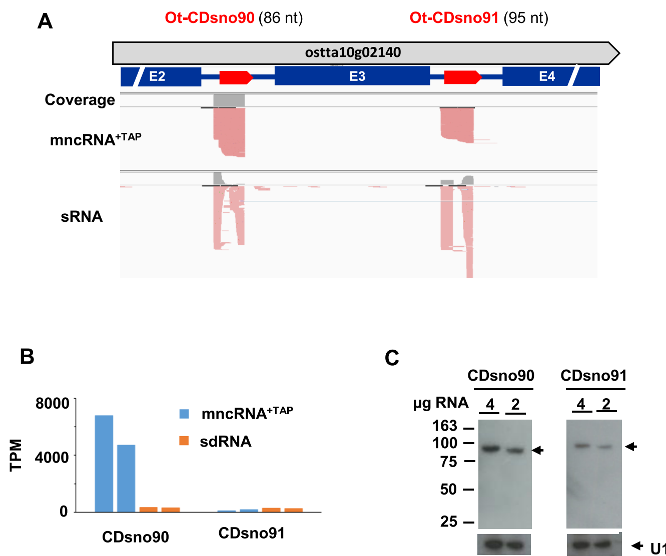

Figure 5. Genomic organization and abundance of Ot−CDsno90 and Ot−CDsno91. (A) Intronic localization of Ot−CDsno90 and Ot−CDsno91. The thick red arrows indicate the snoRNAs. The thick gray arrow indicates the host gene. Blue rectangles indicate the exons flanking the introns. (B) Normalized read counts (TPM) of Ot−CDsno90 and Ot−CDsno91 replicates (blue bars) and to sdR90 and sdR91 (orange bars) respectively. (C) Northern blots analysis using specific probes to assess the abundance of Ot−CDsno90 and Ot−CDsno91, respectively (See Figure 3D for details of labeling).

