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tRNA Splicing: An RNA World Add-on or an Ancient Reaction?

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Introns are encoded in the genes for tRNA in organisms from all three kingdoms of life. Their removal is an essential step in the maturation of tRNA precursors. In Bacteria, introns are self-splicing and are removed by a group 1 splicing mechanism (Kuhsel et al. 1990; Reinhold-Hurek and Shub 1992; Biniszkiewicz et al. 1994). In Eukaryotes and Archaea, intron removal is mediated enzymatically by proteins. Recent progress in understanding both eukaryotic and archaeal tRNA splicing has revealed that the two processes, previously thought to be unrelated, are in fact similar. Insight gained from the comparison has provided a clearer understanding of intron recognition, the catalysis of intron removal, and has given new insight into the evolution of the tRNA splicing process.

INTRON-CONTAINING tRNA

tRNA Introns in Eukaryotes

Intervening sequences (introns) were discovered 20 years ago in the yeast genes for the tyrosine-inserting non-sense suppressor tRNA (Goodman et al. 1977) and for $tRNA^{Phe}$ (Valenzuela et al. 1978). With the completion of the yeast genome, it is known that of the 274 yeast tRNA genes 61, or 20%, contain introns. Table 1 lists the tRNAs that contain introns. PCR cloning of tRNAs from higher eukaryotes has revealed a similar distribution of intron-containing tRNA (Stange and Beier 1986; Green et al. 1990; Schneider et al. 1993). The introns in all of the genes are small (14–60 bases), and they are all located in the same position, one base to the 3′ side of the anticodon (Fig. 1A). Structure probing revealed that the common "cloverleaf" tertiary structure seen in the crystal structure of $tRNA^{Phe}$ is maintained in intron-containing tRNAs and that the intron, including the splice sites, is the most exposed region of the molecule

Table 1 Yeast tRNA precursors containing introns

tRNA	Intron length (nucleotides)	No. of genes
$tRNA^{Ser}_{CGA}$	19	1
$tRNA^{Ser}_{GCU}$	19	4
$tRNA^{Lys}_{UUU}$	23	7
$tRNA^{Pro}_{UGG}$	31,30,33	7,2,1
$tRNA^{Trp}_{CCA}$	34	6
$tRNA^{Phe}_{GAA}$	18,19	3,7
$tRNA^{Leu}_{CAA}$	32,33	8,2
$tRNA^{Ile}_{UAU}$	60	2
$tRNA^{Leu}_{UAG}$	19	3
$tRNA^{Tyr}_{GUA}$	14	8
		Total = 61
	Total tRNA genes = 274	

(Swerdlow and Guthrie 1984; Lee and Knapp 1985). This led to a proposed model for the tertiary structure of pre-tRNA shown in Figure 1B. More recent experiments by Tocchini-Valentini and coworkers (Baldi et al. 1992) have demonstrated that a conserved base pair (the A-I base pair) between a base of the 5′ exon (position 32) immediately following the anticodon stem and a base in the single-stranded loop of the intron (position –3) is required for correct excision at the 3′ splice site (Fig. 1A). Thus, the distinctive feature in eukaryotic intron-containing pre-tRNAs is the position of the intron and the presence of the A-I base pair.

tRNA Introns in Archaea

tRNA introns are found in every archaeal species that has been studied (Thompson et al. 1989; Kleman-Leyer et al. 1997; Lykke-Anderson and Garrett 1997; Klenk et al. 1998). Although they are often similar, archaeal introns are different from their eukaryotic counterparts. Comparative sequence analysis of a host of archaeal introns has revealed that the 5′ and 3′splice sites are always located in a 3-nucleotide bulge separated by a 4-bp helix: the bulge-helix-bulge (BHB) motif (Fig. 2) (Thompson et al. 1989). Intron length is variable, from a short intron of 33 nucleotides found in the $tRNA^{Met}$ gene of *Methanococcus jannaschii*, to 105 nucleotides of the $tRNA^{Trp}$ gene of *Haloferax volcanii* (Daniels et al. 1985), but the intron is required to base-pair with the 5′ exon to allow formation

