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Review

tRNase Z: the end is not in sight

B. Späth, G. Canino and A. Marchfelder*

Molekulare Botanik, Universität Ulm, Albert-Einstein-Allee 11, 89069 Ulm (Germany),
Fax: +49-731-5022626, e-mail: anita.marchfelder@uni-ulm.de

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Abstract. Although the enzyme tRNase Z has only recently been isolated, a plethora of data has already been acquired concerning the enzyme. tRNase Z is the endonuclease that catalyzes the removal of the tRNA 3′ trailer, yielding the mature tRNA 3′ end ready for CCA addition and aminoacylation. Another substrate cleaved by tRNase Z is the small chromogenic phosphodiester bis(*p*-nitrophenyl)phosphate (bpNPP), which is the smallest tRNase Z substrate known so far. Hitherto the biological function as tRNA 3′-end processing enzyme has been shown only in one prokaryotic and one eukaryotic organism, respectively. This review summarizes the present information concerning the two tRNase Z substrates pre-tRNA and bpNPP, as well as the metal requirements of tRNase Z enzymes.

Keywords. tRNase Z, metallo-β-lactamases, bpNPP, tRNA processing, ribonuclease.

The tRNase Z enzymes

The generation of an accurate tRNA 3′ end is vital for CCA addition and subsequent aminoacylation (Fig. 1). In many bacteria, most eukaryotes and all archaea tRNA 3′-end processing is catalyzed by the endonuclease tRNase Z, which cleaves the phosphodiester directly 3′ to the discriminator nucleotide (the discriminator is the unpaired base immediately preceding the CCA motif) leaving a 3′ hydroxyl group on the tRNA 3′ end and a 5′ phosphate on the trailer. tRNase Z proteins exist in two forms, a long form of 750–930 amino acids only present in eukarya (tRNase Z^L) and a short form of 280–360 amino acids (tRNaseZ^S), which is present in all three domains. The N-terminal part of the long proteins usually contains a target sequence that potentially routes the protein to mitochondria or chloroplasts. The C-terminal part of the long tRNase Z enzymes has high sequence similarity to the short tRNase Z proteins and contains the conserved His motif. In addition, there is a low sequence similarity between the N-terminal part of the long tRNase Z enzymes and the short tRNase Z proteins, with the N-terminal part containing a His motif but a pseudo-His motif [1]. Because of these sequence similarities, it was proposed that the gene for the long tRNase Z enzymes evolved by duplication of the short tRNase Z gene [1]. This proposal is indirectly corroborated by the fact, that all short tRNase Z enzymes are active as homodimers [2–5].

The structures for three bacterial tRNase enzymes (*Bacillus subtilis, Escherichia coli* and *Thermotoga maritima*) have been solved [2, 3, 6]. All three structures show the same basic metallo-β-lactamase fold with a core of two seven-stranded β-sheets flanked on each side by three α-helices.

Former reviews have given a general overview of the tRNase Z family [7, 8] and a recent one deals with the bacterial tRNase Z enzymes and the latest structure models [9]. Therefore, here, we summarize recent data

* Corresponding author.

