Mammalian 2',3' cyclic nucleotide phosphodiesterase (CNP) can function as a tRNA splicing enzyme *in vivo*

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ABSTRACT

Yeast and plant tRNA splicing entails discrete healing and sealing steps catalyzed by a tRNA ligase that converts the 2',3' cyclic phosphate and 5'-OH termini of the broken tRNA exons to 3'-OH/2'-PO₄ and 5'-PO₄ ends, respectively, then joins the ends to yield a 2'-PO₄, 3'-5' phosphodiester splice junction. The junction 2'-PO₄ is removed by a tRNA phosphotransferase, Tpt1. Animal cells have two potential tRNA repair pathways: a yeast-like system plus a distinctive mechanism, also present in archaea, in which the 2',3' cyclic phosphate and 5'-OH termini are ligated directly. Here we report that a mammalian 2',3' cyclic nucleotide phosphodiesterase (CNP) can perform the essential 3' end-healing steps of tRNA splicing in yeast and thereby complement growth of strains bearing lethal or temperature-sensitive mutations in the tRNA ligase 3' end-healing domain. Although this is the first evidence of an RNA processing function *in vivo* for the mammalian CNP protein, it seems unlikely that the yeast-like pathway is responsible for animal tRNA splicing, insofar as neither CNP nor Tpt1 is essential in mice.

Keywords: 3' end-healing; RNA 2',3' cyclic phosphodiester; tRNA ligase

INTRODUCTION

Intron-containing tRNAs are encoded in all archaeal and eukaryal genomes. The intron is usually located in the anticodon loop of the pre-tRNA, and must be removed precisely for the tRNA to function in protein synthesis. Two incisions of the pre-tRNA at the exon–intron borders yield 2',3' cyclic phosphate and 5'-OH termini at both break sites (Peebles et al. 1983). The cleavages are performed by a tRNA splicing endonuclease that recognizes the fold of the pre-tRNA. The structure and mechanism of the splicing endonuclease are conserved among archaea and lower and higher eukaryal species (Trotta et al. 1997; Li et al. 1998; Paushkin et al. 2004; Xue et al. 2006).

By contrast, the tRNA repair phase of the splicing pathway differs in archaea and eukarya. The ends of cleaved archaeal tRNAs are rejoined in a single-step reaction entailing attack of the 5'-OH on the 2',3' cyclic phospho-

diester to form a 3',5' phosphodiester at the splice junction (Zofalova et al. 2000; Salgia et al. 2003). The archaeal protein that catalyzes the tRNA sealing reaction is not known. Yeast tRNA splicing occurs by an entirely different biochemical route. A single multifunctional tRNA ligase enzyme, Trl1, first heals and then seals the ends of the broken tRNA half-molecules. Trl1 performs three reactions: (1) the 2',3' cyclic phosphate of the proximal tRNA half-molecule is hydrolyzed to a 3'-OH, 2'-PO₄ by a cyclic phosphodiesterase (CPD); (2) the 5'-OH of the distal half-molecule is phosphorylated by a GTP-dependent polynucleotide kinase; and (3) the 3'-OH, 2'-PO₄, and 5'-PO₄ ends are sealed by an ATP-dependent RNA ligase to form a spliced tRNA containing an unconventional 2'-PO₄, 3'-5' phosphodiester at the splice junction (Greer et al. 1983). The 2'-PO₄ at the splice junction is ultimately removed by the 2'-phosphotransferase Tpt1 (Spinelli et al. 1999).

Yeast Trl1 consists of an N-terminal ligase domain (amino acids 1–388) and a C-terminal end-healing domain (amino acids 389–827). The ligase domain is an ATP-dependent strand-sealing enzyme that belongs to the ligase family exemplified by bacteriophage T4 RNA ligase 1. The end-healing domain is composed of distinct kinase and CPD modules (Fig. 1). The kinase module resembles T4 polynucleotide kinase (Wang et al. 2002, 2006). The T4 and