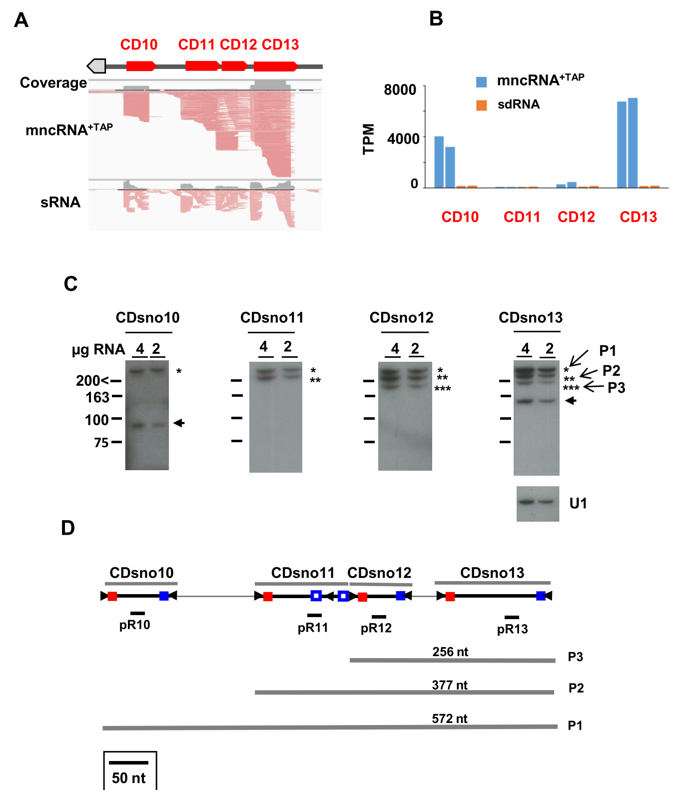

Figure 6. Genomic organization and expression of polycistronic cluster 1. (A) Organization and read profile of cluster 1. The thick red arrows indicate the position and the orientation of the clustered Ot–snoRNA. (B) Normalized read counts of the 4 Ot–snoRNAs predicted in cluster 1. TPM corresponding to replicates from mncRNA+TAP (blue bars) and sRNA (orange bars) libraries are shown, respectively. (C) Northern blot analysis of Ot–snoRNAs from cluster 1. Conditions were similar to Figure 3D, with the following exceptions. The blots were hybridized with four different probes (indicated in panel 3D) to detect the predicted mature Ot–snoRNAs. The black arrows indicate the position of the predicted mature Ot–snoRNAs. P1 (*), P2 (**) and P3 (***) indicate the intermediate products, schematized in panel D. The length marker 200 < indicates that the length of size bands longer than 200 nts could not be determined precisely in the 10% polyacrylamide gel used here (see 'Materials and Methods' section). All northern blots were exposed for 15 h without intensifying screening except for Ot−CDsno10 which was exposed with an intensifying screen. (D) Detailed structure of cluster 1 drawn to scale. The position of mature Ot−CDsno10 to Ot−CDsno13 predicted from their read profiles are indicated. Red and blue filled squares indicate C and D boxes, respectively. Open squares represent divergent D' boxes. Black filled arrowheads represent Inverted Repeats flanking terminal C and D boxes. Note that Ot−CDsno11 has a divergent CD−box snoRNA structure. The positions of the different probes (pR10–pR13) are indicated. P1, P2 and P3 represent the predicted 'intermediates' revealed by the northern blot.

the level of Ot−CDsno53 was overestimated by transcriptomic quantification, may be due to 'PCR overamplification' produced by some transcripts (48). No signal was detected with a specific probe for Ot−CDsno8, even after several days of exposure with an intensifying screen in concordance with its low level of expression (result not shown).

U1, a highly structured snRNA, is among the most abundant ncRNAs in eukaryotic cells (36,47). We compared the level of Ot−CDsno53 to *O. tauri*'s U1, of 163 nt in length. The northern blot showed that U1 was also highly abundant, the signal intensity being even slightly stronger than Ot−CDsno53 (Figure 3D), contradictory to U1 quantification of reads (Figure 2B) and clearly showed that its level of accumulation was underestimated, as it occurs for highly structured RNAs with strong secondary structures (47).

Northern blot analysis confirmed however the very high abundance of Ot−CDsno53, contrasting with the low level of Ot−CDsno8. Considering that the expression of an intronic snoRNA depends on the expression of its host gene, we compared the levels of expression of Ot−CDsno53 and Ot−CDsno8 host genes respectively, using published *O. tauri* mRNA transcriptomic data (23). Cultures and RNA extractions for the mRNA transcriptomes were done in the same conditions as those used to prepare Ot−mncRNA transcriptomes. Expression profiles, analyzed over 25 h, including a day and night period, revealed they were both expressed at a moderate and comparable level, even though these genes had different expression profiles (Figure 4D). Clearly, no correlation could be made between the high accumulation Ot−CDsno53 and the expression level of its host gene, which is only moderately expressed. Overall, these data suggest that high stability is important for Ot−CDsno53 abundance. This hypothesis was reinforced by our observation that globally, most abundant C/D box Ot–snoRNAs (more than 500 TPM) have the highest snoRNA/sdRNAs ratios (>5-fold), while the bulk of C/D box Ot– snoRNAs have lower snoRNA/sdRNAs ratios (Supplementary Figure S3).

Expression of intronic snoRNAs

We extended this analysis by comparing the accumulation levels of different Ot–snoRNA candidates hosted in consecutive introns of the same host gene. Ot−CDsno90 and

Ot−CDsno91 are two C/D box snoRNAs nested, respectively, in the second and third introns of ostta10g02140, a gene predicted to encode a nucleoporin (Figure 5A). Remarkably, although both are encoded at equimolar levels in the pre-mRNA precursor, Ot−CDsno90 was highly accumulated (∼5733 TPM) whereas Ot−CDsno91 was much less abundant (161 TPM) (Figure 5B). Northern blot with specific probes to each of them gave signals from single bands at their expected sizes (Figure 5C). Based on signal intensities Ot−CDsno90 would be much more abundant than Ot−CDsno91 (Figure 5C). Differences of abundance between those two snoRNAs correlates with a high ratio (17.4) of Ot−CDsno90/sdR90 and a low ratio (0.5) Ot−CDsno91/sdR91 (Figure 5B).