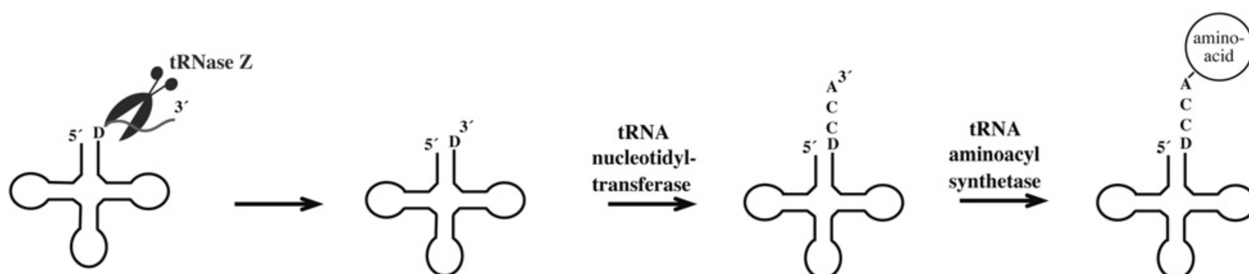

Figure 1. Maturation of tRNA precursors in which CCA is not encoded. Precursor tRNAs are transcribed with additional sequences 5′ and 3′ of the tRNA, which have to be removed to yield a functional tRNA. The tRNA 3′ end is generated by the endonuclease tRNase Z. After maturation of the tRNA 3′ end, the CCA sequence is added by tRNA nucleotidyltransferase. Some eukaryal and archaeal tRNA genes contain introns, which are removed by the splicing endonuclease (not shown). Several nucleotides of the tRNA have to be modified before the functional tRNA is complete. Subsequently, the amino acid can be added to the last nucleotide of the CCA sequence by the respective aminoacyl tRNA synthetase and the aminoacyl tRNA is then ready to engage in protein biosynthesis (D: Discriminator nucleotide, the nucleotide 5′ to the CCA sequence).

concerning the metal requirements as well as recent results concerning the two substrates of this enzyme: precursor tRNAs (Fig. 1) and the small phosphodiester bis(*p*-nitrophenyl)phosphate (bpNPP) (Fig. 2).

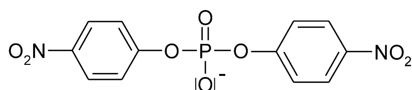

Figure 2. Structure of the small phosphodiester bis(*p*-nitrophenyl)phosphate (bpNPP), which is the smallest tRNase Z substrate identified so far.

tRNase Z enzymes belong to the metallo-β-lactamase family

tRNase Z enzymes belong to the superfamily of metallo-β-lactamases. The unifying character of this family is the "β-lactamase" fold, which consists of a α-β/β−α structure. In addition to this structural fold, all metallo-β-lactamases contain five motifs; these are highly conserved in active members of the superfamily and participate in metal coordination and the hydrolysis reaction: motif 1 consists of an aspartate, and motif 2 is typified by HxHxDH (only the first histidine and the aspartate are invariant); these first two motifs are located at the end of two β-strands. Motif 3 (histidine), motif 4 (aspartate) and motif 5 (histidine) are also found at the end of β-sheets (note that not all positions of the five motifs are invariant) [10].

The conservation of the structural fold and the metal coordinating framework suggest the presence of a unifying catalytic mechanism for a whole range of hydrolytic reactions. The metallo-β-lactamase fold represents an ancient conserved structure that forms the basis of several catalytic activities in all the three domains and functions both as an active enzyme as well as in structural and regulatory roles devoid of enzymatic activity. It was first described for metal-containing β-lactamases, hydrolytic enzymes that confer bacterial resistance to β-lactam antibiotics [11]. Members of this protein superfamily possess a wide variety of substrates. Most of the substrates have an ester linkage and a negative charge in common. They include among others class B β-lactamases (substrate: lactams), glyoxalase II (substrate: *S*-D-lactoylglutathiones), aryl sulfatases (substrate: aryl sulfates), cytidine monophosphate-*N*-acetyl neuraminic acid (CMP-NeuAc) hydrolases (substrate: cytidine monophospho-*N*-acetylneuraminate), cAMP phosphodiesterases (substrate: cAMP), and PhnP protein (substrate: phosphonate derivates, involved in phosphonate metabolism). A separate subgroup within the metallo-β-lactamase family is the β-CASP family consisting solely of proteins that interact with nucleic acids (see below). Although the tRNase Z enzymes also act on nucleic acids they are not part of this group but have been grouped together with PhnP proteins because of sequence similarities [10].

One feature providing the broad substrate spectrum of this enzyme family is the addition of new domains to the metallo-β-lactamase domain as seen in the mRNA 3′-end processing endonuclease CPSF-73 (cleavage-polyadenylation specificity factor 73), where the β-CASP domain is attached to the metallo-β-lactamase domain. The metallo-β-lactamase domain contains the catalytic site and the β-CASP domain seems to mediate the substrate specificity. A similar arrangement has been found in the tRNase Z enzymes. Next to the metallo-β-lactamase domain they contain a domain unique for the tRNase Z enzymes, the exosite. Exosite is a term that describes a protein element outside the active site that participates in substrate binding. In the tRNase Z enzymes, the exosite is a flexible arm that protrudes from the main protein

body. The exosite is involved in pre-tRNA binding but separated from the site of hydrolysis.