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FIGURE 1. Rat CNP complements *ts* mutations in yeast and plant tRNA ligase. (*Top* panel) The tRNA ligases of yeast (Trl1) and plant (AtRNL) are composed of three discrete catalytic domains: an N-terminal ligase module (magenta), a central 5'-OH polynucleotide kinase module (yellow), and a C-terminal RNA 2',3' cyclic phosphodiesterase (CPD) module (cyan). The positions of the P-loop motif at the kinase active site and the two HxT motifs that comprise the CPD active site are depicted above the yeast polypeptide. Rat CNP is composed of a C-terminal catalytic fragment (CF; cyan) that includes two HxT motifs, plus an N-terminal extension (yellow) that includes a putative P-loop motif. An alanine substitution for the first HxT histidine (indicated by a red asterisk) of Trl1 (H673A) or AtRNL (H999A) elicits a temperature-sensitive growth defect in yeast. (*Bottom* panel) Complementation by rat CNP was demonstrated by spotting on selective agar plates serial 10-fold dilutions of *trl1Δ pTRL1-H673A (ADE2 CEN)* or *trl1Δ AtRNL1-H999A (TRP1 CEN)* yeast cells that had been transformed with a *CEN TRL1* plasmid (positive control) or with 2μ plasmids bearing genes encoding wild-type CNP, a catalytically inert CNP mutant, or CNP-CF. Cells transformed with the empty 2μ vector served as a negative control. The plates were photographed after incubations for 3 d at 30°C, 35°C, or 37°C, 4 d at 23°C or 25°C, or 5 d at 20°C.

yeast kinases belong to the P-loop phosphotransferase superfamily and contain the signature NTP-binding "GKT" motif (Fig. 1). The CPD component belongs to the 2H phosphotransferase superfamily defined by two "HxT" motifs (Mazumder et al. 2002; Nasr and Filipowicz 2000). Trl1-like proteins composed of the same three modules in the same linear order are present in other fungi and in kinetoplastid protozoa (Wang and Shuman 2005). An *Arabidopsis* tRNA ligase ortholog (AtRNL) is a 1104-amino acid polypeptide with a similar tripartite domain structure (Fig. 1; Englert and Beier 2005; Wang et al. 2006). AtRNL is capable of performing all three essential splicing reactions in vivo in yeast cells that lack Trl1 (Wang et al. 2006).

A longstanding mystery of tRNA biology concerns the mechanism of tRNA exon ligation in animal cells and the identity of the enzymes involved. Initial studies demonstrated a direct tRNA end-joining reaction in human cell extracts in which the 2',3' cyclic phosphate is retained as a

3'-5' phosphodiester at the splice junction of the mature tRNA (Filipowicz and Shatkin 1983; Laski et al. 1983). Later work suggested that human cells also have a second yeast-like pathway of tRNA splicing (Zillman et al. 1991) in which the junction 3'-5' phosphate in mature tRNA derives from ATP and the starting 2',3' cyclic phosphate is converted to a 2'-PO₄ that is ultimately removed. The existence of a yeast-like tRNA repair system in metazoa is consistent with the presence of Tpt1-type phosphotransferase and RNA-specific 5'-OH polynucleotide kinase activities in human cells (Spinelli et al. 1998; Shuman and Hurwitz 1979). However, no Rnl1-type RNA sealing enzyme has been identified in a metazoan organism, and there is no apparent homolog of Rnl1 or the Trl1 ligase domain in any metazoan proteome.

Solving the "division of labor" problem for the two distinct human tRNA exon-joining pathways hinges on identifying the relevant enzymes. A recent advance was the attribution of RNA 5'-OH kinase activity to the human Clp1 protein (Weitzer and Martinez 2007). The crystal structure of yeast Clp1 revealed a central P-loop phosphotransferase module to which ATP was bound (Noble et al. 2007). Available evidence argues against yeast Clp1 being the catalyst of the 5' end-healing step of yeast tRNA splicing, insofar as Trl1 itself has the requisite kinase function

and kinase-inactivating mutations in Trl1 are lethal in vivo (Sawaya et al. 2003). However, the situation in human cells might be different. Human Clp1 can phosphorylate the 5'-OH end of cleaved pre-tRNA and, based on siRNA knockdown experiments, Clp1 is suggested to be an agent of mammalian tRNA splicing (Weitzer and Martinez 2007). This model, although attractive, remains to be tested by generating a mammalian cell line or organism in which Clp1 is either deleted or mutated in its active site.

The observation that RNA repair systems from heterologous sources can complement the catalytic function of one or all of the domains of yeast Trl1 (Schwer et al. 2004; Wang et al. 2006) provides a means to find or validate new proteins that can catalyze tRNA splicing reactions in vivo. Here we apply this approach to show that mammalian 2',3' cyclic nucleotide phosphodiesterase (CNP), a protein that has been studied extensively as a major constituent of myelin in the central nervous system (Braun et al. 2004),

can perform the 3' end-healing step of tRNA splicing in yeast. CNP is a member of the 2H phosphotransferase superfamily and is adept at hydrolyzing the O3'-P bond of 2',3' cyclic nucleotides to yield a nucleoside 2'-phosphate product.

