. snoRNA genes are in capital letters; sequences of exons are in bold and italic; boxes C/D and C'/D' are boxed with solid and dashed lines, respectively. A bar is drawn over sequences complementary to rRNA and arrows indicate nucleotides involved in the terminal stem.

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Z113 is the only H/ACA box snoRNA found in the clusters. It appears homologous to maize snoR2 by comparative analysis. In maize, snoR2 is accompanied by two other C/D box snoRNAs, U49 and U14, and this clustering has been found several times (28). Intriguingly, rice Z113 is positioned in a similar genomic context, with Z112 (the cognate gene of U49) upstream and Z114 (the homolog of U14) downstream, but there are also other different genes within the cluster.

Positive identification of specific snoRNAs from the clusters

To confirm that the expected snoRNAs were transcribed from the gene clusters and processed from a polycistronic precursor, reverse transcription analyses were carried out with RNA isolated from partially purified nuclei from *O.sativa* cells. These experiments employed six oligonucleotide primers, designed and synthesized to pair with the coding regions of six novel snoRNA genes, Z102, Z104, Z105, Z106, Z107 and Z109, which were selected from clusters II, V and VI, respectively. As shown in Figure 5, a major cDNA product consistent with the expected size was observed in each case. The snoRNAs yielded signals of different intensities under the same experimental conditions, possibly reflecting different cellular abundances. These appear to correlate with gene number. For example, in cluster V, two nearly identical copies of Z104 are present, while only one copy of Z106 is present, and the band representing the cDNA product of Z104 is stronger than that representing Z106. Intriguingly, the signal due to Z105 does not accumulate at the same level as that due to Z106, and is even stronger than that due to Z104, suggesting that other copies of Z105 might exist in the rice genome. In fact, we have found another copy of the Z105 snoRNA gene on rice chromosome I by searching the growing rice DNA database (our unpublished result). Another possible explanation for the different signal levels of snoRNAs is differences among snoRNA gene promoters. However, at present little is known concerning plant snoRNA gene promoters and differential expression.



Figure 2. Detection of snoRNA polycistronic precursors by RT–PCR. (A) Schematic representations of clusters II and IV. The coding regions of snoRNAs are shown as black boxes. Arrowheads represent primer pairs used for the detection of the precursors by RT–PCR. The lengths of the expected products are given. (B) Detection of polycistronic precursors. Lane DNA, positive control PCR performed on rice total DNA; lane RT–PCR, PCR amplification after reverse transcription of rice total RNA; lane PCR-Co, control PCR performed on rice total RNA without reverse transcription; lane M, molecular weight marker (pBR322 digested with *Hae*III and 5'-end labeled with [γ^{-32} P]ATP). PCR amplification products exhibiting the expected size are indicated by arrows.

Analyses of the intergenic sequences in the gene clusters

All of the snoRNA genes in the clusters are arranged in a headto-tail fashion and are closely linked. The sizes of the intergenic regions range from 46 to 456 bp. Sequence analyses have shown that all of them are rich in uridine, with >40% and even as high as 61% in one case (the spacer between Z108 and Z109 in cluster VI). This phenomenon is similar to the one observation for the snoRNA gene clusters from A.thaliana (29). However, in addition to being U-rich, the sequences of the intergenic spacers of each cluster are very different from each other. Although hairpin-like structures can be deduced for the spacers with long sequences with the RNA folding program, some small spacers in the cluster (for example the spacer between Z109 and Z110) do not tend to form any stable secondary structure. Moreover, no conserved motif was found among the folded spacers. Thus, unlike the hairpin secondary structure with a tetranucleotide loop that is recognized by RNase III in yeast (23), the spacers in rice snoRNA gene clusters may adopt other structures that contribute to the signal for processing of the polycistronic transcripts. In addition, since the polycistronic transcript of plant snoRNAs can be efficiently processed from both intronic and non-intronic contexts, the intergenic regions of the clusters in both contexts may share a common processing mechanism that remains to be elucidated.

