Healing for destruction: tRNA intron degradation in yeast is a two-step cytoplasmic process catalyzed by tRNA ligase Rlg1 and 5'-to-3' exonuclease Xrn1

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In eukaryotes and archaea, tRNA splicing generates free intron molecules. Although ~600,000 introns are produced per generation in yeast, they are barely detectable in cells, indicating efficient turnover of introns. Through a genome-wide search for genes involved in tRNA biol-ogy in yeast, we uncovered the mechanism for intron turnover. This process requires healing of the 5’ termini of linear introns by the tRNA ligase Rlg1 and destruction by the cytoplasmic tRNA quality control 5’-to-3’ exonuclease Xrn1, which has specificity for RNAs with 5’ monophosphate.

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nuclear RTD 5'-to-3' exonuclease Rat1 affect tRNA intron turnover. We conclude that tRNA intron decay is a cytoplasmic two-step process that is catalyzed by the 5'-to-3' nuclelease Xrn1 and tRNA ligase Rlg1.

Results and Discussion

The 5'-to-3' exonuclease Xrn1 degrades tRNA introns

To identify unknown gene products involved in tRNA biogenesis, degradation, and subcellular trafficking, we conducted an unbiased genome-wide screen of yeast genes using the S. cerevisiae MATα haploid deletion collection (results of this screen will be presented separately) [Winzeler et al. 1999, Giaever et al. 2002]. One of the primary goals of our screen was to identify gene products involved in tRNA intron turnover.

To conduct this screen, we assessed the impact of each gene product upon tRNAileUAU, which contains the largest tRNA intron (60 nucleotides [nt]) in yeast, by Northern analysis [Wu et al. 2013]. We found that cells deleted for XRN1 accumulate high levels of free tRNAileUAU intron compared with wild-type cells. We verified this result using a probe complementary to the nucleotides 1–37 of the 5' exon and nucleotides 1–30 of the intron of tRNAileUAU [Fig. 1A [probe 1], B [lanes 1,2]]. Similar results were obtained by analysis of xrn1Δ cells attained from the independent MATα collection [Fig. 1B, lane 3; Giaever et al. 2002]. These results were further confirmed using another probe [Fig. 1A, probe 2] that hybridizes solely to the 60-nt tRNAileUAU Intron [Fig. 1C]. Accumulation of introns was suppressed when Xrn1 was exogenously expressed in xrn1Δ cells from a multicopy plasmid, whereas xrn1Δ cells transformed with vector alone accumulated introns [Fig. 1D, lanes 3,4]. We also assessed intron levels for four additional RNAs encoded by tRNALeuCAU, tRNAArgUUU, tRNAProCCA, and tRNAProUGG genes using probes complementary solely to the entire intron of each tRNA. Deletion of XRN1 results in accumulation of all tested tRNA introns [Supplemental Fig. S1]. Quantitative analysis of the signal intensity of tRNA introns compared with initial transcripts demonstrated 2.5-fold to 11-fold increases in xrn1Δ cells compared with control cells [Supplemental Fig. S1]. These data provide evidence that the 5'-to-3' exonuclease Xrn1 affects tRNA intron turnover in yeast.

5' monophosphorylation is requisite prior to intron degradation by Xrn1

It is known that Xrn1 specifically hydrolyzes RNA molecules with a 5' monophosphate group [Stevens 1980]. However, tRNA splicing generates intron molecules with a 5' hydroxyl group [Knapp et al. 1979]. Therefore, we hypothesized that the 5' termini of nascent spliced introns must be phosphorylated prior to degradation by Xrn1 [Fig. 2A].