RESULTS AND DISCUSSION

Mammalian CNP—An enzyme in search of a function

Mammalian CNP is a 400-amino acid polypeptide composed of a C-terminal catalytic fragment (CF) and a 150-amino acid N-terminal extension (Fig. 1; Lee et al. 2001). CNP constitutes 4% of total myelin protein in the central nervous system. CNP is expressed at lower levels outside the central nervous system, and it has been found to associate with mitochondria and cytoskeletal proteins (Bifulco et al. 2002; Lee et al. 2005, 2006). Homozygous CNP-knockout mice were viable, and showed no overt abnormalities up to 4 mo of age (Lappe-Siefke et al. 2003). Myelin in young knockout mice was of normal ultrastructure and protein composition, except for the absence of CNP. Older knockout mice developed progressive motor deficits and died prematurely because of diffuse brain axonal swelling and neurodegeneration leading to hydrocephalus (Lappe-Siefke et al. 2003). It is not known whether the late onset of neural disease is caused by an absence of the CNP protein (e.g., as a structural component of myelin or the cytoskeleton) or an absence of CNPase enzymatic activity. Indeed, there is an outstanding conundrum that 2',3' cyclic nucleotides are not present in appreciable amounts in mammalian cells, implying either that the catalytic activity of CNP is a red herring or that 2',3' cyclic mononucleotides are not the physiological substrate (Braun et al. 2004).

X-ray and NMR structures of the catalytic fragment (CF) highlighted a bilobar fold composed of two repeated $\alpha + \beta$ modules related by pseudotwofold symmetry (Kozlov et al. 2003; Sakamoto et al. 2005). The two HxT motifs are located at the active site. Based on these and other 2H protein structures (Hofmann et al. 2000, 2002; Kato et al. 2003; Kozlov et al. 2007), it is proposed that the histidines and threonines coordinate the scissile phosphodiester and promote its hydrolysis by transition-state stabilization and general acid-base catalysis. It has been appreciated for some time that mammalian CNP is biochemically and structurally akin to the CPD domain of yeast tRNA ligase. In fact, CNP can convert a 2',3' cyclic phosphodiester RNA terminus to a 3'-OH, 2'-PO₄ product in vitro (Filipowicz et al. 1983). The analogy is further strengthened by the fact that the N-terminal extension of CNP includes a prototypal P-loop NTP-binding motif (Fig. 1), much like the kinase-CPD domains of yeast Trl1 and the plant ortholog AtRNL.

Rat CNP performs the 3' end-healing step of tRNA splicing in vivo

To query whether mammalian CNP can act as a tRNA splicing enzyme, we expressed the full-length CNP protein in *Saccharomyces cerevisiae* under the control of a constitutive promoter on a 2 μ plasmid and asked whether CNP could complement the temperature-sensitive growth defect caused by the H673A mutation in the proximal HxT motif of the CPD module of yeast Trl1 (Sawaya et al. 2003). The *H673A* yeast strain failed to form colonies at $\geq 35^\circ\text{C}$; growth at nonpermissive temperatures was revived by a plasmid borne copy of wild-type *TRL1*, but not by an empty plasmid vector (Fig. 1). The instructive finding was that rat CNP restored growth at the restrictive temperature, signifying that the mammalian enzyme could act in lieu of Trl1 CPD in tRNA splicing (Fig. 1). Additional experiments showed that rat CNP could not complement a temperature-sensitive mutation in the kinase domain of yeast Trl1 (data not shown; also see below), implying that CNP is unable to perform the 5' end healing step of the tRNA splicing pathway, notwithstanding the presence of a P-loop motif in its N-terminal segment. Deletion of the N-terminal domain of rat CNP did not affect its ability to complement the CPD function of yeast Trl1, i.e., expression of the C-terminal catalytic fragment (CF) of rat CNP sufficed for growth of the *H673A* strain at elevated temperatures (Fig. 1).

The plant tRNA ligase AtRNL can provide all of the essential tRNA healing and sealing functions in a yeast *trl1 Δ* null background. The H999A mutation in the first HxT motif of the AtRNL CPD module elicits a temperature-sensitive growth defect in yeast, akin to the equivalent change in the CPD active site of Trl1 (Fig. 1; Wang et al. 2006). This growth defect was reversed by introduction of a 2 μ plasmid expressing either full-length CNP or the CNP catalytic fragment, but not by the empty vector control (Fig. 1). A key finding was that a catalytically defective CNP mutant (H230A–T232A–H309A–T311A) containing alanine substitutions for the histidines and threonines of both HxT motifs was unable to complement growth of the *AtRNL–H999A* strain at restrictive temperature (Fig. 1).