An intronic snoRNA gene cluster is conserved in Gramineae

Five intronic snoRNA gene clusters have been identified from rice so far, together with our previous report of a snoRNA gene cluster in the first intron of the rice Hsp70 gene, which contains four C/D box and two H/ACA box snoRNAs (33; our unpublished results). To demonstrate the conservation of intronic snoRNA gene clusters in plants, we have systematically studied the first intron of the Hsp70 gene, a well conserved protein coding gene in plants, from two subspecies of rice, i.e. *O.sativa* ssp. *indica* and *O.sativa* ssp. *japonica*, and the wild



Figure 3. Predicted methylation guide duplexes between snoRNAs and rRNAs. 2'-O-ribose methylation sites homologous to those of yeast or vertebrates are shown by filled circles and novel methylation sites predicted from the present work are depicted by open circles. Boxes D and/or D' are indicated. (A) snoRNAs with one complementarity to rRNAs; (B) snoRNAs with two complementarities to rRNAs.

rice (Oryza rufipogan). The results showed that both the sequences and the order of the six snoRNA genes in the cluster are highly conserved (data not shown). To further study the divergence and distribution of the cluster, the first intron of the Hsp70 gene from Z.caduciflora (Gramineae) was cloned and sequenced (Fig. 6). The sequence similarity of the first introns of the Hsp70 genes between O.sativa and Z.caduciflora is 66%. Five snoRNA genes can be easily spotted from the sequence of the first intron of the Z.caduciflora Hsp70 gene. All of them exhibit extensive homology to their counterparts in O.sativa and are organized in a similar order in spite of extensive divergence of the intergenic sequences. However, in the region where D1 was expected, only the 3'-half of the snoRNA gene is found, while the 5'-half, including one of the two key motifs, the C box, and the 5' terminal repeat, is completely missing. Therefore, the partial sequence of D1 in Z.caduciflora may be a pseudogene. This result demonstrates that the intronic snoRNA gene cluster in the first intron of the Hsp70 gene is conserved between rice and Z.caduciflora and may extend to all plants of the Gramineae family.

DISCUSSION

Intronic snoRNA gene clusters most likely predominate in the genome of rice

Rice, after *A.thaliana*, is the second plant whose genome has been chosen to be fully sequenced. The rapid progress of the rice genome project will certainly provide a solid base for further studies of rice and other crop grasses. In addition to the

sequence itself, rice has already become a good source for the identification of new genes. In this study, we took advantage of the growing rice DNA database in an effort to shed new light on snoRNA gene organization. So far, seven clusters including 33 snoRNA genes have been identified, and a novel gene organization, i.e. intronic snoRNA gene clusters, was first revealed in rice. Indeed, among the seven clusters, five are located within introns of protein-coding genes. This relatively high proportion of intronic snoRNA gene clusters suggests that this novel gene organization may be prevalent in the rice genome. Our results also reveal that the intronic snoRNA gene cluster in the Hsp70 gene is conserved among rice, wild rice and Z.caduciflora, and probably in many other plants of the Gramineae family. On the other hand, we have identified 35 snoRNA gene clusters from the complete genome of A.thaliana, among which three are intronic snoRNA gene clusters (40; our unpublished results). To our knowledge, no intronic snoRNA gene cluster has been found in yeast or animals so far. Therefore, this gene organization may be unique to plants. The relative scarcity of intronic snoRNA gene clusters in the dicotyledon A.thaliana may reflect a genome that is particularly small and compact. Most introns in A.thaliana are of small size (~170 bp on average) (41), which is merely long enough to accommodate a single snoRNA gene at most. Conversely, the rice genome, estimated to be 430 Mb, is more than three times larger than that of A.thaliana (~120 Mb). Thus, it is not surprising that a large number of intronic snoRNAs in clusters are found in rice and other crops with large genome sizes.

