The novel RNase P in action

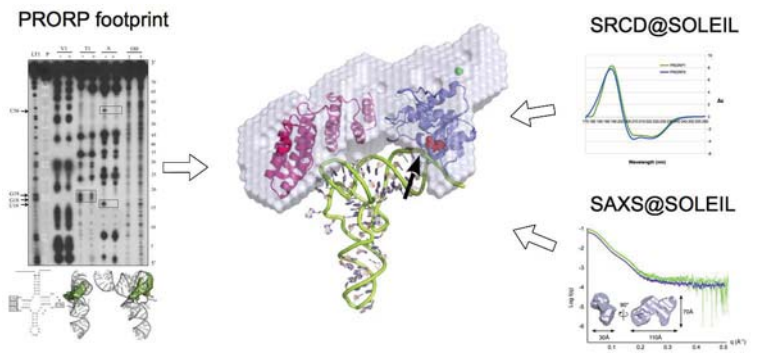
A novel type of RNase P was recently identified which is totally deprived of catalytic RNA. This proteinaceous RNase P (or PRORP) is found in the organelles of many eukaryotes and in the nucleus of some eukaryotes including plants. In order to characterize the architecture of PRORP enzymes and to determine how they bind to pre-tRNAs to perform their 5' maturation we combined biochemical and biophysical approaches. The resulting model of a functional PRORP:substrate complex suggests a tRNA recognition mode similar to that of the ribonucleoproteic RNase P.

5' tRNA maturation in mitochondria

Transfer RNAs (tRNAs) are key actors of protein synthesis: they play the role of adapter molecules during the translation of messenger RNAs by the ribosome into protein sequences. They are produced as precursors with leading and trailing sequences that need to be processed. Their 5' maturation is catalyzed by a ubiquitous enzyme called RNase P. Until recently all known RNase P were ribonucleoproteins, the catalytic activity of the enzyme being held by an RNA molecule. In 2008, a new type of RNase P only composed of proteins was identified in human mitochondria [1] that corresponds to a novel family of nucleases called PRORP for "Proteinaceous RNase P". The group of Philippe Giegé (Institut de Biologie Moléculaire des Plantes, IBMP, Strasbourg) demonstrated that the model plant *Arabidopsis thaliana* possesses three PRORP proteins. PRORP1 is localised in both mitochondria and chloroplasts whereas PRORP2 and PRORP3 are active in the nucleus [2,3]. A collaboration was initiated between two neighbouring institutes in Strasbourg (IBMP and IBMC) to examine these enzymes from *A. thaliana* and a combination of biochemical and biophysical approaches was used to gain a first structural and functional insight into tRNA recognition and maturation by PRORPs.

PRORP: an integrated structural study

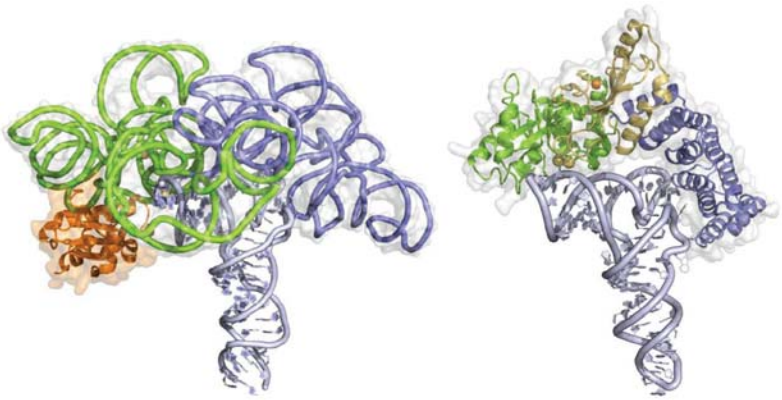
PRORP sequences are characterized by the presence of pentatricopeptide repeat (PPR) motifs and a metallonuclease domain proposed to hold the catalytic center. Because no structure of a close homologue was known at the time we started this study, comparative modeling was carried out on separate domains. We then performed synchrotron radiation circular dichroism (SRCD) on the DISCO beamline to validate the models based on their 2D structure content and to test the conformational stability of PRORP samples prior to further investigations. The presence of a zinc binding motif between the two main domains was demonstrated by site directed mutagenesis of putative zinc chelating residues in association with inductively coupled plasma mass spectrometry. Small angle X-ray scattering (SAXS) data collected on the SWING beamline confirmed the two domain organization of PRORPs and helped place them with respect to each other (Figure ❶).



❶ A first glance at a PRORP:tRNA complex. PRORP2 of *A. thaliana* was built by comparative modelling guided by SRCD and SAXS data, and the resulting model was docked onto a pre-tRNA substrate based on footprint analysis. The RNA cleavage position is indicated by an arrow.

Probing PRORP:tRNA interface

To position PRORP on its RNA substrate, the latter was subjected to RNase digestion in the presence of the enzyme. The protection footprint (Figure ❶) defined the interaction interface and the PRORP enzyme was docked accordingly onto the 3D structure of a pre-tRNA. This model of the maturation complex reveals that eukaryotes have evolved PPR proteins to recognize pre-tRNAs in a similar way as the ribonucleoproteic RNase P reminiscent from the ancient *RNA world* (Figure ❷). Although the scenario of this convergent evolution remains to be established, as well as the precise catalytic mechanism of tRNA maturation, this study is a first step towards the detailed characterization of the PRORP family.



❷ Classical ribonucleoproteic RNase P (left, PDB id: 3Q1R) and PRORP2 (right, model based on PDB id: 4G26) share the same pre-tRNA binding mode [4].

SWING & DISCO beamlines

ASSOCIATED PUBLICATION

Structural insights into protein-only RNase P complexed with tRNA

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