To consolidate the inference from these initial experiments that the 2',3' cyclic phosphodiesterase activity of rat CNP can replace the CPD activity of yeast or plant tRNA ligases, we tested the ability of CNP to substitute for lethal mutations in the CPD active site of AtRNL (Wang et al. 2006). Yeast *trl1 Δ* strains carrying *TRL1* on a *CEN URA3* plasmid were transformed with *CEN TRP1* plasmids bearing lethal *T1001A* and *H1060A* alleles of *AtRNL*. We introduced into these strains *HIS3* plasmids expressing either wild-type AtRNL, full-length rat CNP, the active CNP catalytic fragment, or the catalytically defective CNP mutant. *AtRNL–T1001A* and *AtRNL–H1060A* were unable to sustain growth on medium containing the drug 5-FOA,

which selects against the *URA3 TRL1* plasmid (Fig. 2, vector). Growth on 5-FOA was restored by a wild-type *AtRNL* gene, as expected. The salient findings were that CNP and the catalytic fragment complemented the lethal CPD mutations, whereas the catalytically defective mutant did not (Fig. 2).

CNP complementation of new conditional mutations in the Trl1 healing domain

To obtain additional genetic tools to study the end-healing steps of tRNA splicing, we mutagenized the kinase-CPD segment of the full-length yeast *TRL1* gene and screened for alleles that conferred a temperature-sensitive growth phenotype. This resulted in the isolation of five new *trl1-ts* mutants that grew at 25°C and 30°C, but failed to grow at 35°C or 37°C (Fig. 3). Each of the conditionally defective Trl1 polypeptides contained between two and four amino acid substitutions within the kinase-CPD domain. To delineate which, if any, of the new *ts* mutants were defective uniquely for CPD activity, we tested them for complementation by mammalian CNP. Growth of three of the five *trl1-ts* strains at restrictive temperature was fully restored by introduction of a CNP expression plasmid (Fig. 3). The

L734P-N773D double mutant that was complemented by CNP contained lesions that mapped exclusively within the CPD module, consistent with a singular defect in CPD activity at restrictive temperature. The *N514D-V748E* mutant rescued by CNP has one lesion in the CPD domain (V748E) and one in the kinase module (N514D); we surmise that the valine-to-glutamate change is the likely culprit for the growth defect. The *K449E-P589L-L780P* mutant has one lesion in the CPD domain (L780P) and two lesions in the kinase module (K449E P589L); its complementation by CNP implicates the L780P change in the growth phenotype. The diagnostic value of the complementation test was underscored by the finding that the *S44P-I487V* mutant, which has two lesions in the kinase domain, was not rescued by CNP (Fig. 3). CNP also failed to complement a quadruple mutant containing two changes in the kinase module (S556L D591G) plus two changes in the CPD module (L730P G786C). We performed additional experiments testing complementation of the new *trl1-ts* strains by a plasmid encoding the Trl1-H673A protein, which has an isolated defect in the CPD active site and cannot support growth at 37°C. *TRL1-H673A* complemented only one of the five *trl1-ts* strains (data not shown), that being *S44P-I487V*, which has mutations exclusively in the kinase module and could not be rescued by CNP.

FIGURE 2. Rat CNP complements lethal mutations in plant tRNA ligase. The plant tRNA ligase and rat CNP polypeptides are shown in the top panel with the CPD-inactivating alanine mutations at T1001 and H1060 of *AtRNL* highlighted in medium gray. Yeast *trl1Δ* p360-*TRL1* (*URA3 CEN*) cells containing *CEN TRP1* plasmids bearing lethal *AtRNL* alleles *T1001A* (left panel) or *H1060A* (right panel) were transformed with 2μ *HIS3* plasmids bearing genes encoding either wild-type CNP, a catalytically inert CNP mutant, or CNP-CF. Control cells were transformed with the empty 2μ *HIS3* vector (negative control) or a *CEN HIS3* plasmid bearing wild-type *AtRNL* (positive control). *Trp⁺ His⁺* transformants were selected at 30°C and then streaked on agar medium containing 0.75 mg/mL 5-fluoro-orotic acid (a drug that selects against the *URA3* plasmid with the wild-type *TRL1* gene). The plates were photographed after 4 d at 30°C.