A similar result on the differential accumulation of all other snoRNAs (two or three) located on consecutive introns of a host gene was observed. In all cases a higher abundance of a particular intronic snoRNA was correlated with a high ratio of snoRNA over sdRNAs (Supplementary Figure S4).

These data reinforce the hypothesis that the stability snoRNAs is a major determinant of their abundance and is not correlated with the level of expression of the host mRNA.

Expression of clustered snoRNAs

Next, we compared the abundance of snoRNAs encoded by gene clusters. In plants, yeast and other species transcription of these snoRNA clusters produces a polycistronic snoRNA precursor (pre-snoRNA) which is rapidly processed to liberate the individual snoRNAs (49–51). In *O. tauri*, final mature snoRNAs often exhibited large differences in their accumulation even though they were present at equimolar ratio in the pre-snoRNAs. Cluster 1 encoded four distinct C/D box snoRNAs (Ot−CDsno10 to Ot−CDsno13) predicted on the basis of their read profiles together with the position of C/D boxes flanked by the terminal IRs in the cluster genomic sequence (Figure 6A and D). Ot−CDsno13 is the homolog of U14 which is conserved in all eukaryotes (1,4). Both Ot−CDsno13 (6872 TPM) and Ot−CDsno10 (3593 TPM) were highly accumulated, whereas a lower level was recorded for Ot−CDsno12 (359 TPM) and Ot−CDsno11 (58 TPM) (Figure 6B). The high levels of Ot−CDsno13 and Ot−CDsno10 also correlated high ratios of Ot−CDsno13/sdR13 (45.7) and Ot−CDsno10/sdR10 (23.1), while these ratios were much lower for Ot−CDsno12/sdR12 (3.1) and Ot−CDsno11/sdR11 (0.7) (Figure 6B). A northern blot with specific probes (pR10–pR13) showed a strong signal migrating at the expected size for mature Ot−CDsno13 (Figure 6C). Additional fragments of larger size hybridized with probe pR13, as well as the other probes, and represent additional snoRNA forms produced by cluster 1 (see below).

Ot−CDsno10's probe also detected a signal at the expected size (Figure 6C). However, no signal was detected for the predicted Ot−CDsno11 and Ot−CDsno12, even after long autoradiogram exposure (Figure 6C). These results confirmed the differential accumulation of Ot−CDsno10 and Ot−CDsno13, in agreement with transcriptomic quantifications. We cannot exclude that Ot−CDsno11 and Ot−CDsno12 also accumulate individually, as suggested by transcriptomic read profiles, but their levels would be below the sensitivity of the northern blot.

A similar result was obtained for other clusters, showing large differences in the accumulation of the different snoRNAs produced from the same polycistronic precursor (Supplementary Figures S5 and 6, data not shown). A global analysis of all Ot–snoRNA abundances versus Ot–snoRNA/sdRNA ratios clearly shows a significant positive correlation ($\rho = 0.55$, P-value $< 2.2e^{-16}$) (Supplementary Figure S7A) specially at higher levels of Ot–snoRNAs (>100 TPM) ($\rho = 0.66$, P-value $< 2.2e^{-16}$) (Supplementary Figure S7B).

These data revealed the differential accumulation of snoRNAs when nested in pre-mRNA introns or in polycistronic precursors, and further suggested that stability is an important determinant of their abundance in the cells.