Cluster	Chromosome	snoRNA	Modification (homology)	Location	Accession no.
I 6	6	Z100a	SSU Am623 (yeast snR47)	2nd intron of rpS20 gene	AJ320255
		Z100b			AJ320256
		Z100c			AJ307912
		Z100d			AJ307913
П 1	1	Z101	SSU Am440 (human U16) LSU Cm1849 (human U39)	Spacer between two protein coding genes	AJ307914
		Z102	LSU Am2925 (at snoR18)		
			SSU Am162 (human U44)		AJ307915
	Z103h	No complementarity (at snoR28)		AJ307916	
III	III 3	Z103a		2nd intron of NADH dehydrogenase gene	AJ307917
	Z103b			AJ307918	
		Z103c			AJ307919
		Z103d			AJ307920
IV 1	1	Z103e		2nd intron of rpL30 gene	AJ307921
		Z103f			AJ307922
		Z103g			AJ307923
V	3 Z104a LSU Gm2275 (yeast snR75) Intron of an unknown protein	Intron of an unknown protein	AJ307924		
		Z104b	LSU Am2268 (human U15)		AJ307925
		Z105	LSU Am1364 (at snoR7)		AJ307926
		Z106	LSU Am625 (human U18)		AJ307927
VI 2	2	Z107	LSU Um2396 (at R87)	Spacer between two protein coding genes	AJ307928
		Z108	SSU Gm392 (mouse Z51)		AJ307929
		Z109	LSU Am2902 (at snoR31)		AJ307930
		Z110	LSU Um2641 (at Z27)		AJ307931
		Z111	SSU Um582 (yeast snR77)		AJ307932
		Z112	LSU Cm2869 (human U49)		AJ320263
		Z113	(maize snoR2)		AJ320264
		Z114a	SSU Cm418 (human U14)		AJ315478
		Z114b			AJ315479

Table 1. Localization and constitution of the six snoRNA gene clusters

Some features of the intronic snoRNA gene clusters

There are usually multiple copies of each snoRNA gene in plants (29,31,32). Although snoRNA genes can be found both in independently transcribed and intronic clusters, the sequences from different copies of a snoRNA gene are very conserved in spite of their different locations. However, compared with the richness of components in non-intronic clusters, the constitution of most rice intronic snoRNA clusters is relatively monotonous. All three intronic snoRNA gene clusters in A.thaliana are also made up of multiple copies of a single species of snoRNA gene (our unpublished results). This implies that frequent duplication has occurred within introns during the evolution of the plant genome. However, rice snoRNA cluster V contains two additional species of snoRNA genes besides isoforms of one species, and the snoRNA cluster located in the first intron of the Hsp70 gene consists of six snoRNAs, which belong not only to different species but also to different types, C/D box and H/ACA box. This shows that the structure of intronic snoRNA gene clusters can be more complex than initially thought. Leader et al. (28) have successfully expressed an independently transcribed cluster of maize from both non-intronic and intronic contexts in tobacco protoplasts. This result demonstrates that the processing of snoRNA polycistronic transcripts is independent of splicing (28,30). However, the processing signals in polycistronic transcripts that are recognized by endonucleases and exonucleases in plants have not yet been elucidated. The discovery of different kinds of intronic snoRNA gene clusters provides impetus for further studies of the expression and processing of intronic snoRNA in plant.



Figure 4. Determination of rRNA methylation sites predicted by the novel snoRNAs. Lane 1, control reaction at 500 mM dNTP; lane 2, primer extension at 4 μ M dNTP; lanes A, C, G and T, the rDNA sequence ladder. The sites of ribose methylation in rRNA were revealed by RT pauses at low dNTP concentrations. Arrows indicate potential methylation sites predicted by the novel snoRNAs.



Figure 5. Reverse transcription analyses of snoRNAs. The experiments were carried out with 10 μ g rice RNA and 5'-end labeled primers specific to candidate snoRNAs, as described in Materials and Methods. Lane M, molecular weight markers (pBR322 digested with *Hae*III and 5'-end labeled with [γ -³²P]ATP).



Figure 6. Sequence comparison of the first intron of the Hsp70 genes from *O.sativa* and *Z.caduciflora*. (A) Schematic diagrams of gene organization. The relative positions of the genes are drawn to scale with the exception of the region between exon 1 and C2, which is shown by a wavy line. Exons are shown as black boxes and snoRNA genes as open boxes with solid lines. The open box with dashed lines indicates the D1 pseudogene. (B) Alignment of the two sequences. snoRNA genes are in capital letters and boxed and the name of each gene is shown. Sequences of exons are in bold capital letters. Nucleotide identities are indicated by hyphens and those absent in either sequence by asterisks. The size of each intron is given.

