

## Structure, function and evolution of armless mitochondrial tRNAs

Transfer RNAs (tRNAs) play an important role in protein biosynthesis. They are key partners in the ribosome-translation machinery, where they deliver the cognate amino acid to the growing peptide chain. tRNAs have a widely conserved structure. The secondary structure resembles a cloverleaf, which is composed of 4 arms: the amino acid accepting arm, the D-arm, the anticodon arm and the T-arm. The tertiary structure resembles the letter L. The secondary and tertiary structures are recognized by numerous maturation and modification enzymes as well as specific aminoacyl-tRNA synthetases that charge the tRNAs with their cognate amino acid.

In eukaryotes the tRNA maturation and aminoacylation processes are not restricted to the nucleus but are also found in organelles such as chloroplasts and mitochondria. Mitochondria encode an additional set of tRNAs on their own genome and possess their own translation machinery.

Many mitochondrial tRNAs have a reduced size and typical structural elements are partial or completely lost. An extreme case of these minimalized tRNAs was found in the mitochondria of nematodes. These newly discovered tRNAs are missing both the D-arm and the T-arm. They were therefore called "armless" tRNAs. The roundworm *Romanomermis culicivorax* is a typical representative that possesses such armless tRNAs.

The aim of this work is the characterization of armless tRNAs. In particular, their structural properties and their functionality, in regard to their interaction with partner proteins, such as the tRNA nucleotidyltransferase (CCA-adding enzyme) and an aminoacyl-tRNA synthetase (aaRS), were investigated. First, the structure of two tRNAs was analyzed by means of enzymatic and chemical probing, nuclear magnetic resonance spectroscopy (NMR), and small angle X-ray scattering (SAXS). The results of these analysis show that armless tRNAs form a hairpin-like secondary structure composed of two helices with classical Watson-Crick base pairs. The helices are separated by a central bulge that does not contain any specific internal tertiary base pairings. Due to the designed 3D structure, we suppose an increased intrinsic flexibility of armless tRNAs which may facilitate interaction with partner proteins.

In this study, two recombinant proteins from *R. culicivorax*, the CCA-adding enzyme and the mitochondrial ArgRS, were first expressed and purified. Sequence and structure of both proteins resemble more their eukaryotic than their bacterial homologues, especially those from other nematodes. However, we were also able to detect structural changes for both enzymes, which could indicate an adaptation to armless tRNAs.

The results show that the CCA-adding enzyme recognizes and processes both armless and classical tRNAs. Aminoacylation tests were performed with the purified ArgRS enzyme. However, the enzyme did recognize neither armless tRNAs nor classical tRNAs as substrates under the tested conditions.