Nucleic acid interacting metallo-β-lactamases: the β-CASP enzymes

The β-CASP subfamily (named for metallo-β-lactamase, CPSF, Artemis, Snm1, Pso2) groups enzymes that interact with nucleic acids. All members of this subgroup contain the β-CASP domain next to the metallo-β-lactamase domain. Artemis [12], Snm1 and Pso2 [13] are involved in DNA repair, while CPSF-73 is the endonuclease cleaving mRNA 3′ ends to allow subsequent polyadenylation. Alignment of β-CASP enzymes revealed three conserved motifs. Motif A is characterized by an acidic amino acid (D or E) after a stretch of hydrophobic residues typical of a β-strand structure. Motif B includes a histidine at the end of an amphiphilic β-strand structure followed by an α-helical structure. C-terminal to this last α-helix, and at the end of another predicted β-strand, a conserved histidine (motif C) can be found in all the sequences of the β-CASP family. Exceptions are several sequences including those of the Artemis/SNM1/PSO2 group in which this histidine is most often substituted by a valine. The motif C histidine is conserved in CPSF-73 proteins and in almost all CPSF-100 proteins and thus may play an important role in the specificity towards RNA targets. The three conserved polar amino acids are all located at the end of predicted β-strands, like all of the zinc-binding residues of canonical metallo-β-lactamases.

RNA processing metallo-β-lactamases: the CPSF proteins

The only other RNA processing enzyme of the metallo-β-lactamase family identified so far besides the tRNase Z enzymes is the CPSF-73 protein (Fig. 3). Eukaryotic messenger RNA precursors undergo cleavage and polyadenylation at the 3′ end, and recent analyses showed that the CPSF protein catalyses mRNA 3′ cleavage [14–17]. The recently solved structure of CPSF-73 shows two domains, a metallo-β-lactamase domain and a β-CASP domain. The N-terminal sequence (amino acids 1–208) as well as part of the C-terminal sequence (amino acids 395–460) form the metallo-β-lactamase domain [14]. A similar arrangement is found in the tRNase Z enzymes, suggesting that this structure is unique to the RNA interacting enzymes. Residues between the N-terminal (1–208) and C-terminal domain (395–460) concur with the β-CASP domain. Both proteins have a very high affinity for Zn^{2+} ions ([14], and Späth and Marchfelder, unpublished). In addition, CPSF-73 and tRNase Z have a similar zinc-binding mode that differs from the binding mode of canonical metallo-β-lactamases [14]. The tRNase Z proteins have been grouped with PhnP proteins only by their sequence similarities [10]. Since alignment of parts of the CPSF-73 and tRNase Z proteins shows a sequence similarity of 35% (Fig. 3) and because of the structural and functional similarities between the β-CASP enzyme CPSF-73 and the tRNase Z enzymes, we propose that both enzymes should be placed in the same subgroup. If functional aspects are used to generate subgroups in the metallo-β-lactamase family, all nucleic acid interacting proteins should be assigned to one subgroup. Thus Artemis, SNM1, PSO2, CPSF-73 and the tRNase Z proteins could be put into the nucleic acid interacting protein group (or short NAIP).

Metal cofactors of tRNase Z

Classically the metallo-β-lactamase proteins are defined to require one to two Zn^{2+} ions per monomer for full activity, but there are some members of the family that require other metal ions for the catalytic activity. Glyoxalase II (glxII) for example utilizes zinc, iron and manganese ions in various ratios [18, 19]. The redox enzymes rubredoxin-oxygen oxidoreductase (ROO) [20] and flavorubredoxin (FIRd) [21] contain a bi-iron site.

Mutational studies identified key residues of the zinc site

Mutational studies concerning the binuclear metal binding site were performed with the *E. coli* tRNase Z (EcoTrz) [22, 23], *T. maritima* tRNase Z (TmaTrz) [24], *Drosophila melanogaster* tRNase Z^L (DmeTrz^L) [25] and *Arabidopsis thaliana* tRNase Z (AthTRZ^{S1}) [26].