Implications for 3' end healing in tRNA splicing and RNA repair

Here we provided genetic evidence that mammalian CNP is an RNA repair enzyme *in vivo* and is capable of performing the 3' end-healing step of yeast tRNA splicing. Mammalian CNP is structurally homologous to the CPD domain of yeast and plant tRNA ligases and the outcomes of the cyclic phosphodiesterase reaction (conversion to a 2'-PO₄) are the same. The presence of a 2'-PO₄ at the healed 3'-OH RNA end is required *in vitro* and *in vivo* for strand sealing by the ligase component of yeast Trl1 (Schwer et al. 2004; Keppetipola et al. 2007).

The RNA end-healing function of CNP could be relevant to either of two well-documented cases of programmed RNA breakage and repair in mammals: (1) tRNA splicing and (2) mRNA splicing during the endoplasmic reticulum unfolded protein response (UPR) (Ron and Walter 2007). tRNA ligase is the enzyme responsible for nonspliceosomal splicing of *HAC1* mRNA in the yeast unfolded protein response pathway (Sidrauski et al. 1996; Gonzalez et al. 1999). ER stress-induced cleavage of the *HAC1* mRNA by endonuclease Ire1 excises an intron and leaves 2',3' cyclic phosphate and 5'-OH termini at the cleavage sites. After healing and sealing by Trl1, the *HAC1* translation reading frame is altered so as to replace the C-terminal peptide of the uninduced form of Hac1 (encoded in the intron) with a different segment encoded in the second exon. The induced

FIGURE 3. Test of CNP complementation of new *ts* mutations in the Trl1 end-healing domain. Five new *trl1-ts* alleles bearing the indicated missense mutations in the kinase-CPD domain were isolated as described under Materials and Methods. The amino acid changes that map within the CPD module are underlined. The *ts* growth defect was verified by spotting serial 10-fold dilutions of *S. cerevisiae trl1Δ* cells bearing the indicated *CEN TRP1 TRL1* plasmids on YPD agar and incubating the plates at the temperatures specified. Each of the *trl1-ts* strains was tested for restoration of growth at restrictive temperatures after transformation with a 2 μ *HIS3* plasmid expressing rat CNP as described in Figure 1. Complementation of wild-type growth was scored as +++.

form of Hac1 is a transcription factor that activates certain nuclear genes in response to ER stress, especially genes that specify ER chaperones. The mammalian UPR also involves induced cleavage and unconventional splicing of an mRNA encoding a transcription factor, XBP1 (Calton et al. 2002). The identity of the mammalian mRNA repair enzymes in the UPR is not known.

The likely existence of two different RNA repair pathways in mammalian cells (yeast-type and archaeal-type) raises the interesting issue of whether they are functionally redundant or dedicated to a particular physiological setting. For example, one pathway might perform tRNA splicing while the other specializes in mRNA splicing in the UPR. The key feature of the yeast-type pathway is the need for end healing prior to sealing. The benign effects of completely deleting CNP in mice seem to exclude an essential role for the yeast-type splicing pathway, if one assumes that CNP, which is the source of all measurable 2',3'-cyclic phosphodiesterase activity in mouse brain (Lappe-Siefke et al. 2003), is the only available source of 3' end-healing for the yeast-like splicing pathway. This scenario would relegate the yeast-type pathway in mammals to either no role, or a functionally redundant role, in both tRNA splicing and the UPR. It is safe to assume that tRNA splicing is required for cell viability and would be lethal if disrupted in the mouse. The UPR is required for proper development of the pancreas and the immune system, neither of which appeared to be affected in CNP-knockout mice. If the yeast-type pathway is redundant, then other mammalian homologs of yeast-type tRNA repair proteins might also not be essential. This is the case for mammalian Tpt1, the ortholog of the yeast phosphotransferase that removes the 2'-PO₄ from the tRNA splice junction (Harding et al. 2007). The major challenge now is to identify the protein catalysts of the direct tRNA ligation pathway in mammalian cells (and archaea). Yeast complementation might afford a means to do so by screening mammalian cDNA libraries for functional complementation of a *trl1Δ* strain.