Unusual snoRNAs produced from polycistronic clusters

Northern blot analysis with the pR13 probe (Figure 6D) revealed three larger RNA forms in addition to mature Ot−CDsno13 (Figure 6C). These larger bands could correspond to different snoRNA precursor intermediates, P1, P2 and P3 (Figure 6D). This hypothesis was confirmed by hybridization with Ot−CDsno10, Ot−CDsno11 and Ot−CDsno12 specific probes (Figure 6D). The pR12 probe specifically hybridized to the P1, P2 and P3 RNA forms recognized by pR13 (Figure 6C). The pR11 probe recognized only P1 and P2, while pR10 recognized only P1 and the mature Ot−CDsno10 (Figure 6C). This pattern of hybridization for the different probes fitted perfectly with the snoRNA structural organization predicted by the position of the terminal C/D boxes flanked by the IRs, as proposed for cluster 1 (Figure 6D).

These data confirmed that P1, P2 and P3 may represent distinct polycistronic precursor intermediates of the mature Ot−CDsno16. However, this was surprising given that pre-snoRNA processing is extremely rapid and intermediates of processing do not accumulate in the cells (49–51). In *O. tauri* cells, predicted intermediates were extremely abundant, nearly as much as the mature Ot−CDsno13, which is highly expressed (Figure 6C), revealing that these 'intermediate precursors' were stable products.

A similar situation was observed for snoRNAs from other polycistronic clusters. One example was the C/D box snoRNAs Ot−CDsno19 and Ot−CDsno24 encoded by cluster 2 (Supplementary Figure S5A). The northern blot with a specific probe for Ot−CDsno19 (a canonical C/D box snoRNA targeting the rRNA), did not detect a signal at the expected size of 114 nt, but detected a larger fragment (>200 nt) (Supplementary Figure S5C). This could not be predicted based on its read profile (Supplementary Figure S5A) and indicated that Ot−CDsno19 would be rather a longer snoRNA with 5′ or/and 3′ extension, beyond the terminal C/D boxes.

In the case of Ot−CDsno24, a C/D box targeting rRNA, northern blot detected three major products, two large fragments (>200 nt) and a smaller fragment of ∼75−80 nt (Supplementary Figure S5C). One of the large fragments likely

represented the mature Ot−CDsno24, which is 208 nt long (Supplementary Figure S5A and D). We could not determine the size of these large fragments precisely due to the low resolution of the upper part of the gel, precluding resolution of size differences. The ∼75 fragment could be a smaller C/D box snoRNA which can be predicted by the reads profile, produced by processing of Ot−CDsno24 (Supplementary Figure S5A). The weaker signal at ∼163 nt corresponds to U1, used as a control (see legend of Supplementary Figure S5D).

A third example is shown for cluster 10. Six Ot–snoRNAs were initially predicted based on the read profile of this cluster (Supplementary Figures S5A and B). However additional 'subproducts' were produced by processing of some of the predicted Ot–snoRNAs. One example was Ot−CDsno103 where 3 C/D box snoRNAs were produced according to their reads' profiles, fitting perfectly with the position of the terminal IRs and C/D boxes in the parent Ot−CDsno103 (Supplementary Figure S6C).

These data clearly showed that polycistronic clusters produce long snoRNAs which accumulate at high levels in *O. tauri,* that can in turn be processed to produce shorter forms of Ot–snoRNAs which accumulate at variable levels.