To test this hypothesis, we treated total small RNAs isolated from xrn1Δ cells with calf intestinal phosphatase (CIP), which catalyzes hydrolysis of 5' phosphate groups from RNA, and/or a Terminator 5'-phosphate-dependent exonuclease [TEX], which specifically degrades RNAs with a single 5' phosphate [Fig. 2A; Patrick et al. 2009]. If the 5' ends of tRNA introns that accumulate in xrn1Δ cells have a terminal phosphate, CIP treatment could lead to a change in electrophoretic mobility; in contrast, TEX treatment would result in intron degradation. 5.8S rRNA and 5S rRNA, which harbor 5' monophosphate and triphosphate [Maxam et al. 1977; Henry et al. 1994], respectively, served as positive and negative controls, and their sensitivity/resistance to TEX treatments were as anticipated [Fig. 2B]. Treatment of RNAs with CIP resulted in faster migration of the introns [Fig. 2B, lane 2] compared with mock-treated samples [Fig. 2B, lane 1], and TEX treatment resulted in intron degradation [Fig. 2B, lane 3]. Introns from samples first treated with CIP to remove the 5' phosphate were resistant to subsequent degradation by TEX [Fig. 2B, lane 4]. Interestingly, end-matured intron-containing pre-tRNAs are resistant to TEX, suggesting that their secondary structure inhibits Terminator exonuclease. Together, the data demonstrate that tRNA introns are 5' monophosphorylated prior to degradation by Xrn1; these results motivated a search for the kinase that phosphorylates tRNA introns.

The tRNA ligase Rlg1 phosphorylates tRNA introns prior to degradation by Xrn1

A well-characterized yeast RNA kinase is the tRNA ligase Rlg1. Rlg1 contains three enzymatic activities
required for tRNA ligation: CPDase, polynucleotide kinase, and ATP-dependent ligase activities. Prior in vitro studies proposed assembly of a SEN–Rlg1 complex for concerted tRNA splicing and ligation (Greer 1986). Moreover, Rlg1 mutant cells were found to accumulate an oligonucleotide of the same size as the tRNAIle intron (Phizicky et al. 1992). Therefore, we considered the possibility that free introns generated by SEN splicing may also be substrates of Rlg1.

If Rlg1 is required for intron degradation, loss of RLG1 function should cause accumulation of unphosphorylated introns. Since RLG1 is an essential gene for cell viability, there is no rlg1Δ strain in the yeast deletion collections. We therefore used ts mutants of RLG1, rlg1-4, and rlg1-10 (Phizicky et al. 1992). RNAs isolated from rlg1-4 cells were compared with RNAs from wild-type and xrn1Δ cells by Northern analysis using two probes, 2 and 3, which hybridize to the intron and the 5′ 9′ exon of tRNAIleUAU, respectively (Fig. 3A; Supplemental Fig. 2A). Two very low abundant species of introns with different mobilities were detected from RNAs isolated from wild-type cells grown at 23°C or after a 2-h shift to 37°C (Fig. 3B, lanes 1,3). RNA from rlg1-4 ts cells grown at 23°C (Fig. 3B, lane 5) show results similar to wild-type cells, but after a 2-h shift to the nonpermissive temperature (37°C), there was significant accumulation of tRNA exons (Fig. 3B, lane 7; Supplemental Fig. S2, lane 4), as previously reported (Phizicky et al. 1992). Likewise, tRNAs isolated from rlg1-10 also accumulated tRNA exons (Supplemental Fig. S2B, lane 6). Importantly, both rlg1-4 and rlg1-10 also accumulated tRNA introns (Fig. 3B, lane 7; Supplemental Fig. S2B, lanes 4,6), demonstrating that Rlg1 is required for tRNA intron turnover. In contrast to xrn1Δ cells that accumulate the 5′ monophosphorylated introns with slower mobility (Fig. 3B, lanes 9,11), the introns that accumulate in rlg1 ts mutants had faster mobility (Fig. 3B, lane 7; Supplemental Fig. S2B, lanes 4,6), supporting the idea that the introns accumulating in rlg1 ts mutants lack 5′ phosphate.

If Rlg1 phosphorylates introns prior to Xrn1-mediated degradation, the double mutant of RLG1 and XRN1 genes would accumulate unphosphorylated introns. We therefore generated a rlg1-4 xrn1Δ strain. At 23°C, the rlg1-4 xrn1Δ cells (Fig. 3, lane 13) show the same phenotype as xrn1Δ cells—accumulation of introns with slow mobility. However, after a 2-h shift to 37°C, rlg1-4 xrn1Δ cells also accumulate introns with faster mobility (Fig. 3B, lane 15). The data are consistent with the hypothesis that when cells lose RLG1, introns fail to be properly phosphorylated and thus cannot be degraded by Xrn1, supporting the notion that Rlg1 acts upstream of Xrn1 in intron turnover.