MATERIALS AND METHODS

Yeast vectors for expression of rat CNP

A 1.2-kb DNA fragment spanning the rat CNP ORF was amplified by PCR to introduce an EcoRI site immediately upstream of the ATG codon and an XhoI restriction site downstream of the TGA stop codon. The fragment was inserted into pYX232 (2 μ *TRP1*), a shuttle vector in which expression of CNP is under the transcriptional control of the constitutive *TPI1* promoter to generate the p232-CNP plasmid. The expression plasmids for CF (catalytic fragment, amino acids 151–400) and the catalytically defective CNP mutant (H230A–T232A–H309A–T311A) were generated similarly. All clones were sequenced to exclude the presence of unwanted mutations. The DNA fragments spanning the *TPI1*–CNP expression cassettes were inserted into pRS423 (*HIS3* 2 μ).

New temperature-sensitive mutations in the Trl1 end-healing domain

We generated a library of mutated *TRL1* alleles (*TRL1**) by PCR amplification of the gene segment encoding the kinase-CPD domain (amino acids 375–827) under conditions that promote nucleotide misincorporation. In brief, four PCR amplification reactions were performed in parallel in mixtures containing 2 mM MgSO₄, 0.1 mM MnCl₂, 50 μ M of one dNTP, and 200 μ M of the three other dNTPs. The PCR-amplified DNAs were pooled, digested with XhoI and SpeI, and then inserted into a *CEN TRP1 TRL1* plasmid in lieu of the corresponding wild-type restriction fragment. A p(*CEN TRP1 TRL1**) library harvested from a pool of ~100,000 bacterial transformants was introduced into a *S. cerevisiae trl1Δ* p(*CEN URA3 TRL1*) strain. Trp⁺ yeast colonies selected at 25°C were subjected to two rounds of replica-plating at 25°C on medium containing 0.75 mg/mL 5-fluoroorotic acid (FOA) to select for loss of the *URA3 TRL1* plasmid. About 1200 FOA-resistant *trl1Δ* p(*CEN TRP1 TRL1**) strains were arrayed on YPD agar master plates. Replica-plates were tested in parallel for growth at 25°C and 35°C. Plasmids were recovered from candidate *trl1-ts* strains that failed to grow at 35°C and amplified by transformation in *Escherichia coli*. Individual p(*CEN TRP1 TRL1**) plasmids were reintroduced into *trl1Δ* p(*CEN URA3 TRL1*) cells, which were subjected to plasmid shuffle and then screened for temperature-sensitive growth. Five confirmed

trl1-ts alleles were then sequenced completely to identify the coding changes implicated in conferring the conditional phenotype. In each case, missense mutations were identified within the targeted kinase-CPD segment (Fig. 3).

