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

Jingyan Wu[1,2,3] and Anita K. Hopper[1,3,4]

[1]Department of Molecular Genetics, [2]Graduate Program in Plant Cellular and Molecular Biology, [3]Center for RNA Biology, The Ohio State University, Columbus, Ohio 43210, USA

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 biology 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.

Supplemental material is available for this article.

Received May 2, 2014; revised version accepted June 3, 2014.

tRNAs are essential for translation in all kingdoms of life. In addition, tRNAs play important roles in other cellular events, such as protein degradation, apoptosis, cellular response to stress, and cancer (Phizicky and Hopper 2010). tRNA biogenesis involves numerous post-transcriptional processes, including removal of 5′ leaders and 3′ trailers, addition of 3′ CCA nucleotides and nucleoside modifications, and, for tRNAs encoded by intron-containing genes, splicing of introns (Phizicky and Hopper 2010).

Splicing of introns is essential for cells to generate complete sets of tRNAs to decode all codons. The percentage of tRNA genes that harbor introns varies significantly, from 0% in bacteria to 5% in mice and humans to >60% in some archaea (Genomic tRNA Database, http://lowelab.ucsc.edu/GtRNAdb). In yeast *Saccharomyces cerevisiae*, 59 (21.5%) of 274 tRNA genes from 10 different isodecoder tRNA families contain an intron (Phizicky and Hopper 2010).

In yeast and vertebrates, tRNA splicing is catalyzed by the heterotetrameric tRNA splicing endonuclease (SEN) complex, yielding the 5′ half with a 2′, 3′-cyclic phosphate, the intron with a 5′ hydroxyl and 2′, 3′-cyclic phosphate, and the 3′ half with a 5′ hydroxyl (Knapp et al. 1979; Trotta et al. 1997; Paushkin et al. 2004). In vertebrates, tRNA splicing occurs in the nucleus (Melton et al. 1980; Lund and Dahlberg 1998; Paushkin et al. 2004), whereas it is a cytoplasmic process in yeast, since the SEN complex is located on the outer surface of mitochondria (Yoshihisa et al. 2003). Despite the difference in localization, the structure and function of the SEN complex are conserved from yeast to humans (Trotta et al. 1997; Paushkin et al. 2004).

In yeast and plants, after splicing, the 5′ and 3′ halves are ligated through a series of enzymatic reactions that require three activities of the tRNA ligase Rlg1 (Greer et al. 1983; Phizicky et al. 1986; Englert and Beier 2005): (1) a cyclic phosphodiesterase (CPDase) activity that opens the 2′, 3′ cyclic phosphate of the 5′ exon, (2) a polynucleotide kinase activity that phosphorylates the 5′ end of the 3′ exon, and (3) an ATP-dependent ligase activity that joins the two exons together. Finally, the 2′-phosphate remaining at the ligation junction is removed by the phosphotransferase Tpt1 (Culver et al. 1993) to form the mature tRNA.

Although much is known about the ligation of two tRNA halves, little is known about the fate of the other splicing product, the free tRNA intron. In contrast to the high abundance of mature tRNAs, free introns are barely detectable, indicating that they are degraded rapidly after splicing. To date, two tRNA degradation pathways have been identified in yeast as tRNA quality control mechanisms. The nuclear surveillance pathway provides one mechanism; pre-tRNA$^{Met}_{i}$ lacking m^1A_{58} and pre-tRNAs with aberrant 3′ ends are polyadenylated by the TRAMP complex and further degraded by the nuclear exosome (Kabada et al. 2004; Copela et al. 2008; Ozanick et al. 2009). The other pathway, the rapid tRNA decay (RTD) pathway, destroys mature tRNAs that lack certain combinations of modifications or tRNAs that bear unstable T or acceptor stems (Chernyakov et al. 2008; Whipple et al. 2011).

Although turnover pathways for aberrant precursor and mature tRNAs have been studied extensively, little is known about degradation of tRNA introns. Since ~3,000,000 tRNAs are produced per generation in yeast (Waldron and Lacroute 1975) and >20% are intron-containing tRNAs, an enormous amount of tRNA introns (~600,000) is produced during each generation. Cells may need to efficiently degrade tRNA introns to generate nucleotides or prevent introns from interacting with other RNAs and RNA-binding proteins and perhaps inhibiting their functions. There is precedent for this, as cytoplasmic accumulation of mRNA intron lariats in human cells sequesters and suppresses TDP-43, a key factor involved in amyotrophic lateral sclerosis (Armakola et al. 2012).