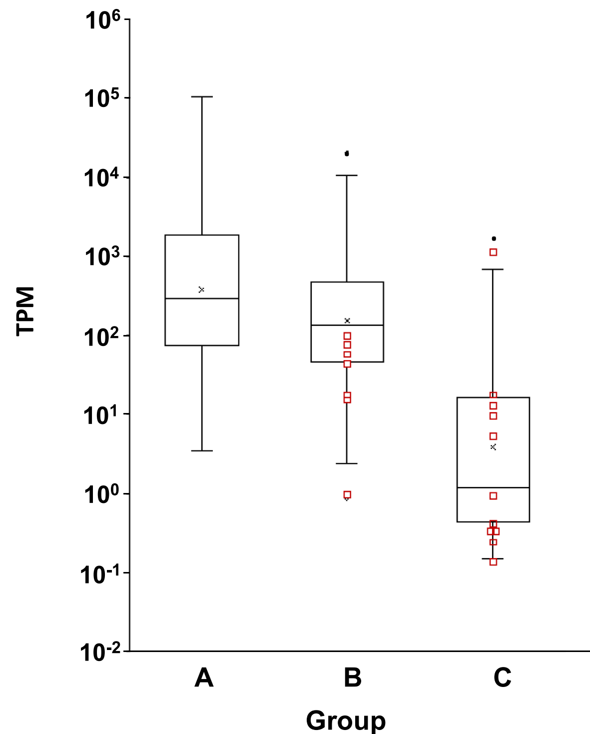

Figure 7. Conservation and abundances of Ot–snoRNAs**.** Group A: Ot–snoRNAs conserved in distant species (human, yeast and plants) Group B: Ot–snoRNAs conserved in Mamiellophyceae Group C: Ot–snoRNAs specific to *Ostreococcus tauri.* Red small squares indicate position of orphan Ot–snoRNAs. This figure was produced using data extracted from Supplementary Tables S4–6

The MRP snoRNA

Ot−MRP (246 nt) is the homolog of MRP RNA, a unique snoRNA distinct from C/D and H/ACA boxes snoRNAs. It is an essential subunit of the MRP endonuclease implicated in processing of pre-rRNA precursors (42). Ot−MRP is located in an intergenic region (Supplementary Figure S7A and 8) and it is not capped (Supplementary Table S3) suggesting that it is transcribed by RNA pol III, as in mammals. This is supported by the presence of a T−stretch terminator (44), at the 3′end of the gene (Supplementary Table S3).

The transcriptomic data suggested that Ot−MRP had a low level of accumulation (∼5–10 TPM) (Supplementary Figure S8B) which would be hardly detected by northern blot. This was surprising because this snoRNA is abundant in eukaryotic cells, as most RNA pol III transcripts (47). To resolve this conflict, we made a northern blot of total RNA with a specific probe for Ot−MRP, revealing a strong signal at the expected size (Supplementary Figure S8C). Clearly, the abundance of Ot−MRP is largely underestimated by the transcriptomic quantification, likely due to the strong secondary structure of this conserved snoRNA (42), as previously observed for U1 snRNA (Figure 4A).

Conservation and predicted functions of *O. tauri* snoRNAs

The extreme differences in Ot–snoRNA abundances might be linked to their relative conservation in other species or to their functions. We thus searched for homologs of Ot–snoRNAs, either by identification of functional homolog in distant species, targeting the same rRNA or snRNA residues reported in snOPY, a snoRNA orthological database (34) or by identification of structural homologs by Infernal/Rfam analysis and by BLASTN alignment of Ot–snoRNA sequences with genomic sequences from algal species (see 'Materials and Methods' section).

Three groups, A, B and C, could be distinguished based on their conservation (Figure 7). Group A was composed of 54 Ot–snoRNAs homologs of snoRNAs found in mammals, yeast or plants (Supplementary Table S4). All of them were predicted to target the corresponding rRNA or snRNA residues which are conserved in these species (Supplementary Table S6). This group had the highest levels of accumulation, including 13 belonging to the top 20 most abundant snoRNAs (Figure 7 and Supplementary Table S6). Group B (Figure 7) was composed of 55 Ot–snoRNAs which had homologs in other species of Mamiellophyceae (Supplementary Table S5). Group B has a similar level of accumulation to group A (Figure 7). Among them 7 belonged to the top 20 most abundant Ot–snoRNAs (Supplementary Table S6). Notably, within this group of Mamiellophyceae-specific snoRNAs, seven were orphans, including the abundant Ot–CDsno19 (273 TPM) (Supplementary Table S6). Group C, was composed of 22 Ot–snoRNAs specific to *O. tauri* (Supplementary Tables S5 and 6). Twelve of them were predicted to target rRNA or snRNA and 10 were orphan snoRNAs (Supplementary Table S6). These had a much lower level of accumulation than the conserved Ot–snoRNAs (Figure 7). However, five of them accumulated much above the average level of this group (Supplementary Table S6). In particular, we note the high abundance of the orphan snoRNA Ot–CDsno131, a C/D box snoRNA with an unusual structure (Supplementary File 3).