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REFERENCES

- Bifulco, M., Laezza, C., Stingo, S., and Wolff, J. 2002. 2',3'-Cyclic nucleotide 3'-phosphodiesterase: A membrane-bound, microtubule-associated proteins and membrane anchor for tubulin. *Proc. Natl. Acad. Sci.* **99**: 1807–1812.
- Braun, P.E., Lee, J., and Gravel, M. 2004. 2',3'-Cyclic nucleotide 3'-phosphodiesterase: Structure, biology and function. *Myelin Biol. Disord.* **1**: 499–521.
- Calfon, M., Zeng, H., Urano, F., Till, J.H., Hubbard, S.R., Harding, H.P., Clark, S.G., and Ron, D. 2002. IRE1 couples endoplasmic reticulum load to secretory capacity by processing the XBP-1 mRNA. *Nature* **415**: 92–96.
- Englert, M. and 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. doi: 10.1093/nar/gki174.
- Filipowicz, W. and Shatkin, A.J. 1983. Origin of splice junction phosphate in tRNAs processed by HeLa cell extract. *Cell* **32**: 547–557.
- Filipowicz, W., Konarska, M., Gross, H.J., and Shatkin, A.J. 1983. RNA 3'-terminal phosphate cyclase activity and RNA ligation in HeLa cell extract. *Nucleic Acids Res.* **11**: 1405–1418. doi: 10.1093/nar/11.5.1405.
- Gonzalez, T.N., Sidrauski, C., Dörfler, S., and Walter, P. 1999. Mechanism of non-spliceosomal mRNA splicing in the unfolded protein response pathway. *EMBO J.* **18**: 3119–3132.
- Greer, C.L., Peebles, C.L., Gegenheimer, P., and Abelson, J. 1983. Mechanism of action of a yeast RNA ligase in tRNA splicing. *Cell* **32**: 537–546.
- Harding, H.P., Lkey, J.G., Hsu, H.C., Zhang, Y., Deng, J., Xu, R.M., Dhama, M.J., and Ron, D. 2007. An intact unfolded protein response in *Trpt1* knockout mice reveals phylogenetic divergence in pathways for RNA ligation. *RNA* (this issue). doi: 10.1261/rna.859908.
- Hofmann, A., Zdanov, A., Genschik, P., Ruvinov, S., Filipowicz, W., and Wlodawer, A. 2000. Structure and mechanism of the cyclic phosphodiesterase of Apppr>p, a product of the tRNA splicing reaction. *EMBO J.* **19**: 6207–6217.
- Hofmann, A., Grella, M., Botos, I., Filipowicz, W., and Wlodawer, A. 2002. Crystal structures of the semireduced and inhibitor bound forms of cyclic nucleotide phosphodiesterase from *Arabidopsis thaliana*. *J. Biol. Chem.* **277**: 1419–1425.
- Kato, M., Shirouzu, M., Terada, T., Yamaguchi, H., Murayama, K., Sakai, H., Kuramitsu, S., and Yokoyama, S. 2003. Crystal structure of the 2'-5' RNA ligase from *Thermus thermophilus* HB8. *J. Mol. Biol.* **329**: 903–911.
- Keppetipola, N., Nandakumar, J., and Shuman, S. 2007. Reprogramming the tRNA-splicing activity of a bacterial RNA repair enzyme. *Nucleic Acids Res.* **35**: 3624–3630. doi: 10.1093/nar/gkm110.
- Kozlov, G., Lee, J., Elias, D., Gravel, M., Gutierrez, P., Ekiel, I., Braun, P.E., and Gehring, K. 2003. Structural evidence that brain cyclic nucleotide phosphodiesterase is a member of the 2H phosphodiesterase superfamily. *J. Biol. Chem.* **278**: 46021–46028.
- Kozlov, G., Denisov, A.Y., Pomerantseva, E., Gravel, M., Braun, P.E., and Gehring, K. 2007. Solution structure of the catalytic domain of RICH protein from goldfish. *FEBS J.* **274**: 1600–1609.
- Lappe-Siefke, C., Goebbels, S., Gravel, M., Nicksh, E., Lee, J., Braun, P.E., Griffiths, I.R., and Nave, K.A. 2003. Disruption of Cnp1 uncouples oligodendroglial functions in axonal support and myelination. *Nat. Genet.* **33**: 366–374.
- Laski, F.A., Fire, A.Z., RajBhandary, U.L., and Sharp, P.A. 1983. Characterization of tRNA precursor splicing in mammalian extracts. *J. Biol. Chem.* **258**: 11974–11980.
- Lee, J., Gravel, M., Gao, E., O'Neill, R.C., and Braun, P.E. 2001. Identification of essential residues in 2',3'-cyclic nucleotide 3'-phosphodiesterase. *J. Biol. Chem.* **276**: 14804–14813.
- Lee, J., Gravel, M., Zang, R., Thibault, P., and Braun, P.E. 2005. Process outgrowth in oligodendrocytes is mediated by CNP, a novel microtubule assembly myelin protein. *J. Cell Biol.* **170**: 661–673.
- Lee, J., O'Neill, R.C., Park, M.W., Gravel, M., and Braun, P.E. 2006. Mitochondrial localization of CNP2 is regulated by phosphorylation of the N-terminal targeting signal by PKC: Implications of a mitochondrial function for CNP2 in glial and non-glial cells. *Mol. Cell. Neurosci.* **31**: 446–462.
- Li, H., Trotta, C.R., and Abelson, J. 1998. Crystal structure and evolution of a transfer RNA splicing enzyme. *Science* **280**: 279–284.
- Mazumder, R., Iyer, L., Vasudevan, S., and Aravind, L. 2002. Detection of novel members, structure-function analysis and evolutionary classification of the 2H phosphodiesterase family. *Nucleic Acids Res.* **30**: 5229–5243. doi: 10.1093/nar/gkf645.
- Nasr, F. and Filipowicz, W. 2000. Characterization of the *Saccharomyces cerevisiae* cyclic nucleotide phosphodiesterase involved in the metabolism of ADP-ribose 1'',2''-cyclic phosphate. *Nucleic Acids Res.* **28**: 1676–1683. doi: 10.1093/nar/28.8.1676.
- Noble, C.G., Beuth, B., and Taylor, I.A. 2007. Structure of a nucleotide-bound Clp1-Pcf11 polyadenylation factor. *Nucleic Acids Res.* **35**: 87–89. doi: 10.1093/nar/gkl1010.
- Paushkin, S.V., Patel, M., Furia, B.S., Peltz, S.W., and Trotta, C.R. 2004. Identification of a human endonuclease complex reveals a link between tRNA splicing and pre-mRNA 3' end formation. *Cell* **117**: 311–321.
- Peebles, C.L., Gegenheimer, P., and Abelson, J. 1983. Precise excision of intervening sequences from precursor tRNAs by a membrane-associated yeast endonuclease. *Cell* **32**: 525–536.
- Ron, D. and Walter, P. 2007. Signal integration in the endoplasmic reticulum unfolded protein response. *Nat. Rev. Mol. Cell Biol.* **8**: 519–529.
- Sakamoto, Y., Tanaka, N., Ichimiya, T., Kurihara, T., and Nakamura, K.T. 2005. Crystal structure of the catalytic fragment of human brain 2',3'-cyclic nucleotide 3'-phosphodiesterase. *J. Mol. Biol.* **346**: 789–800.
- Salgia, S.R., Singh, S.J., Gurha, P., and Gupta, R. 2003. Two reactions of *Haloferax volcanii* RNA splicing enzymes: Joining of exons and circularization of introns. *RNA* **9**: 319–330.
- Sawaya, R., Schwer, B., and Shuman, S. 2003. Genetic and biochemical analysis of the functional domains of yeast tRNA ligase. *J. Biol. Chem.* **278**: 43298–43398.
- Schwer, B., Sawaya, R., Ho, C.K., and Shuman, S. 2004. Portability and fidelity of RNA-repair systems. *Proc. Natl. Acad. Sci.* **101**: 2788–2793.
- Shuman, S. and Hurwitz, J. 1979. 5'-Hydroxyl polyribonucleotide kinase from HeLa cell nuclei. Purification and properties. *J. Biol. Chem.* **254**: 10396–10404.