Substitution of any of the metal ligands significantly decreases the catalytic rate for all residues, confirming the hypothesis that there is an absolute requirement of an intact metal binding site for activity towards pre-tRNA [24–27] and bpNPP [23]. None of AthTRZ^{S1} variants mutated in the potential metal binding residues were able to process pre-tRNAs. Only the bridging AthTRZ^{S1}-D185G variant showed weak activity compared to the wild-type enzyme [26]. Extending these results, Späth et al. found that none of the metal-binding mutants were able to hydrolyze the artificial substrate bpNPP (Späth and Marchfelder, unpublished). Additionally, it was observed

Figure 3. Alignment of the conserved metallo-β-lactamases motifs (motif I–V) from human cleavage-polyadenylation specificity factor 73 (CPSF-73) and the two human tRNase Z enzymes, tRNase Z^S and tRNase Z^L. Identical amino acids are shaded in black, similar residues in gray.

that the pre-tRNA cleavage activity and bpNPP activity of the 20 different $AthTRZ^{S1}$ variants analyzed did not always correlate. Of 12 $AthTRZ^{S1}$ variants without bpNPP activity, 4 still mature pre-tRNA to some extent, leading to the suggestion that different amino acids are important for tRNA processing and bpNPP cleavage or alternatively that some of these amino acids are of importance for adjusting the correct bpNPP position in the active site (Späth and Marchfelder, unpublished).

A striking example for the different behavior towards the two substrates bpNPP and pre-tRNA is the $AthTRZ^{S1}$ variant R252G. This variant processes pre-tRNA with only 26 % of the wild-type activity, but shows a five times better affinity and tenfold higher turnover rate towards bpNPP than the wild-type enzyme (Späth and Marchfelder, unpublished). Co-crystallization of BsuTrz together with tRNA showed that amino acid R252 (BsubTrz: R273) acts as a bridge between tRNA and enzyme, proposing that this arginine is the best candidate for sensing the presence of the tRNA in the dimer and initiating the conformational changes to stabilize this region [2].

Another interesting observation concerning the metal requirement of the tRNase Z protein is the rescue of metal-depleted $AthTRZ^{S1}$ variants by the addition of Mn^{2+}. Whereas $AthTRZ^{S1}$ variants H133L and D185G are inactive in both pre-tRNA processing and bpNPP cleavage in the presence of Mg^{2+}, they regain their pre-tRNA processing activity, but not the bpNPP activity, by addition of Mn^{2+} (Späth and Marchfelder, unpublished). It is possible that these variants do not have a properly folded catalytic site and that, upon addition of manganese, the catalytic site rearranges in such a way that at least pre-tRNA processing can occur. A similar Mn^{2+} rescue was shown for TmaTrz variants [27]. However, the metal content of TmaTrz has not been determined at any point and the enzyme was not metal depleted before the addition of these metal ions. Therefore, it is not clear whether the recombinant TmaTrz isolated from *E. coli* already contained some of the metal ions required for activity.

There is only one non-metal ligating residue close to the HXHXDH motif that significantly affects the catalytic rate when substituted, S521 in $DmeTrz^L$ [25] (T62 in BsuTrz, Fig. 4). This residue precedes the first histidine in the His motif. In this position the hydroxyl group presented by Ser or Thr is conserved throughout the entire metallo-β-lactamase family [10]. Ser or Thr seem to act as a second-shell ligand of one of the metal ions influencing the ligand-metal interaction [28]. Another conserved residue outside of the His motif is also essential for processing, D25 in TmaTrz (D37 in BsuTrz) [24]. In the crystal structure of BsuTrz, D37 is central to an H-bond network in the second shell around the metals and forms H bonds to