Taken together, these data revealed a revealed clear differences in the abundances of Ot–snoRNAs that are conserved with other species compared with the Ot–snoRNAs that were found only in *O. tauri*. In addition, it revealed some orphan Ot–snoRNAs specific to Mamiellophyceae or to *O.tauri*, with a high levels of accumulation, suggesting that they may have important roles in these species.

DISCUSSION

The snoRNA gene family in *Ostreococcus tauri*

We presented here the first study of the snoRNA family in a marine unicellular alga. We identified 131 novel Ot–snoRNAs, to which must be added U3, the only snoRNA that had been annotated in the *O. tauri* genome (22). Hence, at present Ot–snoRNAs represent the largest family of ncRNAs identified in a marine alga.

Most of Ot–snoRNAs were predicted to target modification of rRNA nucleotides, which are essential for ribosome biogenesis. Their expression must be coordinated with rRNA synthesis. Eukaryotes use different strategies to achieve this coordination. In mammals, most snoRNAs are intronics, with an important fraction nested in genes related to ribosome biogenesis (14). In plants and in the unicellular alga *C. reinhardtii*, most snoRNAs are encoded by polycistronic gene clusters which ensure their coordinated expression (14,15). In yeast, which also has a small compact genome with few introns, most snoRNAs are encoded by intergenic genes driven from their own promoter (14). The coordinated expression of these genes is ensured by the Tbf1, a transcriptional regulator that binds to their promoter (52). Therefore, the organization of the *O. tauri* snoRNAs genes appears to be quite unique among eukaryotes as it combines the intronic organization of animals with the clustered organization of plants (Table 1).

Another distinctive feature of Ot–snoRNAs is that, apart from 3 exceptions, they are encoded by single copy genes (Supplementary Table S2). This substantially differs from plants and *Chlamydomonas reinharditii,* where most snoRNAs are encoded by multigenic families. These arose by extensive gene duplication during evolution, producing multiple snoRNA isoforms targeting new rRNA methylation sites (15,53). In *O. tauri* this did not occur, probably because snoRNA gene duplications were constrained by its small and highly compact genome, nevertheless preserving the 'essential' snoRNA complement required to produce the rRNA modification profile necessary for proper ribosome biogenesis. Indeed, the genome size seems to be an important issue in *O. tauri*, the smallest living eukaryote. This might be an important evolutionary adaptation for reduction of its overall cell size, to optimize the surface to volume ratio in this marine phytoplankter. The genome of *O. tauri* is roughly same size as yeast's genome, but has more than 8000 genes, including all the photosynthetic components, versus ~6000 genes in the yeast genome. This notion is further suggested by the observation that in contrast to most other eukaryotes, where rDNA genes encoding the cytoplasmic rRNA are present in hundreds of copies, more than 150 rDNA copies in yeast (1), in *O. tauri* there are only 3 copies of rDNA genes (22).

In sum, the combination of intronic snoRNAs, many of them nested in genes implicated in ribosome biogenesis or RNA processing (Supplementary Table S3) together with the presence of numerous clustered snoRNAs controlled by a common promoter, was an 'evolutionary trick' to optimize the coordinated expression of this essential snoRNA complement at a minimal cost for the development of complex regulatory circuits.

Beyond the canonical snoRNAs

Northern blots revealed unusual snoRNA structures produced by polycistronic loci, that could not be predicted solely based on their transcriptomic profiles. This was shown for cluster 1 which produces large P1, P2 and P3 forms that accumulate to very high levels (Figure 5). Multiple forms were also observed arising from other clusters (Supplementary Figures S5 and 6)

In yeast and plants, clustered snoRNA genes are transcribed by RNA pol II producing a single 'polycistronic' precursor, rapidly processed into individual units (49–51). The high levels of accumulation of P1, P2 and P3 snoRNA precursors suggest they are stable products. This suggest that these longer structures including multiple snoRNAs could have additional functions, beyond the canonical rRNA modification function of the individual snoRNAs released form the polycistronic transcript.