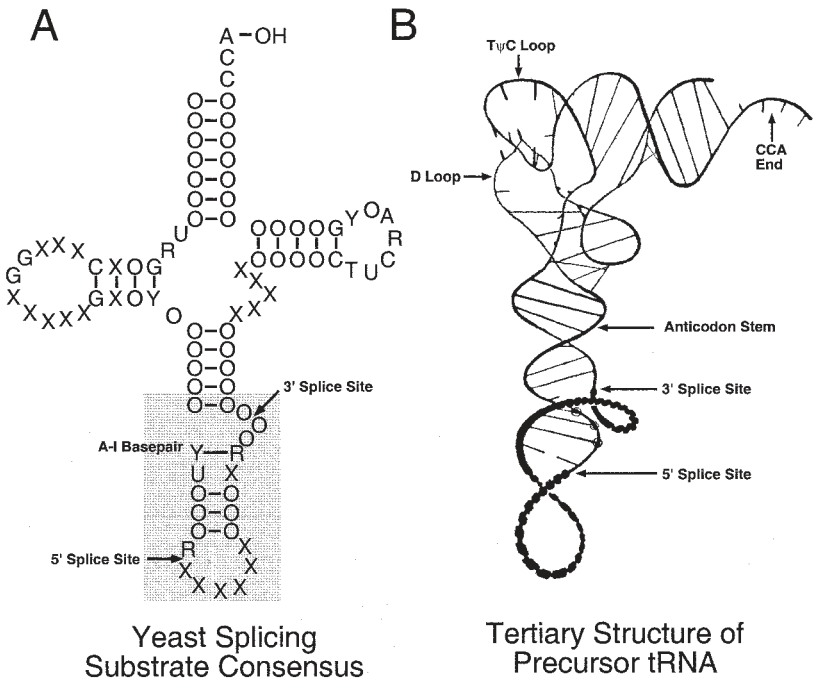

Figure 1 (*A*) Consensus of yeast pre-tRNA (Ogden et al. 1984). (O) Nonconserved base in a region of conserved length or secondary structure; (X) nonconserved base in a region of variable length or secondary structure. (G,C,A,U) Conserved base among the pre-tRNAs. (Y and R) Conserved pyrimidines and purines, respectively. The conserved anticodon-intron (A-I) base pair is depicted. (*B*) Model of the tertiary structure of pre-tRNAs based on the crystal structure of yeast $tRNA^{Phe}$. Intron is indicated in bold, dashed lines. (Reprinted, with permission, from Lee and Knapp 1985.)

of the BHB motif. In general, the introns reside one base 3′ to the anticodon, but introns have been found elsewhere in the tRNA molecule. One such example is that of the $tRNA^{Pro}$ gene of *Methanobacterium thermoautotrophicum*, where there are two introns in the anticodon stem that must be removed in an obligate order to produce the mature tRNA (C.R. Trotta, unpubl.). Another repository for introns in Archaea is the rRNA genes. In fact, one of the earliest discovered archaeal introns, the 622-nucleotide intron of the 23S rRNA of *Desulfurococcus mobilis*, was shown through extensive biochemical characterization to be removed by the archaeal tRNA splicing system with dependence on the BHB motif (Kjems and Garrett 1985; Kjems et al. 1989).

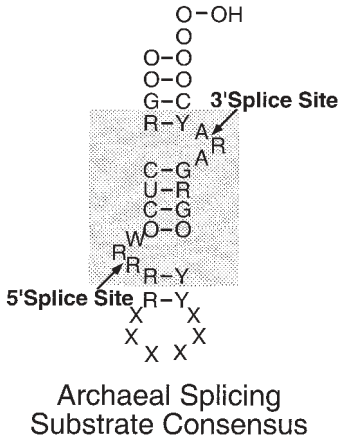

Figure 2 Consensus archaeal substrate. Positions depicted as in Fig. 1A. The bulge-helix-bulge (BHB) motif is shaded in gray.