The introns that contain a snoRNA gene cluster are exceptionally large as compared with other introns in the same host gene, even if the sequence of the snoRNA gene is not considered. It therefore seems that it is not simply the internal snoRNA cluster that makes an intron large. It remains to be elucidated whether there is a length threshold for an intron to contain a snoRNA gene cluster or whether introns without snoRNA gene clusters have become smaller during evolution.

Diversity of snoRNA gene organization

One main goal of studying snoRNAs is to elucidate their diverse modes of gene organization in various organisms. Earlier studies focused mainly on yeast and vertebrates and revealed two distinct genomic organizations (1). In yeast, a majority of snoRNA genes are dispersed as independently transcribed singlets, while in vertebrates, most snoRNA genes are located within introns. The latter organization was later also found in yeast, but in only a few cases (1,22). The discovery of clustered snoRNAs, which are transcribed under an upstream promoter, in yeast and plants adds a third mode, i.e. snoRNA gene clusters, to the diversity of snoRNA gene organization. This gene organization implies the transcription of snoRNAs as polycistronic precursors and a splicing-independent processing pathway involving endonucleolytic cleavage as well as exonucleolytic trimming to release individual snoRNAs (23,30,42). In this study we report intronic snoRNA gene clusters as a novel polycistronic organization. In contrast to independently transcribed clusters, the biosynthesis of multiple snoRNAs from an intronic polycistron depends absolutely on transcription from the promoter of a protein-coding gene and processing of the mRNA precursor.

snoRNA gene clusters, either non-intronic or intronic, permit the coordinated expression of multiple or different snoRNAs at high efficiency. The latter further permits coordinated regulation of the expression of snoRNAs and the host gene. Interestingly, in both plants and mammals, numerous snoRNAs can be produced simultaneously from a mRNA precursor of a host gene despite the different arrangements. We notice that in plants, usually only one intron of a host gene encodes multiple snoRNAs, whereas in mammals a host gene may contain numerous snoRNA sequences, but never more than one snoRNA per intron (1,42). The adoption of two different modes of snoRNA gene organization may reflect intrinsic differences in gene expression and evolution between plants and vertebrates.

The finding of snoRNA gene clusters in various organisms suggests an ancient origin of this gene organization (29). It is not yet clear whether this is also true of intronic snoRNA gene clusters, which are so far unique to plants. More detailed analyses of the rice genome and comparative studies using data from other organisms may provide answers to this important question.

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REFERENCES

- 1. Maxwell,E.S. and Fournier,M.J. (1995) The small nucleolar RNAs. *Annu. Rev. Biochem.*, **35**, 897–934.
- Smith,C.M. and Steitz,J.A. (1997) Sno storm in the nucleolus: new roles for myriad small RNPs. *Cell*, 89, 669–672.
- Omer,A.D., Lowe,T.M., Russell,A.G., Ebhardt,H., Eddy,S.R. and Dennis,P.P. (2000) Homologs of small nucleolar RNAs in Archaea. *Science*, 288, 517–522.
- Gaspin, C., Cavaille, G., Erauso, G. and Bachellerie, J.P. (2000) Archaeal homologs of eukaryotic methylation guide small nucleolar RNAs: lessons from the *Pyrococcus* genomes. J. Mol. Biol., 297, 895–906.
- Dennis, P.P., Omer, A. and Lowe, T. (2001) A guided tour: small RNA function in Archaea. *Mol. Microbiol.*, 40, 509–519.