Here we uncovered the mechanism for tRNA intron decay in yeast. Through a genome-wide screen for novel gene products involved in tRNA biology, we found that tRNA introns accumulate in cells lacking the cytoplasmic RTD 5′-to-3′ exonuclease Xrn1. Degradation of introns via Xrn1 requires healing of tRNA intron 5′ termini by the tRNA ligase Rlg1. Neither defects in 3′-to-5′ nucleases nor a temperature-sensitive (ts) mutation of the

[*Keywords*: tRNA splicing; tRNA ligation; intron turnover; Xrn1; Rlg1; 5′ to 3′ exonuclease]
[4]Corresponding author
E-mail hopper.64@osu.edu
Article is online at http://www.genesdev.org/cgi/doi/10.1101/gad.244673.114.

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′ nuclease 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 MATa* 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 tRNA intron turnover.

To conduct this screen, we assessed the impact of each gene product upon $tRNA^{Ile}_{UAU}$, 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 $tRNA^{Ile}_{UAU}$ 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 $tRNA^{Ile}_{UAU}$ (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 usng another probe (Fig. 1A, probe 2) that hybridizes solely to the 60-nt $tRNA^{Ile}_{UAU}$ 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 $tRNA^{Leu}_{CAA}$, $tRNA^{Lys}_{UUU}$, $tRNA^{Trp}_{CCA}$, and $tRNA^{Pro}_{UGG}$ 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

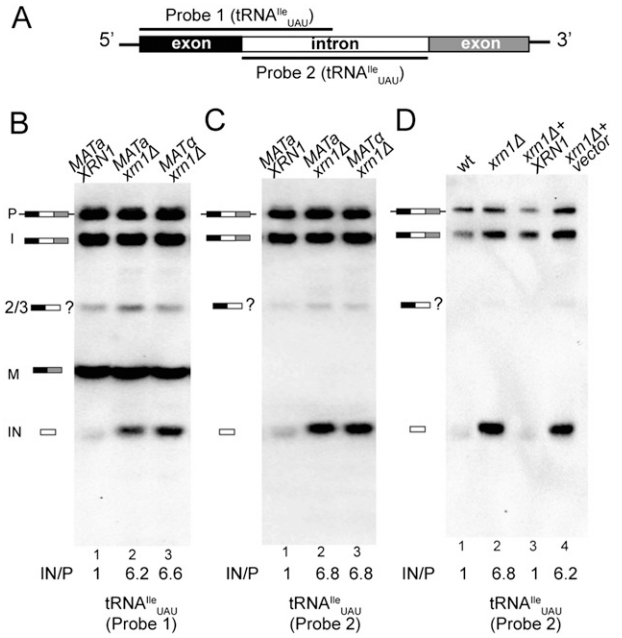

Figure 1. Deletion of the *XRN1* gene leads to tRNA intron accumulation. (*A*) A schematic view of a primary $tRNA^{Ile}_{UAU}$ transcript with 5′ leader and 3′ trailer, exons, and an intron and the probes used for Northern hybridization. (*B*) Two independent *xrn1Δ* strains (*MATa xrn1Δ* and *MATα xrn1Δ*) accumulate $tRNA^{Ile}_{UAU}$ introns. Northern analysis was performed using probe 1. (*C*) Northern analysis of *MATa* wild-type (wt) and two independent *xrn1Δ* strains using probe 2. (*D*) Complementation of the phenotype of *xrn1Δ* cells by plasmid encoded Xrn1. Northern analysis of RNAs from *MATa* wild-type, *xrn1Δ*, and *xrn1Δ* cells transformed with the multicopy plasmid YEpXrn1 or vector alone were performed using probe 2. Ratios of the signal intensities of introns versus primary tRNA transcripts were calculated and normalized to the wild-type ratio. (P) Primary tRNA transcript (145 nt); (I) end-mature intron containing tRNA (136 nt); (2/3 with a question mark) unknown species, which is likely the 5′ 2/3 splicing intermediate according to its size (97 nt); (M) mature tRNA (76 nt); (IN) intron (60 nt).