THE ENZYMATIC GYMNASTICS OF tRNA SPLICING

The Eukaryotic Pathway

The accumulation of pre-tRNAs in the *rna1-1* mutant (Hopper et al. 1978) provided a source of pre-tRNA for assay of tRNA splicing in vitro (Knapp et al. 1978). The system was employed to work out the pathway of tRNA splicing (Fig. 3). The first step of the reaction is recognition and cleavage of the pre-tRNA substrate at the 5′ and 3′ splice sites by a tRNA splicing endonuclease. The cleavage by the endonuclease at the splice sites results in 2′,3′ cyclic phosphate and 5′-hydroxyl termini (Peebles et al. 1983). The tRNA 5′ and 3′ exons are then the substrate for a series of reactions catalyzed by the tRNA splicing ligase (for a detailed review of the ligation mechanism, see Arn and Abelson 1998). Ligase is a multifunctional enzyme possessing phosphodiesterase, polynucleotide kinase, and RNA ligase activities. In the ligase reaction, phosphodiesterase opens the 2′,3′ cyclic phosphate to give a 2′-phosphate. Next the 5′-hydroxyl is phosphorylated by transfer of the γ-phosphate from an NTP cofactor. Ligase is then adenylylated and the AMP moiety is transferred to the 5′-phosphate on the 3′-exon forming an activated phosphoanhydride. Finally, ligase joins the two exons by catalyzing an attack of the 3′-hydroxyl on the activated donor phosphoanhydride to form a 3′,5′ phosphodiester, 2′-monophosphoester bond with the release of AMP. This results in a pre-tRNA molecule with a 2′-phosphate at the splice junction. Removal of the 2′-phosphate is accomplished by the activity of a 2′-phosphotransferase

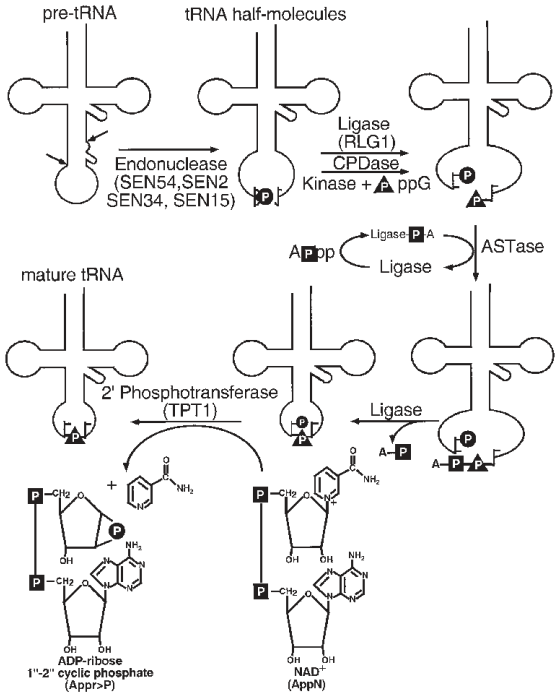

Figure 3 The tRNA splicing pathway of yeast. Gene names are given in parentheses for the proteins of the pathway. (CPDase) Cyclic phosphodiesterase; (ASTase) adenylyl synthetase. See text for details. (Reprinted, with permission, from Abelson et al. 1998.)

that catalyzes the transfer of the phosphate to NAD, releasing nicotinamide and yielding ADP-ribose 1″-2″ cyclic phosphate (Appr>P) and mature tRNA (Fig. 3).

The Archaeal Pathway

Much less is known about the enzymes involved in the processing of archaeal pre-tRNA due to the difficulty in working with extracts from these organisms. As in the eukaryotic pathway, removal of introns requires the function of both an endonuclease and a ligase. The archaeal endonuclease recognizes substrates containing the consensus BHB motif and cleaves at the 5′ and 3′ splice sites to yield 2′,3′ cyclic phosphate and 5′-hydroxyl termini (Thompson et al. 1989). Thus, the cleavage mechanism is identical to the eukaryotic mechanism, providing the earliest clue to the relatedness of the two enzymes (see below). Upon cleavage by the endonuclease, the tRNA half-molecules must be ligated to produce a mature tRNA,

but the ligase activity has yet to be defined. It is clear that the yeast mechanism does not pertain since the γ-phosphate of ATP is not incorporated into spliced tRNA as seen in the yeast mechanism (Kjems and Garrett 1985; Gomes and Gupta 1997).