- Balakin,A.G., Smith,L. and Fournier,M.J. (1996) The RNA world of the nucleolus: two major families of small nucleolar RNAs defined by different box elements with related functions. *Cell*, 86, 823–834.
- 7. Tollervey, D. and Kiss, T. (1997) Function and synthesis of small nucleolar RNAs. *Curr. Opin. Cell Biol.*, **3**, 337–342.
- Tyc,K. and Steitz,J.A. (1989) U3, U8 and U13 comprise a new class of mammalian snRNPs localized in the nucleolus. *EMBO J.*, 8, 3113–3119.
- Ganot, P., Caizergues-Ferrer, M. and Kiss, T. (1997) The family of box ACA small nucleolar RNAs is defined by an evolutionarily defined secondary structure and ubiquitous sequence elements essential for RNA accumulation. *Genes Dev.*, 11, 941–956.
- Eichler, D.C. and Craig, N. (1995) Processing of eukaryotic ribosomal RNA. Prog. Nucleic Acid Res. Mol. Biol., 49, 197–239.
- Lafontaine, D. and Tollervey, D. (1995) Trans-acting factors in yeast prerRNA and pre-snoRNA processing. *Biochem. Cell Biol.*, 73, 803–812.
- Venema, J. and Tollervey, D. (1995) Processing of pre-ribosomal RNA in Saccharomyces cerevisiae. Yeast, 11, 1629–1650.
- Kiss-Laszlo, A., Henry, Y., Bachellerie, J.P., Caizergues-Ferrer, M. and Kiss, T. (1996) Site-specific ribose methylation of preribosomal RNA: a novel function for small nucleolar RNAs. *Cell*, 85, 1077–1088.
- Nicoloso, M., Qu, L.H., Michot, B. and Bachellerie, J.P. (1996) Intronencoded, antisense small nucleolar RNAs: the characterization of nine novel species points to their direct role as guides for the 2'-O-ribose methylation rRNA. J. Mol. Biol., 260, 178–195.
- Ni,J., Tie,A.L. and Fournier,M.J. (1997) Small nucleolar RNAs direct site-specific synthesis of pseudouridine in ribosomal RNA. *Cell*, 89, 565–573.
- Ganot,P.H., Bortolin,M.L. and Kiss,T. (1997) Site-specific pseudouridine formation in eukaryotic pre-rRNA is guided by small nucleolar RNAs. *Cell*, 89, 799–809.
- Tycowski,K.T., You,Z.H., Graham,P.J. and Steitz,J.A. (1998) Modification of U6 splicesomal RNA is guided by another small RNA. *Mol. Cell*, 2, 629–638.
- Ganot, P.H., Jady, B.E., Bortolin, M., Darzcq, X. and Kiss, T. (1999) Nucleolar factors direct the 2'-O-ribose methylation and pseudouridylation of U6 splicesomal RNA. *Mol. Cell. Biol.*, 19, 6906–6917.
- Hüttenhofer, A., Kiefmann, M., Meier-Ewert, S., O'Brien, J., Lehrach, H., Bachellerie, J.-P. and Brosius, J. (2001) RNomics: an experimental approach that identifies 201 candidates for novel, small, non-messenger RNAs in mouse. *EMBO J.*, 20, 2943–2953.
- Jady,B. and Kiss,T. (2001) A small nucleolar guide RNA functions both in 2'-O-ribose methylation and pseudouridylation of the U5 spliceosomal RNA. *EMBO J.*, 20, 541–551.
- Weinstein,L.B. and Steitz,J.A. (1999) Guided tours: from precursors snoRNA to functional snoRNP. *Curr. Opin. Cell Biol.*, 11, 378–384.
- Lowe, T.M. and Eddy, S.R. (1999) A computational screen for methylation guide snoRNAs in yeast. *Science*, 283, 1168–1171.
- Qu,L.H., Henras,A., Lu,Y.J., Zhou,H., Zhou,W.X., Zhu,Y.Q., Zhao,J., Henry,Y., Caizergues-Ferrer,M. and Bachellerie,J.P. (1999) Seven novel methylation guide small nucleolar RNAs are processed from a common polycistronic transcript by Rat1p and RNase III in yeast. *Mol. Cell. Biol.*, 19, 1144–1158.
- Tycowski,K.T., Shu,M.-D. and Steitz,J.A. (1996) A mammalian gene with introns instead of exons generating stable RNA products. *Nature*, 379, 464–466.
- Kiss, T. and Filipowicz, W. (1995) Exonucleolytic processing of small nucleolar RNAs from pre-mRNA introns. *Genes Dev.*, 9, 1411–1424.
- Cavaillé, J. and Bachellerie, J.-P. (1996) Processing of fibrillarinassociated snoRNAs from pre-mRNA introns: an exonucleolytic process exclusively directed by the common stem-box terminal structure. *Biochimie*, 78, 443–456.
- Leader, D.J., Sanders, J.F., Waugh, R., Shaw, P.J. and Brown, J.W.S. (1994) Molecular characterization of plant U14 small nucleolar RNA genes: closely linked genes are transcribed as a polycistronic U14 transcript. *Nucleic Acids Res.*, 22, 5196–5200.
- Leader, D.J., Clark, G.P., Watters, J., Beven, A.F., Shaw, P.J. and Brown, J.W. (1997) Clusters of multiple different small nucleolar RNA genes in plants are expressed as and processed from polycistronic presnoRNA. *EMBO J.*, 16, 5742–5751.
- Qu,L.H., Meng,Q., Zhou,H. and Chen,Y.Q. (2001) Identification of 10 novel snoRNA gene clusters from *Arabidopsis thaliana*. *Nucleic Acids Res.*, 29, 1623–1630.