Long C/D and H/ACA boxes snoRNAs, as well as fusion of C/D and H/ACA boxes sno/scaRNAs, with unusual structures, exerting very different functions have been described in animals (12,54). Another remarkable example is the human telomerase RNA which participates in telomere synthesis. This is not a snoRNA, but its 3′end is characterized by a perfect H/ACA box RNA fold which is assembled into an H/ACA snoRNP (55).

The unusual snoRNA forms described here are, to our knowledge, the first examples reported in the green lineage. They reveal the plasticity of the polycistronic genes to generate a highly versatile group of snoRNAs which might have important functions in *O. tauri* and related species.

Different levels of abundance of Ot–snoRNAs

Quantification of Ot–snoRNA reads revealed an extremely large range in their levels of accumulation (Figures 2A and B). Globally, northern blot analysis supported the transcriptomic data and confirmed the large difference of abundance of the Ot–snoRNAs. Given that snoRNAs have a common basic C/D box or H/ACA box structure, such large differences in transcript abundance of some snoRNAs is puzzling.

The results presented here, focusing on the differential accumulation of C/D box snoRNAs expressed from the same gene host (Figure 5 and Supplementary Figure S4) or from the same polycistronic precursor (Figure 6 and Supplementary Figures S5-6) strongly suggest that stability is an important determinant in the abundance of the C/D box snoRNAs. The large majority of the small RNAs derived from Ot–snoRNAs are likely produced by nonspecific degradation of the parent snoRNAs. However, as discussed below, we cannot exclude that a few of them could have a biological function (discussed below).

The differential stability among C/D box snoRNAs is most likely determined by differential rate on snoRNP assembly on the nascent snoRNAs (3,49). Intronic snoRNAs are produced by processing of the intron released by splicing. This process includes 5′ and 3′ exonucleolytic trimming that produce the mature extremities of the snoRNAs (43,56). The nascent snoRNAs are protected from further trimming by its co-transcriptional assembly with the four nucleolar proteins (2–3,13). In the case of C/D box snoRNAs, whether intronic or polycistronic, this process is initiated by binding of the 15.5 kd protein (Snu13p in yeast) to the K-turn motif formed by C and D elements and the terminal stem (2,57). Thus, the final level of the mature snoRNA is established by a kinetic competition between snoRNP assembly and exonucleolytic degradation on the nascent snoRNA (49). A similar process would occur in *O. tauri* which encodes conserved homologs to all 4 proteins forming the C/D and H/ACA boxes snoRNPs, respectively (Supplementary File 5).

The Ot–snoRNAs and their function in ribosome biogenesis

Most of Ot–snoRNAs were predicted to target modification of rRNA residues. In this context, the excessive expression of some of them, including the top 20 Ot–snoRNAs, is puzzling when compared to most of the other snoRNAs that also target the rRNAs (Figures 2B and 7).

Indeed, a similar observation has been reported for the human snoRNAome, revealing that in human cells the pool of snoRNAs is dominated by a few snoRNAs which are highly abundant (58). Remarkably, seven of the top 20 Ot–snoRNAs (Ot–HAsno1, Ot–HAsno20, Ot–HAsno23, Ot–CDsno44, Ot–CDsno46, Ot–HAsno100 and Ot–HAsno108) are homologous to some of those found in the list of the most abundant snoRNAs in mammals (58).

In humans, 110 residues are modified by *2′-O-ribose* methylation and 100 by pseudouridylation, targeted by snoRNAs. In yeast, with a very compact genome, 55 residues are modified by methylation and 44 by pseudouridylation (4). The number of rRNA nucleotides modified in any marine algal species is unknown, as they have not been mapped. However, the Ot–snoRNAs were predicted to target modification of 112 rRNA residues: 101 by methylation and 11 by pseudouridylation. The low number of pseudouridylated rRNA residues is likely not representative, as usually they are similar to the number of methylated residues. This is due to the low number of H/ACA snoRNAs that we identified, as we discarded several potential candidates because their structures were divergent from canonical features and snoGPS, which has stringent criteria, did not recognized them. Anyway, overall these results suggest that the level of rRNA modification in *O. tauri* is considerably higher than in yeast's rRNAs. Indeed, a similar result was predicted in *C. reinharditii*, where 74 C/D box snoRNAs were predicted to target 2′-O-ribose methylation of 96 rRNA residues (15) much higher than the 55 residues methylated in yeast rRNAs (4).