To test the hypothesis that loss of RLG1 causes accumulation of introns lacking 5′ phosphate, we performed TEX treatment followed by Northern analysis for RNAs isolated from wild-type, rlg1-4, xrn1Δ, and rlg1-4 xrn1Δ cells (Fig. 3). If introns accumulating in rlg1 ts...
mutants are unphosphorylated, they would be resistant to TEX treatment. As anticipated, introns accumulating in rlg1-4 cells after a shift to 37°C were not degraded by TEX [Fig. 3B, lanes 7,8], whereas introns accumulating in xrn1Δ cells are degraded [Fig. 3B, lanes 9–12]. Similar to introns in xrn1Δ cells, introns accumulating in rlg1-4 xrn1Δ cells grown at 23°C are also degraded by TEX [Fig. 3B, lanes 13,14]. However, of the two intron species accumulating in rlg1-4 xrn1Δ cells after shift to 37°C [Fig. 3B, lane 15], only the pre-existing monophosphorylated species was sensitive to TEX treatment [Fig. 3B, lane 16]. As anticipated, the unstructured 5′ tRNA exons bearing 5′ monophosphates that accumulate in rlg1-4 and rlg1-4 xrn1Δ cells upon shift to nonpermissive temperature were degraded by TEX [Fig. 3B, lanes 7,8,15,16]. Together, the data show that loss of RLG1 leads to accumulation of the intron with 5′ hydroxyl, whereas loss of XRN1 causes accumulation of introns with 5′ phosphate. These studies provide evidence that Rlg1 possesses the kinase activity that phosphorylates introns prior to recognition and degradation of introns by Xrn1.

We also analyzed introns of four other tRNAs, tRNA^{ACU}, tRNA^{UUU}, tRNA^{GCC}, and tRNA^{UGG} in the rlg1-4 mutant. Loss of RLG1 lead to a fourfold to 15.6-fold increase in intron levels [Supplemental Fig. S1]. These data suggest that the Rlg1- and Xrn1-mediated tRNA intron degradation pathway is generally used for all tRNAs.

Prior to vitro studies of Rlg1 substrate specificity showed that, in addition to catalyzing ligation of tRNA halves, Rlg1 is able to phosphorylate or ligate the ends of artificial linear RNA substrates [Phizicky et al. 1986; Apostol et al. 1991]. Our finding that Rlg1 phosphorylates introns indicates that linear molecules can be the substrates for Rlg1 kinase activity in vivo. On the other hand, the question arises as to whether Rlg1 ligase activity is able to join the intron ends to generate circular introns. Rlg1 is capable of circularizing artificial substrate in vitro [Phizicky et al. 1986], and stable circular introns formed by 3′,5′-phosphodiester linkage have been reported for certain archaea [Salgia et al. 2003]. However, since TEX is able to effectively degrade introns, it would appear that the majority of introns in yeast are linear, although there may be minor pools of circular introns that are beyond our detection level. In addition, we did not detect elevated levels of end-matured intron-containing tRNAs or 2/3 molecules consisting of an exon and an intron in xrn1Δ cells, indicating that Rlg1 does not join the spliced introns and exons back together even in the presence of high intron levels, whereas it clearly is able to phosphorylate free introns.

Although yeast and plants use Rlg1 and the 2′ phosphotransferase Tpt1 for tRNA ligation, vertebrates appear to contain two distinct tRNA ligation pathways: a direct ligation pathway and a yeast-like ligation pathway. In the first, a 3′-to-5′ ligase activity is used to directly join the 3′ phosphate of the 5′ exon to the 5′ hydroxyl of the 3′ exon [Popow et al. 2012]. In the second, hClp1, a component of the mammalian SEN complex, catalyzes phosphorylation of the 5′ end of the 3′ exon of tRNAs, although the ligase is not identified [Zillmann et al. 1991; Weitzer and Martinez 2007, Ramirez et al. 2008]. New studies reported that CLP1 mutation leads to severe neurodegenerative disease, and fibroblasts from human patients accumulate linear introns with a 5′ hydroxyl, although intron accumulation was not observed in vitro analysis [Karaca et al. 2014, Schaffer et al. 2014]. We wonder whether introns are targets of CLP1 in vertebrate cells and whether it is possible that defects in tRNA intron turnover might contribute to the neurological phenotypes caused by CLP1 mutations.

