Protist, Vol. 164, 748–761, September 2013
http://www.elsevier.de/protis
Published online date 28 August 2013

Protist

ORIGINAL PAPER

Signal Recognition Particle RNA in Dinoflagellates and the Perkinsid *Perkinsus marinus*

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Submitted March 24, 2013; Accepted July 23, 2013
Monitoring Editor: Frank Seeber

In dinoflagellates and perkinsids, the molecular structure of the protein translocating machinery is unclear. Here, we identified several types of full-length signal recognition particle (SRP) RNA genes from *Karenia brevis* (dinoflagellate) and *Perkinsus marinus* (perkinsid). We also identified the four SRP S-domain proteins, but not the two Alu domain proteins, from *P. marinus* and several dinoflagellates. We mapped both ends of SRP RNA transcripts from *K. brevis* and *P. marinus*, and obtained the 3′ end from four other dinoflagellates. The lengths of SRP RNA are predicted to be ∼260-300 nt in dinoflagellates and 280-285 nt in *P. marinus*. Although these SRP RNA sequences are substantially variable, the predicted structures are similar. The genomic organization of the SRP RNA gene differs among species. In *K. brevis*, this gene is located downstream of the spliced leader (SL) RNA, either as SL RNA-SRP RNA-tRNA gene tandem repeats, or within a SL RNA-SRP RNA-tRNA-U6-5S rRNA gene cluster. In other dinoflagellates, SRP RNA does not cluster with SL RNA or 5S rRNA genes. The majority of *P. marinus* SRP RNA genes array as tandem repeats without the above-mentioned small RNA genes. Our results capture a snapshot of a potentially complex evolutionary history of SRP RNA in alveolates.

Key words: Dinoflagellates; *Perkinsus marinus*; SRP RNA; protein translocation.

Introduction

Protein translocation to the endoplasmic reticulum in eukaryotes, to the thylakoid membrane in chloroplasts, and to the plasma membrane in Archaea and bacteria is mediated by signal recognition particles (SRPs) that bind to the signal peptide of the target protein in order to channel it to its destination. While SRPs in eubacteria consist of one polypeptide (Ffh) bound to an RNA molecule (4.5S or 6S), eukaryote SRPs are ribonucleoprotein complexes formed by one RNA molecule (7SL RNA or SRP RNA) and six distinct proteins (SRP9, SRP14, SRP19, SRP54, SRP68,

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http://dx.doi.org/10.1016/j.protis.2013.07.004

and SRP72) (Rosenblad 2005). The eukaryotic particle contains one S domain responsible for signal recognition, and one Alu domain responsible for translation elongation arrest. In SRPs, the RNA component provides the framework for the proper arrangement of the SRP proteins (Walter and Blobel 1983). Since the first discovery in mammalian cells (Walter and Blobel 1980, 1982), SRP components have been fully characterized for only a few phylogenetic groups. This is because some of the genes encoding these components, especially SRP RNA, share very low similarity in the primary sequences, and are highly diverse in structures. Since standard sequence analysis tools, such as BLAST, are largely ineffective at finding the highly variable SRP gene homologs, several studies (e.g. Regalia et al. 2002) have explored profile searches (PSI-BLAST, HMMER, INFERNAL) and secondary structure prediction methods (PSI-Pred) to investigate phylogenetic distributions of SRPs. The computational analysis has led to the discovery of SRP components in virtually all organisms for which complete or nearly complete genome sequences are available (Rosenblad et al. 2004). Those findings have led to the establishment of a SRP database (Rosenblad et al. 2003), and to a proposed new nomenclature for SRP RNA to standardize cataloging of the SRP components (Zwieb et al. 2005). Recently, Rosenblad et al. (2009) proposed that all the SRPs identified so far should be divided into seven groups: bacterial SRPs with a small (4.5S) SRP RNA, bacterial SRPs with a large (6S) SRP RNA, archaeal SRPs, fungal SRPs (Ascomycota), metazoan SRPs, protozoan SRPs, and plant SRPs.

Dinoflagellates are one of the three lineages of eukaryotes in the crown group Alveolata that also includes apicomplexans and ciliates (Fast et al. 2002). Globally distributed in the aquatic ecosystems, dinoflagellates are one of the most abundant groups of primary producers (50% of species) on the one hand, and micrograzers (50% of species) on the other, in the marine ecosystem. They also play a very important role in marine ecosystems because a group of symbiotic dinoflagellates (*Symbiodinium* spp.) are indispensable to the growth of coral reefs. In addition, diverse dinoflagellate species and populations proliferate to form harmful or toxic algal blooms, and dinoflagellates are the most important contributors of marine biotoxins.

As eukaryotes, dinoflagellates possess a number of bizarre genetic and cytological features (Hackett et al. 2004; Lin 2011). They have immense and wide-range genomes, 1-80 times the size of the human haploid genome (3-250 pg DNA; see Hou and Lin 2009 and references therein). Dinoflagellates divide with closed mitosis and extranuclear spindles, and their chromosomes are permanently condensed (Spector 1984). Extensive mRNA editing occurs to transcripts of dinoflagellate mitochondrial and chloroplast genes (Lin et al. 2002, 2008). mRNA *cis*-splicing is uncommon among dinoflagellates, and deviates from the universal GT/AG rule (Bachvaroff and Place 2008; Palmer 1996); but spliced leader RNA *trans*-splicing is widespread (Zhang et al. 2007). Until recently (Lin et al. 2010; Roy and Morse 2012), dinoflagellates were incorrectly thought to lack histones (e.g. Rizzo 2003), likely due to the low abundances of these proteins, and the functions of these proteins are still uncertain.

Perkinsus marinus is a pathogenic alveolate causing dermo disease in oysters in Atlantic estuaries of the western hemisphere from the USA to Brazil (da Silva et al. 2013; Villalba et al. 2004), as well as the Pacific Gulf of California (Cáceres-Martinez et al. 2012). Although its exact phylogenetic position is not yet entirely clear, our recent investigation on the spliced leader (SL) RNA gene structure, intron prevalence, and phylogenetic position led us to conclude that although the genus-*Perkinsus* clade shares commonalities with dinoflagellates, it is an independent alveolate lineage (Perkinsozoa) positioned between the phyla Apicomplexa and Dinoflagellata (Zhang et al. 2011).

To date, the molecular structure of the protein translocating machinery has not been studied in either dinoflagellates or perkinsids. In this study, we isolated SRP RNA homologs from several dinoflagellate species and identified four types of SRP RNA from the *P. marinus* genome project sequence. We also identified the four S-domain binding SRP protein genes from the *P. marinus* genome sequence and the existing dinoflagellate databases. By RNA blot, primer extension and rapid amplification of cDNA 3′ ends (3′ RACE), we mapped both ends of SRP RNAs in *K. brevis* and *P. marinus*, and the 3′ end of five other dinoflagellates. We also predicted the structures for the full-length SRP RNA sequences obtained.

Results

Detection of SRP RNA in Dinoflagellates and *P. marinus*

Total RNA from five dinoflagellates, *P. marinus*, and *Leishmania tarentolae* were analyzed