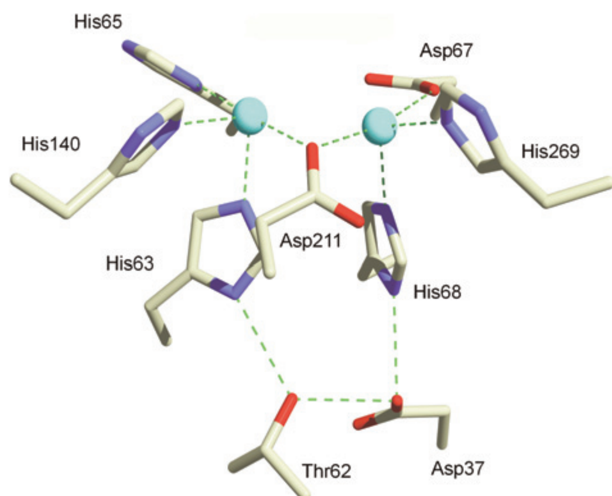

Figure 4. The metal binding site of the *B. subtilis* tRNase Z (BsuTrz). The model is drawn according to the crystal structure of the enzyme [2]. The Zn^{2+} ions are shown as gray spheres. The metal binding site 1 is shown at the left with His65, His140 and His63, binding site 2 is at the right with Asp67, His269 and His68. The zinc coordination bonds and the H bonds are depicted as dashed lines. Thr62 and Asp37 serve as potential second shell ligands.

T62 and the metal ligand H68; D37 is a residue in a disallowed Ramachandran region. In addition, T62 forms an H bond to the metal ligand H63. This H-bond network thereby stabilizes the metal coordination and electronically affects the metal atoms, explaining the importance of this residue [7].

Metal requirements of tRNase Z

The first tRNase Z enzyme analyzed in detail regarding its metal content was EcoTrz. The enzyme was shown to be strictly dependent on zinc ions [5]. No other physiologically relevant divalent ion was able to restore the bpNPP cleavage activity of the metal-depleted enzyme [5]. Another prokaryotic tRNase Z enzyme, BsuTrz, contains two Zn^{2+} ions in its active site [2], whereas TmaTrz shows a coordination of one Zn^{2+} ion (Table 1) [3].

Metal analysis of metal-depleted *A. thaliana* tRNase Z (AthTRZ^{S1}) revealed that 0.8 Zn^{2+} ions are still bound per monomer [29]. Thus, the enzyme itself has a very high affinity for Zn^{2+} since the chelators tetrakis-(2-pyridylmethyl)ethylenediamine (TPEN) and EDTA are not able to remove the residual zinc ions. A similar observation was made for the human cleavage–adenylation specificity factor CPSF-73. Two zinc ions are present in the active site of CPSF-73 even without the addition of zinc to the crystallization solution, indicating that this site might possess an extremely high affinity for zinc ions that makes it impossible to remove it [14].

Table 1. Overview of the metal ion content in the active site of metallo-β-lactamases. Only proteins, for which the metal content was determined by the electron density of the crystal structure of these proteins, are shown. The short tRNase Z enzymes are homodimers and thus contain two active sites, one in each monomer.

Enzyme	Metal ions/active site	Reference
BsuTrz	2 Zn^{2+} (only in monomer A)	[2]
EcoTrz	2 Zn^{2+} (in each monomer)	[6]
TmaTrz	1 Zn^{2+} (in each monomer)	[3]
ROO	2 Fe^{2+}	[20]
CPSF-73	2 Zn^{2+}	[14]
AHL-lactonase	2 Zn^{2+}	[51, 52]

ROO: rubredoxin-oxygen oxidoreductase, AHL-lactonase: *N*-acyl-L-homoserine lactone hydrolase.

The pre-tRNA-processing activity of the chelator-treated AthTRZ^{S1} enzyme (which still contains zinc ions but is not active on pre-tRNAs) could be rescued by the addition of Mg^{2+}, Mn^{2+} or Ca^{2+} [4, 26]. These analyses of the tRNase Z enzymes reveal a metal binding site that is typical for transition metal incorporation and clearly differs from the binding sites of magnesium-binding proteins. The metal-binding site 1 of tRNase Z is composed of three histidine residues, whereas the second binding site consists of two His and one Asp (Fig. 4). An additional Asp serves as a bridging ligand (Fig. 4). In contrast, the magnesium-binding site is characterized by a higher carboxylate content and the Mg^{2+} ion is coordinated to six ligands. Therefore, the main role of Mg^{2+} and Ca^{2+} might be the stabilization of the pre-tRNA and the mediation of a correct positioning of the substrate to the tRNase Z enzyme. Manganese can either replace magnesium or can be used for hydrolysis, as already shown for glxII [18]. It is possible that Mg^{2+} and Ca^{2+} are not directly involved in catalysis by binding to the metal binding site, because the bpNPP activity of chelator-treated AthTRZ^{S1} is not improved by the addition of these metal ions, whereas Zn^{2+} and Mn^{2+} increase the activity three- or fivefold, respectively [29]. On the other hand, different reaction mechanisms of pre-tRNA processing and bpNPP hydrolysis could also explain the different metal requirements. While we now have relevant data about the nature of the metal ion required for tRNase Z activity, we still do not know how many metal ions are bound and how many are required for full enzymatic activity. Wommer et al. [30] reported that dissociation constants (K_D) for Zn^{2+} binding of metallo-β-lactamases are substrate dependent, but tRNase Z enzymes were not included in this study. For several metallo-β-lactamases the presence of substrates induces a decrease of K_D for binding of the first metal ion, whereas the K_D for