tRNA intron degradation is a 5′-to-3′ process in the cytoplasm

Although the above studies provide evidence that turnover of tRNA introns is a two-step process consisting of healing by Rlg1 followed by 5′-to-3′ degradation by Xrn1, they do not eliminate the possibilities that other RNA decay machineries also degrade introns and that this process may occur in other cellular compartments than the cytoplasm. To address these issues, we examined whether Ral1, the nuclear 5′-to-3′ exonuclease Xrn1 ortholog [Johnson 1997], affects intron turnover. We used a ts mutant of RAT1, rat1-1 [Li et al. 2011], since RAT1 is an essential gene that is not contained in the deletion collection. Similar to wild type [Fig. 4A, lanes 3,4], rat1-1 fails to accumulate tRNA introns after a 2-h shift to the nonpermissive temperature 37°C [Fig. 4A, lanes 1,2], arguing that tRNA introns are not Rat1 substrates and supporting the notion that RNA intron degradation is restricted to the cytoplasm in yeast. Since splicing occurs in the nucleus in vertebrates, an intriguing question is: Where does intron degradation occur in vertebrates?

Through our screen of the deletion collection, we found that none of the mutants of the other 5′-to-3′ or 3′-to-5′ RNA decay enzymes accumulated tRNA introns. To verify that they are not involved in tRNA intron turnover, we examined their deletion mutants from the independent MATα deletion collection [Giaever et al. 2002].

Two other 5′-to-3′ RNA decay enzymes, Rai1, which is predominately localized to the nucleus [Xue et al. 2000],
Intron levels of tRNALeu are decreased by Rlg1-mediated healing step prior to degradation catalyzed by Xrn1. Assessment of other nuclear or cytoplasmic 5′-to-3′ and 3′-to-5′ decay pathways provides evidence that intron turnover is a 5′-to-3′ process in the cytoplasm [Fig. 5].

Materials and methods

Yeast strains

*S. cerevisiae* strains in the MATa/BY4741 background (MATa his3Δ1 leu2Δ0 met1Δ0 ura3Δ0) and/or the MATα/scarb4742 background (MATα his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0) were used: wild type, xrn1Δ, rai1Δ, dxo1Δ, ski2Δ, ski3Δ, ski7Δ, and ski8Δ [Open Biosystems]. rlg1-4 and rlg1-10 ts strains were previously described [Phizicky et al. 1992]. The XRNI gene was deleted from the *rlg1-4* strain by gene replacement with a bacterial hygromycin B resistance gene (Hph) to construct *rlg1-4 xrn1Δ*. The ts *rai1-1* strain was a gift from Dr. Charlie Boone [Li et al. 2011].

CIP treatment

Twenty micrograms of small RNAs was treated with CIP (New England Biolabs) for 1 h at 37°C according to the manufacturer’s protocol. The reaction was terminated by phenol extraction and ethanol precipitation.

TEX treatment

Ten micrograms of small RNAs was treated with TEX (Epi-centre) for 1 h at 30°C according to the manufacturer’s protocol. The reaction was terminated by phenol extraction and ethanol precipitation.

Northern analysis

Small RNAs were isolated by phenol extraction from cultures grown at the permissive temperature (23°C) to 0.4 OD600. The ts mutants were shifted for 2 h to the nonpermissive temperature (37°C) before RNA extraction. Seven micrograms of small RNAs was separated by electrophoresis through a 10% polyacrylamide gel and transferred onto a Hybond N+ membrane. tRNAs were detected using DIG-labeled probes as described [Wu et al. 2013]. The membranes were exposed to a Lumi-Imager [Boehringer Mannheim], and the signal intensity of each band was quantified as absolute integrated light units (BLU) using LumiAnalyst version 3.0 software.

Conclusions

Here we report the discovery of the mechanism of intron turnover in yeast [Fig. 5]. It is a process catalyzed by a Rlg1-mediated healing step prior to degradation catalyzed by Xrn1. Assessment of other nuclear or cytoplasmic 5′-to-3′ and 3′-to-5′ decay pathways provides evidence that intron turnover is a 5′-to-3′ process in the cytoplasm [Fig. 5].
Armakola M, Higgins MJ, Figley MD, Barnarda SJ, Scarborough EA, Díaz Z, Fang X, Shorter J, Krogan NJ, Finkbeiner S, et al. 2012. Inhibition of RNA lariat debranching enzyme suppresses TDP-43 toxicity in ALS disease models. Nat Genet 44: 1302–1309.