CHARACTERIZATION OF THE tRNA SPLICING ENZYMES

The Eukaryotic tRNA Endonuclease

The first step of tRNA splicing is the recognition and cleavage of the splice sites by the tRNA splicing endonuclease. Utilizing the in vitro system for tRNA splicing, the endonuclease was shown to be nuclear-membrane-associated (Peebles et al. 1983). The detergent, Triton X-100, at 0.9% is required to release the activity from the membrane fraction. In a particularly difficult purification, the endonuclease was purified one million fold from a yeast nuclear membrane preparation to homogeneity and appeared to be a αβγ trimer of 54-kD, 44-kD, and 34-kD subunits (Rauhut et al. 1990). Later, a fourth subunit of 15-kD was discovered (Trotta et al. 1997). Thus, the enzyme is a αβγδ heterotetramer. Cloning of the genes encoding each of these protein subunits was accomplished by both genetics and protein chemistry. Ho et al. (1990) screened a bank of temperature-sensitive mutants and isolated a yeast mutant that accumulated 2/3 tRNA precursor molecules that were not cleaved at the 5′ splice site. The gene, SEN2 (*s*plicing *en*donuclease) (Winey and Culbertson 1988), was cloned by complementation, and antibodies to the product were shown to recognize the 44-kD subunit of the purified splicing endonuclease. Development of a simpler affinity purification utilizing a tagged SEN2 gene allowed preparation of sufficient material to identify the other three gene products, SEN54, SEN34, and SEN15 (Trotta et al. 1997).

Two of the subunits, SEN2 and SEN34, contain a homologous domain of approximately 130 amino acids. SEN2 contains the only transmembrane sequence and presumably mediates membrane association of the enzyme. All four subunits contain a nuclear localization sequence. Two-hybrid interactions were detected between Sen54-Sen2 and Sen34-Sen15. Since the enzyme is a stable tetramer, interaction must occur between these two presumed heterodimers although they are not detected in the two-hybrid assay (Trotta et al. 1997).

The Eukaryotic tRNA Ligase

The purification of the soluble tRNA ligase from yeast was simpler and quickly led to the isolation of a 95-kD polypeptide and the cloning of its

essential gene, RLG1 (Phizicky et al. 1986, 1992). This single polypeptide carries out all three of the steps in the ligase mechanism (Fig. 3). Interestingly, partial proteolysis of the protein resulted in three fragments that were shown to contain distinct activities supporting a domain-like structure of the enzyme (Xu et al. 1990). The amino-terminal fragment is adenylylated at lys-114, and sequence comparisons have shown this lysine residue to be equivalent to the active lysine of the T4 RNA ligase (Xu et al. 1990; Apostol et al. 1991). The carboxy-terminal fragment contains the cyclic phosphodiesterase activity. The central domain then would be the likely locus of the kinase activity; however, an enzyme deleted in this domain paradoxically retains ligase activity. The enzyme has two nucleotide-binding sites, for GTP and for ATP (Belford et al. 1993). GTP is employed in the kinase step and ATP is used for the formation of the activated adenylyl-RNA intermediate. The in vivo function of such a complex NTP requirement is unclear, but it has been suggested that such a requirement could couple the splicing reaction to transcription and/or translation (Belford et al. 1993).

The complexity of the domain structure of ligase raises interesting questions as to the origin of this protein. The yeast tRNA ligase is mechanistically and structurally related to phage T4 RNA ligase and polynucleotide kinase (Apostol et al. 1991). The phage enzymes together contain the three activities present in the tRNA ligase, but in addition, T4 polynucleotide kinase contains a phosphatase that removes the 2′-phosphate (Walker et al. 1975). Thus, T4 RNA ligase and T4 polynucleotide kinase can ligate pre-tRNA half-molecules produced by endonuclease, yielding a product that does not contain a 2′-phosphate.