binding of the second metal ion increases. These results led to the suggestion that the mono-zinc enzymes are the physiologically relevant species in the presence of substrates, whereas the metal-free state might dominate for some of the enzymes in the absence of substrates [30]. The K_D for binding of Zn^{2+} ions to L1, a metallo-β-lactamase of the pathogenic strain *Stenotrophomas maltophilia,* are similar and led to the conclusion that L1 is active in its binuclear state [31]. This conclusion was confirmed in the same year by the observation that L1 indeed required both Zn^{2+} for full activity [32].

Zinc isothermal titration calorimetry (ITC) and zinc activity titration of apo-EcoTrz suggested full bpNPP cleavage activity with an average zinc content of one zinc ion per monomer [21]. The homodimeric Trz enzymes can display two binding modes for one metal ion per monomer (*i.e.*, two metal ions per dimer): either one subunit binds both metal ions or each subunit has one metal ion bound. The former case is true for the *B. subtilis* tRNase Z, as shown by the crystal structure in which two zinc ions were found in the A subunit [2]. The latter case has been found in TmaTrz, where each subunit contains one Zn^{2+} ion, as shown by crystallography [3].

It is as yet not clear how the single metal ion is positioned in the binuclear binding site since it could bind either to site 1 or site 2 (Fig. 4). Several studies showed that the single metal ion is distributed between both binding sites [33–35] but the functional role of metal ion translocation in mononuclear metallo-β-lactamase enzymes still remains to be resolved [36]. Thus, to date, there are still a lot of open questions about the nature and position of metal ions and the catalytic mechanism in metallo-β-lactamase-proteins and especially in tRNase Z enzymes.

Present data for the tRNase Z enzymes clearly show that they are metal dependent [5, 26, 29] and, depending on the kind of substrate cleaved, either Zn^{2+} [5, 29] and Mn^{2+} can increase the cleavage activity [29] (for bpNPP), or Zn^{2+} and Mg^{2+}, Ca^{2+} or Mn^{2+} ([26], Späth and Marchfelder, unpublished data) are required (for pre-tRNA processing), respectively. The proposed role of the metal ion for tRNase Z enzyme function is predominantly to polarize the phosphate group of the substrate [2].