Chang JH, Jiao X, Chiba K, Oh C, Martin CE, Tollervey D. 2003. An NMD pathway in yeast involving Rex1p exoribonuclease activity. Nat Struct Mol Biol 10: 1011–1017.

Chernyakov I, Whipple JM, Kotelawala L, Grayhack EL, Phizicky EM. 2008.Degradation of several hypomodified mature tRNA species in Saccharomyces cerevisiae is mediated by Met22 and the 5′-3′ exonuclease Rat1p and Xm1. Genes Dev 22: 1369–1380.

Copela LA, Fernandez CF, Sherrer RL, Wolin SL. 2008. Competition between the Rex1 exonuclease and the La protein affects both Trf4p-mediated RNA quality control and pre-tRNA maturation. RNA 14: 1214–1227.

Culver GM, McCraith SM, Zillmann M, Kiezck R, Michaela N, LaReau RD, Turner DH, Phizicky EM. 1993. An NAD derivative produced during transfer RNA splicing: ADP-ribose 1′-2′ cyclic phosphate. Science 261: 206–208.

Englert M, Beier H. 2005. Plant tRNA ligases are multifunctional enzymes that have diverged in sequence and substrate specificity from RNA ligases of other phylogenetic origins. Nucleic Acids Res 33: 388–399.

Giaever G, Chu AM, Ni L, Connelly C, Riles L, Veronneau S, Dow S, Earl AM, LaBranche CA, Bussey H, et al. 2002. Functional profiling of the Saccharomyces cerevisiae genome. Nature 418: 387–391.

Greer CL. 1984. Assembly of a tRNA splicing complex: evidence for concerted excision and joining steps in splicing in vitro. Mol Cell Biol 6: 635–644.

Greer CL, Peebles CL, Gegenheimer P, Abelson J. 1983. Mechanism of action of a yeast RNA ligase in tRNA splicing. Cell 32: 537–546.

Henry Y, Wood H, Morrissey JP, Petfalski E, Kearsey S, Tollervey D. 1994. The 5′-3′ mode of hydrolysis. J Biol Chem 269: 3082–3085.

Johnson AW. 1997. Rat1p and Xrn1p are functionally interchangeable exoribonucleases that are restricted to and required in the nucleus and cytoplasm, respectively. Mol Cell Biol 17: 6122–6130.

Kadaba S, Krueger A, Trice T, Kreic AM, Hinnebusch AG, Anderson J. 2004. Nuclear surveillance and degradation of hypomodified initiator tRNAMet in S. cerevisiae. Genes Dev 18: 1227–1240.

Karaca E, Weitzer S, Pehlivan D, Shiraishi H, Gogakos T, Hanada T, Kadaba S, Krueger A, Trice T, Kreic AM, Hinnebusch AG, Anderson J. 2004. Nuclear surveillance and degradation of hypomodified initiator tRNAMet in S. cerevisiae. Genes Dev 18: 1227–1240.

Winzeler EA, Shoemaker DD, Astrumoff A, Liang H, Anderson K, Andre B, Bangham R, Benito R, Boeke JD, Bussay H, et al. 1999. Functional characterization of the S. cerevisiae genome by gene deletion and parallel analysis. Science 285: 901–906.

Wu J, Huang HY, Hopper AK. 2013. A rapid and sensitive non-radioactive method applicable for genome-wide analysis of Saccharomyces cerevisiae genes involved in small RNA biology. Yeast 30: 119–128.

Yue X, Bai X, Lee I, Kallstrom G, Ho J, Brown J, Stevens A, Johnson AW. 2000. Saccharomyces cerevisiae Rat1 [YCL246c] is homologous to human DOM3Z and encodes a protein that binds the nuclear exoribonuclease Rat1p. Mol Cell Biol 20: 4006–4015.

Yoshishisa T, Yunoki-Eaaki K, Ohshima C, Tanaka N, Endo T. 2003. Possibility of cytoplasmic pre-tRNA splicing: the yeast tRNA splicing endonuclease mainly localizes on the mitochondria. Mol Cell Biol 14: 3269–3279.

Zillmann M, Gorovsky MA, Phizicky EM. 1991. Conservation of RNase P RNA-splicing in eukaryotes. Mol Cell Biol 11: 5410–5416.

Phizicky EM, Hopper AK. 2010. tRNA biology charges to the front. Genes Dev 24: 1832–1860.

