Structural study of a tRNA maturase Projet codes : 30 01 675 and 30 01 718

Transfer RNAs are synthesized as part of longer primary transcripts that require processing of both their 3' and 5' extremities in every living organism known. The 5' side is matured by the quasi-universally conserved endonucleolytic ribozyme, RNase P, while removal of the 3' tails can be either exonucleolytic or endonucleolytic. The endonucleolytic pathway is catalysed by an enzyme known as RNase Z, or 3' tRNase. RNase Z is found in the vast majority, if not all, eukaryotes and archaea and in about half of the sequenced bacteria. This tRNA maturase is an essential protein in many of the species tested and mutations in the equivalent gene in humans, *ELAC2*, have been linked to prostate cancer susceptibility. RNase Z cleaves tRNA precursors lacking an encoded CCA motif immediately 3' to the discriminator base (Pellegrini et al., *EMBO J*., 2003). The products of the cleavage reaction are a 3'-matured tRNA ending in the hydroxyl group of the discriminator base and a trailer sequence with a 5' phosphate .

X-ray data were collected from cryo-cooled crystals from a SeMet derivative of native RNase Z and from a complex between a mutant form of RNase Z and $tRNA^{Thr}$ Multiwavelength anomalous diffraction data from the SeMet derivative and data from the complex were collected using a MarCCD detector at beam-line BM30A of the ESRF.

We solved the crystal structure at 2.1 Å resolution of seleno-methionine labelled RNase Z from *Bacillus subtilis (*Li de la Sierra-Gallay I *et al., Nature.,* 2005 *)* by MAD. The structure consists of a dimeric core of metal-dependent β-lactamase domains, each with a protruding flexible arm, for which we proposed a role in tRNA binding. Indeed, deletion of the entire flexible arm of the *E. coli* ortholog of RNase Z (ElaC or RNase BN) was subsequently shown to result in a loss of tRNA binding, without affecting the enzyme's phosphodiesterase activity . In the absence of substrate, the two subunits of RNase Z are not equivalent; only one, the A-subunit, has an active site in which a pair of zinc ions are correctly coordinated for catalysis . Although it was possible that the enzyme only cleaved one tRNA per dimer, mimicking the situation thought to occur in longer forms of RNase Z, the allosteric properties of the short form of the enzyme were better explained by a model in which binding of tRNA led to a conformational change that activated the catalytic site of the B-subunit. Our recently resolved structure of the complex between a mutant form of RNase Z and $tRNA^{Thr}$ at 2.9 Å resolution provide evidence in favor of the model where two tRNA precursors are cleaved per dimer *(*Li de la Sierra-Gallay *et al*. 2006 *soumis*).

References

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