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. Rlgl contains three enzymatic activities

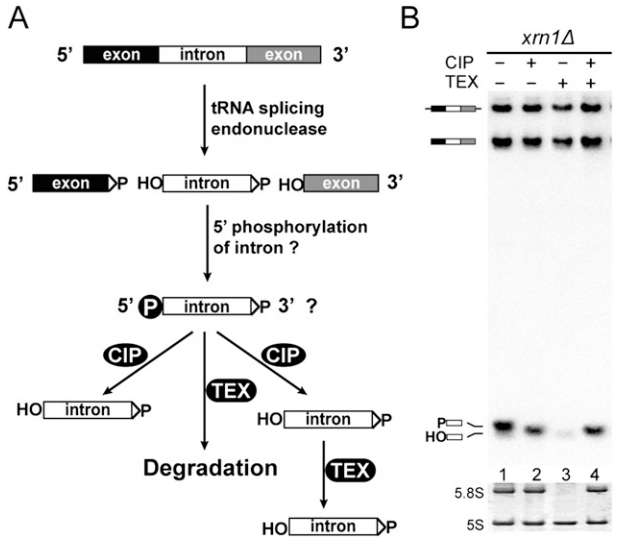

Figure 2. The 5′ end of tRNA introns is monophosphorylated prior to degradation by Xrn1. (*A*) Schematic view of tRNA splicing products and the strategy for determining the 5′ phosphorylation status of introns using CIP and TEX. (*B*) CIP and TEX treatments. Northern analysis of untreated or treated total small RNAs isolated from *xrn1Δ* cells was performed using probe 2. (P, white block) Intron with a 5′ phosphate; (HO, white block) intron with 5′ hydroxyl. 5.8S and 5S served as controls.

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 $tRNA^{Ile}$ 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′ exon of $tRNA^{Ile}_{UAU}$, 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

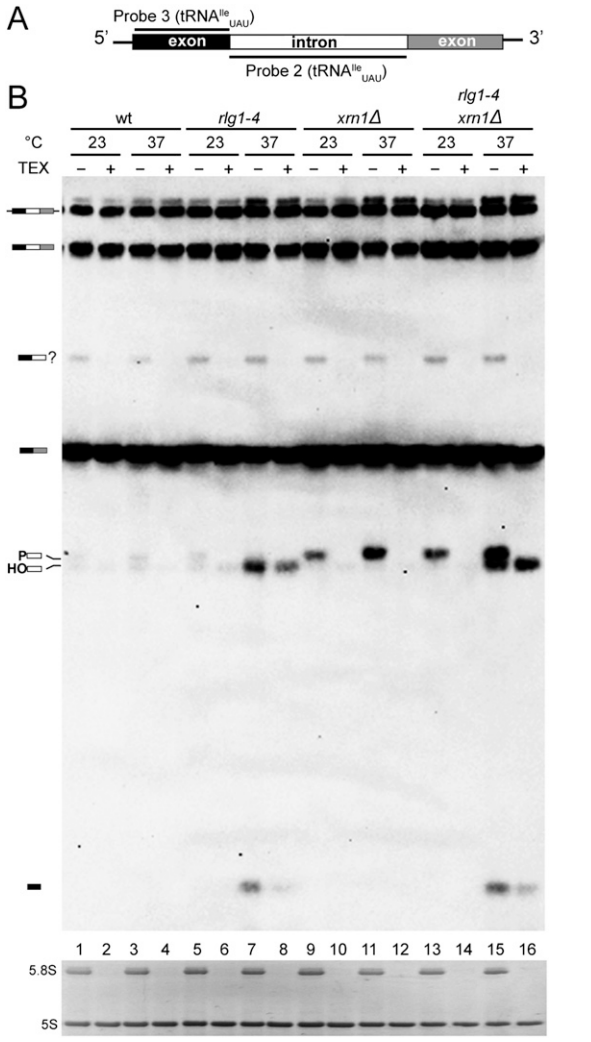

Figure 3. Rlg1 phosphorylates tRNA introns. (*A*) Probes 3 and 2 were used in the Northern blot shown in *B*. (*B*) Northern analysis of TEX-treated and untreated small RNAs from wild-type (wt), *rlg1-4*, *xrn1Δ*, and *rlg1-4 xrn1Δ* cells grown at 23°C or after a 2-h shift to 37°C. (Black block) 5′ exon; other symbols are as described in Figures 1 and 2. 5.8S and 5S rRNAs served as controls.