- Sidrauski, C., Cox, J.S., and Walter, P. 1996. tRNA ligase is required for regulated mRNA splicing in the unfolded protein response. *Cell* **87**: 405–413.
- Spinelli, S.L., Malik, H.S., Consaul, S.A., and Phizicky, E.M. 1998. A functional homolog of a yeast tRNA splicing enzyme is conserved in higher eukaryotes and in *Escherichia coli*. *Proc. Natl. Acad. Sci.* **95**: 14136–14141.
- Spinelli, S.L., Kierzek, R., Turner, D.H., and Phizicky, E.M. 1999. Transient ADP-ribosylation of a 2'-phosphate implicated in its removal from ligated tRNA during splicing in yeast. *J. Biol. Chem.* **274**: 2637–2644.
- Trotta, C.R., Miao, F., Arn, E.A., Stevens, S.W., Ho, C.K., Rauhut, R., and Abelson, J.N. 1997. The yeast tRNA splicing endonuclease: A tetrameric enzyme with two active site subunits homologous to the archaeal tRNA endonucleases. *Cell* **89**: 849–858.
- Wang, L.K. and Shuman, S. 2005. Structure–function analysis of yeast tRNA ligase. *RNA* **11**: 966–975.
- Wang, L.K., Lima, C.D., and Shuman, S. 2002. Structure and mechanism of T4 polynucleotide kinase—An RNA repair enzyme. *EMBO J.* **21**: 3873–3880.
- Wang, L.K., Schwer, B., Englert, M., Beier, H., and Shuman, S. 2006. Structure–function analysis of the kinase-CPD domain of yeast tRNA ligase (Trl1) and requirements for complementation of tRNA splicing by a plant Trl1 homolog. *Nucleic Acids Res.* **34**: 517–527. doi: 10.1093/nar/gkj441.
- Weitzer, S. and Martinez, J. 2007. The human RNA kinase hClp1 is active on 3' transfer RNA exons and short interfering RNAs. *Nature* **447**: 2222–2226.
- Xue, S., Calvin, K., and Li, H. 2006. RNA recognition and cleavage by a splicing endonuclease. *Science* **312**: 906–910.
- Zillman, M., Gorovsky, M.A., and Phizicky, E.M. 1991. Conserved mechanism of tRNA splicing in eukaryotes. *Mol. Cell. Biol.* **11**: 5410–5416.
- Zofalova, L., Guo, Y., and Gupta, R. 2000. Junction phosphate is derived from the precursor in the tRNA spliced by the archaeon *Haloferax volcanii* cell extract. *RNA* **6**: 1019–1030.

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