Phizicky EM, Schwartz RC, Abelson J. 1986. Saccharomyces cerevisiae tRNA ligase. Purification of the protein and isolation of the structural gene. J Biol Chem 261: 2978–2986.

Phizicky EM, Consaul SA, Nehrke KW, Abelson J. 1992. Yeast tRNA ligase mutants are nonviable and accumulate tRNA splicing intermediates. J Biol Chem 267: 4577–4582.

Popow J, Schleiffer A, Martinez J. 2012. Diversity and roles of tRNAs. Cell Mol Life Sci 69: 2657–2670.

Ramirez A, Shuman S, Schwer B. 2008. Human RNA 5′-kinase (hClp1) can function as a tRNA splicing enzyme in vivo. RNA 14: 1737–1745.

Salgia SR, Singh SK, Gurha P, Gupta R. 2003. Two reactions of Haloferax volcanii RNA splicing enzymes: joining of exons and circularization of introns. RNA 9: 319–330.

Schaffer AE, Egges VR, Caglayan AO, Reuter MS, Scott E, Coufal NG, Silhavy JL, Xue Y, Kayserli H, Yasuno K, et al. 2014. CLP1 founder mutation links tRNA splicing and maturation to cerebellar development and neurodegeneration. Cell 157: 651–663.

Steven A. 1980. Purification and characterization of a Saccharomyces cerevisiae exoribonuclease which yields 5′-mononucleotides by a 5′-3′ mode of hydrolysis. J Biol Chem 255: 8080–8085.

Trotta CR, Miao F, Arn EA, Stevens SW, Ho CK, Raulht R, Abelson JN. 1997. The yeast tRNA splicing endonuclease: a tetreric enzyme with two active site subunits homologous to the archaeal tRNA endonucleases. Cell 89: 849–858.

Waldron C, Lacroute F. 1975. Effect of growth rate on the amounts of ribosomal and transfer ribonucleic acids in yeast. J Bacteriol 122: 855–865.

Weitzer S, Martinez J. 2007. The human RNA kinase hClp1 is active on 5′-3′ tRNA exons and short interfering RNAs. Nature 447: 222–228.

Whipple JM, Lane EA, Chernyakov I, D’Silva S, Phizicky EM. 2011. The rapid yeast tRNA decay pathway primarily monitors the structural integrity of the acceptor and T-stems of mature tRNA. Genes Dev 25: 1173–1184.

Waldron C, Lacroute F. 1975. Effect of growth rate on the amounts of ribosomal and transfer ribonucleic acids in yeast. J Bacteriol 122: 855–865.

Weitzer S, Martinez J. 2007. The human RNA kinase hClp1 is active on 5′-3′ tRNA exons and short interfering RNAs. Nature 447: 222–228.

Whipple JM, Lane EA, Chernyakov I, D’Silva S, Phizicky EM. 2011. The rapid yeast tRNA decay pathway primarily monitors the structural integrity of the acceptor and T-stems of mature tRNA. Genes Dev 25: 1173–1184.

Winzeler EA, Shoemaker DD, Astrumoff A, Liang H, Anderson K, Andre B, Bangham R, Benito R, Boeke JD, Bussay H, et al. 1999. Functional characterization of the S. cerevisiae genome by gene deletion and parallel analysis. Science 285: 901–906.

Wu J, Huang HY, Hopper AK. 2013. Rapid and sensitive non-radioactive method applicable for genome-wide analysis of Saccharomyces cerevisiae genes involved in small RNA biology. Yeast 30: 119–128.

Yue X, Bai X, Lee I, Kallstrom G, Ho J, Brown J, Stevens A, Johnson AW. 2000. Saccharomyces cerevisiae Rat1 [YCL246c] is homologous to human DOM3Z and encodes a protein that binds the nuclear exoribonuclease Rat1p. Mol Cell Biol 20: 4006–4015.

Yoshishisa T, Yunoki-Eaaki K, Ohshima C, Tanaka N, Endo T. 2003. Possibility of cytoplasmic pre-tRNA splicing: the yeast tRNA splicing endonuclease mainly localizes on the mitochondria. Mol Cell Biol 14: 3269–3279.

Zillmann M, Gorovsky MA, Phizicky EM. 1991. Conservation of tRNA splicing in eukaryotes. Mol Cell Biol 11: 5410–5416.