Differences between tRNase Z enzymes from different domains and organisms

After the identification of the first tRNase Z genes in *A. thaliana* ($AthTRZ^{S1}$ and $AthTRZ^{S2}$) and *Methanocaldococcus jannaschi* (MjaTrz) in 2002 [37], the search for orthologous proteins in other organisms showed that these proteins are distributed in all three domains of life. tRNase Z is found in all Eukaryotes and Archaea sequenced so far, and is widely distributed among Bacteria. Archaeal and bacterial organisms encode only a single short tRNase Z protein. The only exception is the archaeon *Haloquadratum walsbyi* for which three tRNase Z homologs have been reported [38]. Eukaryotes code for at least one long tRNase Z (as in *Saccharomyces cerevisiae* and *D. melanogaster*) but one long and one short tRNase Z enzymes (*Homo sapiens*), or two long and two short proteins (*A. thaliana*) have also been found (Table 2). The short tRNase Z proteins are active as homodimers, whereas the long ones act as monomers. All recombinant tRNase Z enzymes characterized so far show *in vitro* tRNA precursor-processing activity [22, 24, 37, 39–43] and, generally, the enzyme cleaves the precursor immediately after the discriminator base. However, differences were found in respect to pre-tRNAs containing the CCA motif (Table 3). Whereas $AthTRZ^{S1}$ and MjaTrz recombinant enzymes are not inhibited by the CCA motif [43], the overexpressed enzymes from *Synechocystis* and *B. subtilis* do not process CCA-containing tRNA precursors at all. For *B. subtilis*, the *in vitro* results are in accordance with the *in vivo* observations that, upon repression of the BsuTrz gene, only CCA-less tRNA precursors accumulate [39]. A unique situation has been described for *T. maritima.* In this organism, all tRNA genes with one exception contain the CCA motif. *In vitro* analysis with the recombinant TmaTrz showed that the enzyme can process both forms of the tRNA precursors [24]. Mutational analysis on TmaTrz indicated two residues that could be involved in the recognition of the cleavage site (S31 and T33 in TmaTrz, [24]). According to the recent crystal structure of TmaTrz, these two residues are located at the dimerization interface and are surrounded by amino acids that differ from the well-conserved residues in the corresponding region in the other tRNase Z proteins. The authors conclude that the different amino acids involved in dimer formation could be correlated with the difference in the cleavage site typical of TmaTrz [3]. Reactivity towards the small phosphodiester bpNPP also differs from enzyme to enzyme (see also *Functions of the tRNase Z enzymes* below).

Functions of the tRNase Z enzymes

The majority of the data acquired on tRNase Z originates from *in vitro* experiments. All recombinant long and short tRNase Z enzymes tested so far were found to process tRNA 3′ ends *in vitro* [22, 24, 37,

Table 2. Distribution of short and long tRNase Z enzymes in eukaryotic organisms. In prokaryotes only short tRNase Z enzymes are found, whereas eukaryotes contain long and short tRNase Z proteins. Within the eukaryotic domain the distribution of long and short tRNase Z enzymes varies. Eukaryotic organisms have at least one long tRNase Z protein (like baker's yeast). A comprehensive analysis of the distribution of tRNase Z proteins in bacteria and archaea can be found in [50].

Organism	tRNase Z short	tRNase Z long	References
Schizosaccharomyces pombe	Not encoded	$SpoTrz^L$: NP_595514	[53]
Saccharomyces cerevisiae	Not encoded	$SceTrz^L$: NP_013005	[54]
Neurospora crassa	Not encoded	$NcrTrz^L$: XP_957703	[55]
Caenorhabditis elegans	Not encoded	$CelTrz^L$: NP_001023109	
Drosophila melanogaster	Not encoded	$DmeTrz^L$: AAF99588	[49]
Gallus gallus	Not encoded	$GgaTrz^L$: XP_415584	
Danio rerio	$DreTrz^S$: NP_001003503	$DreTrz^L$: AAH98612	
Arabidopsis thaliana	$AthTRZ^{S1}$: AAG52354 $AthTRZ^{S2}$: AAD25827	$AthTRZ^{L1}$: AAL49818 $AthTRZ^{L2}$: BAC41975	[37]
Mus musculus	$MmuTrz^S$: AAG24919	$MmuTrz^L$: CAI24609	[1]
Homo sapiens	$HsaTrz^S$: AAG24917	$HsaTrz^L$: AAG24441	[1]

Table 3. Comparison of the *in vitro* processing activities of different recombinant tRNase Z enzymes with respect to CCA-containing and CCA-less pre-tRNAs, respectively.

tRNA precursor species	$AthTRZ^{S1}$	MjaTrz	SynTrz	BsuTrz	TmaTrz
CCA-less	Processing 3′ to the discriminator	Processing 3′ to the discriminator	Processing 3′ to the discriminator	Processing 3′ to the discriminator	Processing 3′ to the discriminator
CCA-containing	Processing 3′ to the discriminator	Processing 3′ to the discriminator	No processing	No processing	Processing 3′ to the CCA

Ath: *Arabidopsis thaliana*, Mja: *Methanocaldococcus jannaschi*, Syn: *Synechocystis* sp PCC6803, Bsu: *Bacillus subtilis,* Tma: *Thermotoga maritima*, Trz: tRNase Z.