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, $tRNA^{Leu}_{CAA}$, $tRNA^{Lys}_{UUU,}$ $tRNA^{Trp}_{CCA}$, and $tRNA^{Pro}_{UGG}$ 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 in 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 in vitro analyses (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 Rat1, 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 tRNA 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 predominantly localized to the nucleus (Xue et al. 2000),

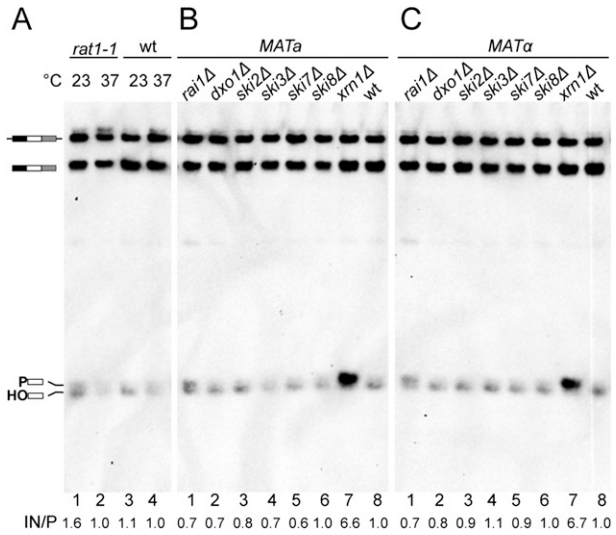

Figure 4. tRNA intron turnover is a 5′-to-3′ cytoplasmic process. (*A*) Northern analysis of RNAs from *rat1-1* and wild-type (wt) cells grown at 23°C or after a shift to 37°C using probe 2. (*B*) Northern analysis of RNAs from *MATa rai1Δ*, *dxo1Δ*, *ski2Δ*, *ski3Δ*, *ski7Δ*, *ski8Δ*, *xrn1Δ*, and wild-type cells. (*C*) Northern analysis of RNAs from *MATα rai1Δ*, *dxo1Δ*, *ski2Δ*, *ski3Δ*, *ski7Δ*, *ski8Δ*, *xrn1Δ*, and wild-type cells. Ratios of the signal intensities of introns versus primary tRNA transcripts were calculated and normalized to the wild-type ratio.