Considering these observations, one possibility is that differential accumulation of Ot–snoRNAs could contribute to ribosome heterogeneity in *O. tauri* cells. Differential expression of snoRNAs in different tissues, or responding to different stimuli, has been observed in higher eukaryotes (13,58). In addition, it has been shown that nucleotide modifications can modulate translation capacity and are an important source for ribosome heterogeneity (1). Furthermore, such rRNA modifications are highly responsive to environmental changes and in response to diseases (4). The scaRNAs targeting modifications of snRNAs, which direct splicing, also show this heterogeneity in their level of modification (59). The differential expression of snoRNAs that target rRNA might therefore be related to regulating the levels of different modifications in the ribosomes of *O. tauri* cells, thereby enabling reactivity to subtle changes in the marine environment.

Finally, an additional explanation would be that the highly abundant snoRNAs (predicted to target rRNAs or scaRNAs) might have additional functions, unrelated to rRNA or snRNA modifications (12,13). One example is human SNORD27, a C/D box snoRNA. This snoRNA has a dual function, targeting both rRNA methylation and regulation of alternative splicing in a pre-mRNA encoding a transcription factor (60). Another important function could be that, in addition to their canonical function, they could be precursor producing small RNAs with regulatory functions. This has been shown in human cells for some snoRNAs that produce miRNAs with regulatory functions (10,11). *Ostreococcus tauri* lacks Dicer and Ago homologs, and hence has no canonical miRNA pathways. However, we cannot exclude that certain small RNAs produced by a Dicer-independent processing of some Ot–snoRNAs, as reported in eukaryotes (12,61–62), might have a regulatory function.

The orphan Ot–snoRNAs

Similar questions arise concerning the role of the 17 orphan Ot–snoRNAs, for which no rRNA or snRNA predicted target was found. Ten of them are specific to *O. tauri*, while the seven others are found in other species of the class of Mamiellophyceae (Supplementary Table S6). Orphan snoRNAs have been found in several species, and could have functions unrelated to rRNA methylation or pseudouridylation. In the last few years, this was shown for some cases in human or in yeast. Some examples of new functions include targeting acetylation of rRNAs (6), regulation of mRNA alternative splicing (8,60) and control of 3′ end mRNA processing (63). Very recently, in human cells the orphan C/D box snoRD97 and SCARNA97 were found to target methylation of elongator $tRNA^{Met}$, despite their apparent intranuclear localization. Remarkably, this methylation prevented the site-specific cleavage of a $tRNA^{Met}$ which is induced by the stress-responsive endoribonuclease angiogenin, protecting $tRNA^{Met}$ integrity in response to stress (9).

Orphan Ot–snoRNAs that accumulate at significant levels could therefore have important functions in these marine algae, perhaps unrelated to rRNA or snRNA modifications. In particular, this could be the case of Ot–CDsno131, specific to *O. tauri*, which is very highly expressed (Supplementary Table S6).

In summary, the identification and characterization of the Ot–snoRNA family in *O. tauri* has revealed new fea-

tures of eukaryotic snoRNA expression that permit different levels of accumulation in a minimum of space, thus well adapted to its very small genome. The work presented here will provide a solid base that should be useful for assessing the impacts of environmental changes and viral infections on a major family of ncRNAs marine microalgae.

DATA AVAILABILITY

The sequence data for the RNA-seq analysis are available in the NCBI sequence read archive with the provisional accession BioProject number PRJNA624281.

SUPPLEMENTARY DATA

Supplementary Data are available at NARGAB Online.

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