Supplement 1. Cleavage site determination for the recombinant RNase Z from Arabidopsis and the RNase Z isolated from wheat. 5’ labelled tRNA precursor was incubated with the RNase Z isolated from wheat (lane wheat) and the recombinant RNase Z from Arabidopsis (lane rnuz). The precursor was partially digested with RNase T1 in lane T1 and partially hydrolyzed with alkali in lane OH. The recombinant RNase Z cleaves the precursor at exactly the same sites as the RNase Z isolated from wheat.
Supplement 2. Cleavage site determination for the recombinant RNase Z from *Methanococcus janaschii* and the RNase Z isolated from *Haloferax volcanii* (Schierling et al. (2002) *J Mol Biol* 316, 895-902). 5’ labelled tRNA precursor was incubated with the RNase Z isolated from *H. volcanii* (lane H. vol) and the recombinant RNase Z from Methanococcus (lane rmjaz). The precursor was partially digested with RNase T1 in lane T1 and partially hydrolyzed with alkali in lane OH. The recombinant RNase Z cleaves the precursor at exactly the same sites as the RNase Z isolated from the halophilic archaeon.

Plant and archael RNase Z enzymes both process the mitochondrial pretRNA\textsubscript{Tyr} between nucleotides 75 and 76. The cleavage site seems to depend from the precursor used since other pretRNAs are cleaved directly 3′ to the discriminator as has been shown for pretRNA\textsubscript{His} and pretRNA\textsubscript{Cys} for the plant RNase Z and for pretRNA\textsubscript{Ala} for the archael RNase Z.