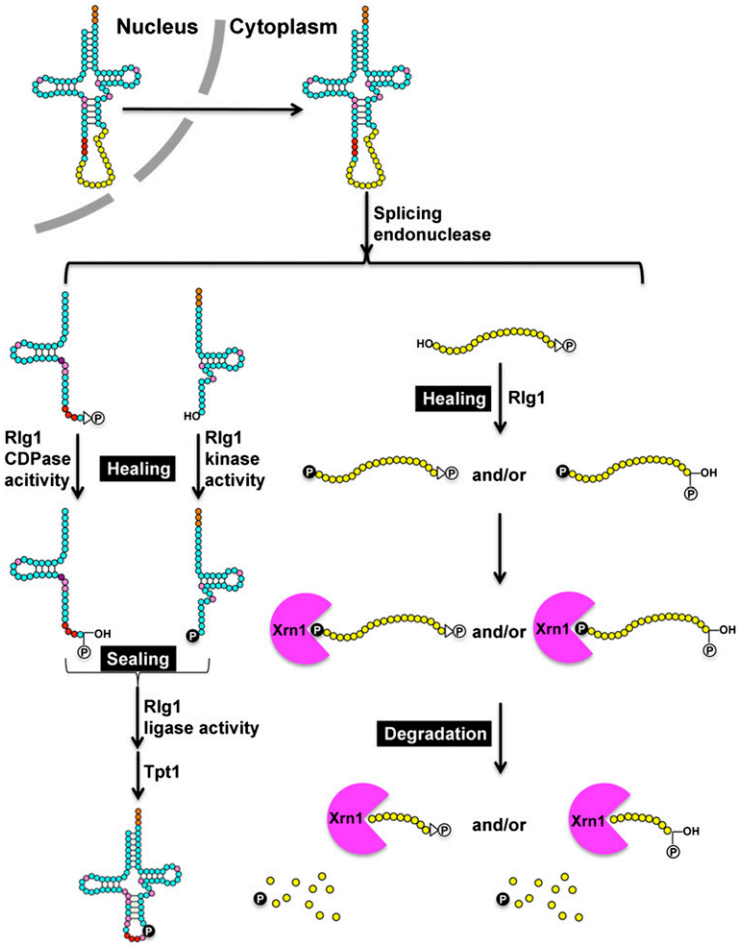

Figure 5. Mechanisms for tRNA splicing, ligation, and intron degradation in yeast. tRNA second structure is illustrated with the following color scheme of circles: exons in light blue, anti-codons in red, introns in yellow, modified nucleotides in pink, and CCA in orange. After nuclear export, end-matured intron-containing tRNAs are spliced by SEN. The CPDase and polynucleotide kinase activity of Rlg1 catalyze healing of the excised ends, and ligase activity catalyzes the sealing of two exons. During intron turnover, the 5′ end of newly spliced introns is phosphorylated by Rlg1, although whether the 3′ end is subjected to Rlg1 CPDase activity is unclear. After Rlg1-mediated phosphorylation, introns are recognized and degraded by Xrn1.

and Dxo1, which is primarily cytoplasmic (Chang et al. 2012), were examined. There is no accumulation of tRNA introns in RNAs from *dxo1Δ* or *rai1Δ* cells (Fig. 4B,C, lanes 1,2). We also examined mutations of subunits of the cytoplasmic exosome, the SKI complex (Mitchell and Tollervey 2003). None of the SKI mutants (*ski2Δ*, *ski3Δ*, *ski7Δ*, and *ski8Δ*) accumulated tRNA introns (Fig. 4B,C, lanes 3–6). Intron levels of $tRNA^{Leu}_{CAA}$ were also examined in all of these mutants, and no accumulation was observed (data not shown). Our data show that the exosome does not contribute to tRNA intron turnover, suggesting that intron turnover may not occur via 3′-to-5′ exonucleases.

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).

Materials and methods

Yeast strains

S. cerevisiae strains in the *MATa*/BY4741 background (*MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0*) and/or the *MATα*/BY4742 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 *XRN1* 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 *rat1-1* 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 (Epicentre) 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 OD_{600}. 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 Boehringer light units (BLU) using LumiAnalyst version 3.0 software.

Oligonucleotides

The sequences of the oligonucleotides used are provided in the Supplemental Material.

Acknowledgments

We thank all A.K.H. laboratory members for excellent scientific interactions. We are grateful for the *rlg1-4* and *rlg1-10* ts mutants and valuable comments from Dr. Eric Phizicky. We also thank Dr. Jane Jackman for inspiring advice on this work. This work was supported by the National Institutes of Health (GM27930 to A.K.H.) and The Ohio State University Pelotonia Graduate Fellowship (to J.W.).

References

Apostol BL, Westaway SK, Abelson J, Greer CL. 1991. Deletion analysis of a multifunctional yeast tRNA ligase polypeptide. Identification of essential and dispensable functional domains. *J Biol Chem* **266:** 7445–7455.

Armakola M, Higgins MJ, Figley MD, Barmada SJ, Scarborough EA, Diaz 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, Kiledjian M, Tong L. 2012. Dxo1 is a new type of eukaryotic enzyme with both decapping and 5′–3′ exoribonuclease activity. *Nat Struct Mol Biol* **19:** 1011–1017.

Chernyakov I, Whipple JM, Kotelawala L, Grayhack EJ, Phizicky EM. 2008. Degradation of several hypomodified mature tRNA species in *Saccharomyces cerevisiae* is mediated by Met22 and the 5′–3′ exonucleases Rat1 and Xrn1. *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, Kierzek R, Michaud 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, Lucau-Danila A, Anderson K, Andre B, et al. 2002. Functional profiling of the *Saccharomyces cerevisiae* genome. *Nature* **418:** 387–391.