39–43]. The long and the short form of the tRNase Z proteins behave differently towards the second standard *in vitro* substrate, the chromogenic molecule bpNPP. Whereas all long tRNase Z enzymes tested so far do not show any activity towards this substrate, all short tRNase Z proteins cleave bpNPP but with different efficiency. The *E. coli* enzyme displays the highest activity towards this substrate [5], similar to the *B. subtilis* tRNase Z (Späth and Marchfelder, unpublished). Considerably lower bpNPP hydrolytic activity was found with the plant tRNase Z^{S1} enzyme ([4], and Späth and Marchfelder, unpublished), and the archaeal enzymes (HvoTrz and PfuTrz) have almost no bpNPP activity [4].

Hitherto the biological function of the tRNase Z enzyme has only been analyzed for two organisms, *B. subtilis* [39] and *D. melanogaster* [41], and has been shown to be tRNA 3′-end processing. Repression of the *B. subtilis* tRNase Z gene results in accumulation of CCA-less tRNA precursors, while CCA-containing tRNA precursors do not accumulate [39]. Knock down of the tRNase Z gene in *D. melanogaster* results in accumulation of 3′ unprocessed pre-tRNAs in the nucleus as well as in mitochondria. *Drosophila* contains only a single tRNase Z gene that codes for a long version. According to the prediction of the respective sorting servers, the N-terminal part of this long protein contains a target sequence for mitochondria, which fits nicely with the observation that mitochondrial tRNA 3′ processing is impeded in the RNAi cells [41]. In addition to tRNA 3′-end processing, several observations suggest that the long tRNase Z enzymes might have additional functions in the cell. In *S. cerevisiae*, the tRNase Z^L gene was found to be essential, but this can not be due to the tRNA 3′-processing function since tRNA 3′ processing in the nucleus is backed up by exonucleases [44] and mitochondrial RNA metabolism is not required upon growth on glucose. A screen for ribonucleases in yeast revealed that the yeast tRNase Z seems to have a role in 35S rRNA processing [45]. Thus, the essential function could be due to rRNA-processing activity. Another study in yeast showed that a tRNase Z mutant can be rescued by complementation with the *REX2* gene [46]. The Rex2p protein is an RNA exonuclease involved in U4 snRNA processing, 5.8S rRNA maturation, U5 snRNA maturation and RNase P RNA processing. In addition, Rex2p has been implicated in spliceosome assembly, DNA repair and cytoskeleton reorganization [46]. Rex2p localizes to mitochondria, suggesting that tRNase Z is not only

involved in RNA processing but may also participate in mitochondrial activities [46]. Kushner and colleagues showed that tRNase Z in *E. coli* is involved in mRNA decay of specific mRNAs [47]. Taken together, these results show that the tRNase Z enzymes might have other RNA substrates besides tRNA precursors.

Knock down of the tRNase Z^L gene in *C. elegans* using RNAi resulted in slowly growing and sterile worms, suggesting a role in cell division [48]. The *Drosophila* tRNase Z is induced by the juvenile hormone, a hormone that is implicated in regulation of cell proliferation and differentiation [49]. Mutations in the gene for the human long tRNase Z seem to cause cancer [1]. These data indicate that an additional function of tRNase Z could be involved in cell cycle functions either directly or indirectly through its RNA-processing activity.

Conclusion and outlook

Even though a lot of information has been acquired in the last 5 years since the discovery of the tRNase Z, we still have a lot to learn about the enzyme. So far only the structure of the bacterial tRNase Z has been determined; structures of the archaeal and eukaryotic short tRNase Z enzymes remain to be solved. Likewise the structure of the long tRNase Z proteins needs to be determined. At the moment only scattered biochemical data are available, which leaves important enzyme characteristics like the reaction mechanism unclear. Furthermore, the limited *in vivo* data about these enzymes tell us little about additional *in vivo* functions and substrates. Further analysis of the function and properties of tRNase Z promises novel insights in the coming years.

Footnote. A uniform nomenclature for the tRNase Z enzymes was proposed earlier [7]. Generally, the enzyme is called tRNase Z (EC 3.1.26.11) and the abbreviation is Trz. To distinguish tRNase Z enzymes from different organisms the first letter of the genus and the first two letters of the species are added. If short and long tRNase Z enzymes exist in one organism they are distinguished by superscript S or L, respectively.

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