- Leader, D.J., Clark, G.P., Watters, J., Beven, A.F., Shaw, P.J. and Brown, J.W.S. (1999) Splicing-independent processing of plant box C/D and box H/ACA small nucleolar RNAs. *Plant Mol. Biol.*, 39, 1091–1100.
- Barneche, F., Gaspin, C., Guyot, R. and Echeverría, M. (2001) Identification of 66 box C/D snoRNAs in *Arabidopsis thaliana*: extensive gene duplications generated multiple isoforms predicting new ribosomal RNA 2'-O-methylation sites. J. Mol. Biol., 311, 57–73.
- Brown,J.W., Clark G.P., Leader D.J., Simpson C.G. and Lowe,T. (2001) Multiple snoRNA gene clusters from *Arabidopsis. RNA*, 7, 1817–1832.
- Qu,L.H., Zhong,L., Shi,S.H., Lu,Y.J., Fang,R. and Wang,Q. (1997) Two snoRNAs are encoded in the first intron of the rice hsp70 gene. *Prog. Nat. Sci.*, 7, 371–377.
- Chomczynski, P. and Sacchi, N. (1987) Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. *Anal. Biochem.*, 162, 732–735.
- 35. Caffarelli,E., Fatica,A., Prisley,S., De Gregorio,E., Fragapane,P. and Bozzoni,I. (1996) Processing of the intron-encoded U16 and U18 snoRNAs: the conserved C and D boxes control both the processing and the stability of the mature snoRNA. *EMBO J.*, **15**, 1121–1131.

- Samarsky, D.A., Fournier, M.J., Singer, R.H. and Bertrand, E. (1998) The snoRNA box C/D motif directs nucleolar targeting and also couples snoRNA synthesis and localization. *EMBO J.*, **17**, 3743–3757.
- Bachellerie, J.P. and Cavaille, J. (1997) Guiding ribose methylation of rRNA. *Trends Biochem. Sci.*, 22, 257–261.
- Tycowski,K.T., Smith,C.M., Shu,M.D. and Steitz,J.A. (1996) A small nucleolar RNA required for site-specific ribose methylation of rRNA in *Xenopus. Proc. Natl Acad. Sci. USA*, 93, 14480–14485.
- Tollervey, D. (1996) Small nucleolar RNAs guide ribosomal RNA methylation. *Science*, 273, 1056–1057.
- Zhou, H., Meng, Q. and Qu, L.H. (2000) Identification of Z2 snoRNA gene cluster from *Arabidopsis thaliana* genome. *Sci. China Ser. C*, 43, 449–453.
- The Arabidopsis Genome Initiative (2000) Analysis of the genome sequence of the flowering plant *Arabidopsis thaliana*. *Nature*, 408, 796–815.
- Bachellerie, J.-P., Cavaille, J. and Qu, L.-H. (2000) Nucleotide modifications of eukaryotic rRNAs: the world of small nucleolar RNA guides revisited. In Carrett, R.A. (ed.), *The Ribosome: Structure*, *Function, Antibiotics and Cellular Interaction*. ASM Press, Washington, DC, pp. 191–203.