Greer CL. 1986. 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′ end of yeast 5.8S rRNA is generated by exonucleases from an upstream cleavage site. *EMBO J* **13:** 2452–2463.

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, Krecic 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, Jhangiani SN, Wiszniewski W, Withers M, Campbell IM, et al. 2014. Human CLP1 mutations alter tRNA biogenesis, affecting both peripheral and central nervous system function. *Cell* **157:** 636–650.

Knapp G, Ogden RC, Peebles CL, Abelson J. 1979. Splicing of yeast tRNA precursors: structure of the reaction intermediates. *Cell* **18:** 37–45.

Li Z, Vizeacoumar FJ, Bahr S, Li J, Warringer J, Vizeacoumar FS, Min R, Vandersluis B, Bellay J, Devit M, et al. 2011. Systematic exploration of essential yeast gene function with temperature-sensitive mutants. *Nat Biotechnol* **29:** 361–367.

Lund E, Dahlberg JE. 1998. Proofreading and aminoacylation of tRNAs before export from the nucleus. *Science* **282:** 2082–2085.

Maxam AM, Tizard R, Skryabin KG, Gilbert W. 1977. Promotor region for yeast 5S ribosomal RNA. *Nature* **267:** 643–645.

Melton DA, De Robertis EM, Cortese R. 1980. Order and intracellular location of the events involved in the maturation of a spliced tRNA. *Nature* **284:** 143–148.

Mitchell P, Tollervey D. 2003. An NMD pathway in yeast involving accelerated deadenylation and exosome-mediated 3′ → 5′ degradation. *Mol Cell* **11:** 1405–1413.

Ozanick SG, Wang X, Costanzo M, Brost RL, Boone C, Anderson JT. 2009. Rex1p deficiency leads to accumulation of precursor initiator tRNAMet and polyadenylation of substrate RNAs in *Saccharomyces cerevisiae*. *Nucleic Acids Res* **37:** 298–308.

Patrick KL, Shi H, Kolev NG, Ersfeld K, Tschudi C, Ullu E. 2009. Distinct and overlapping roles for two Dicer-like proteins in the RNA interference pathways of the ancient eukaryote *Trypanosoma brucei*. *Proc Natl Acad Sci* **106:** 17933–17938.

Paushkin SV, Patel M, Furia BS, Peltz SW, Trotta CR. 2004. Identification of a human endonuclease complex reveals a link between tRNA splicing and pre-mRNA 3′ end formation. *Cell* **117:** 311–321.

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 (t)RNA ligases. *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, Eggens VR, Caglayan AO, Reuter MS, Scott E, Coufal NG, Silhavy JL, Xue Y, Kayserili H, Yasuno K, et al. 2014. CLP1 founder mutation links tRNA splicing and maturation to cerebellar development and neurodegeneration. *Cell* **157:** 651–663.

Stevens A. 1980. Purification and characterization of a *Saccharomyces cerevisiae* exoribonuclease which yields 5′-mononucleotides by a 5′ leads to 3′ mode of hydrolysis. *J Biol Chem* **255:** 3080–3085.

Trotta CR, Miao F, Arn EA, Stevens SW, Ho CK, Rauhut R, Abelson JN. 1997. The yeast tRNA splicing endonuclease: a tetrameric 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 3′ transfer RNA exons and short interfering RNAs. *Nature* **447:** 222–226.

Whipple JM, Lane EA, Chernyakov I, D'Silva S, Phizicky EM. 2011. The yeast rapid 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, Astromoff A, Liang H, Anderson K, Andre B, Bangham R, Benito R, Boeke JD, Bussey 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.

Xue Y, Bai X, Lee I, Kallstrom G, Ho J, Brown J, Stevens A, Johnson AW. 2000. *Saccharomyces cerevisiae* RAI1 (YGL246c) is homologous to human DOM3Z and encodes a protein that binds the nuclear exoribonuclease Rat1p. *Mol Cell Biol* **20:** 4006–4015.

Yoshihisa T, Yunoki-Esaki 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 Biol Cell* **14:** 3266–3279.

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