The 2′-Phosphotransferase

Early investigation into the activity responsible for 2′-phosphate removal at the splice junction demonstrated the requirement for two separate components present in yeast extracts (McCraith and Phizicky 1990). The first component was purified and determined to be the cellular cofactor NAD^+ (McCraith and Phizicky 1991). As described earlier, NAD^+ is the receptor in a reaction that involves the transfer of the 2′-phosphate at the splice junction to the 2″ position of the ribose of NAD^+, yielding the high-energy compound ADP-ribose 1″-2″ cyclic phosphate (Culver et al. 1993). The second component was purified and shown to be a 26.2-kD polypeptide encoded by the TPT1 gene (Culver et al. 1997). Expressed in *Escherichia coli*, the single polypeptide could catalyze the transfer of the 2′-phosphate to NAD^+. The gene was shown to be essential in yeast, and

it was suggested that its essential role could be either removal of the 2′-phosphate from all intron-containing tRNA molecules or generation of the novel molecule Appr>p. To further understand the role of each of these potential functions, a conditional lethal phosphotransferase mutant was generated (Spinelli et al. 1997). Yeast expressing this mutant accumulated tRNA molecules with a 2′-phosphate at the splice junction. Interestingly, these tRNA molecules were undermodified at positions near the splice junction residue, whereas other modifications appeared to be normal. These results suggest that the removal of the 2′-phosphate is essential for correct modification of the residues near the splice junction and that tRNA containing the 2′-phosphate is not a substrate for the modification enzyme and likely inactive in carrying out its function in translation. The results also suggest a temporal order for the maturation of pre-tRNA with splicing and 2′-phosphate removal occurring before modification of certain positions in the tRNA molecule. The TPT1 gene was also found to have homologs in other eukaryotes and, surprisingly, in *E. coli* and some Archaea (Culver et al. 1997; Trotta et al. 1997). The function of this protein in Bacteria and Archaea is unclear, since a source of 2′-phosphates is not apparent (see the discussion on the archaeal ligase mechanism below). *E. coli* does not possess intron-containing tRNA or a eukaryotic-like RNA ligase. It will therefore be interesting to elucidate its role in bacteria, hopefully providing a clue as to the origin of this unique processing event.

The Archaeal tRNA Endonuclease

The endonuclease of members of the Archaea proved to be just as difficult to purify as those of Eukaryotes. Daniels and coworkers chose the halophilic archaeon *Haloferax volcanii* to carry out the purification. The enzyme is present in low abundance and is extremely difficult to stabilize during the purification protocol. When finally purified, the endonuclease of *H. volcanii* proved to be composed of a single 37-kD protein encoded by the EndA gene (Kleman-Leyer et al. 1997). Unlike the heterotetrameric yeast enzyme, the active enzyme is composed of a single subunit shown by gel filtration to behave as a homodimer in solution. The protein was shown to contain a 130-amino-acid domain homologous to the two yeast subunits SEN2 and SEN34. This was the observation that unified the two lines of research and led to the progress described below.

The Archaeal tRNA Ligase

The identity of the archaeal tRNA ligase is at present unknown; however,

with the complete genome sequence of four members of the Archaea, it is clear that there is no homolog of the yeast tRNA splicing ligase (a remarkable statement to be able to make, now common in the genomic sequence era). Thus, it appears that ligation of tRNA half-molecules occurs via a different reaction mechanism from that of the eukaryotic ligation reaction.

All sequenced archaeal genomes do, however, contain an open reading frame encoding a protein homologous to the 2′-5′ ligase of *E. coli* characterized by E. Arn (Arn and Abelson 1996). This protein functions in vitro as an RNA ligase joining tRNA half-molecules to form a 2′-5′ phosphodiester bond at the splice junction. The exact mechanism of this reaction is unknown, but it does not require the addition of exogenous ATP. Thus, it is tempting to speculate that the homolog found in Archaea may function to ligate the tRNA half-molecules of the splicing reaction. 2′-5′ linkages have not been detected in tRNA, but it is possible that the archaeal ligase has an altered specificity and catalyzes the formation of a 3′-5′ linkage. Clearly, it will be interesting to further investigate the archaeal homologs of the 2′-5′ ligase.

tRNA Splicing in Higher Eukaryotes

Like the yeast tRNA introns, the position of the introns in higher eukaryotes is conserved and the cleavage reaction operates in a manner identical to the yeast tRNA splicing endonuclease as exemplified by the *Xenopus* endonuclease. Ligation of the half-molecules to yield mature tRNA has been shown to proceed through a mechanism similar to the yeast ligase (Konarska et al. 1981; Schwartz et al. 1983; Zillmann et al. 1991). Using HeLa cell extracts, Zilmann et al. (1991) have demonstrated the incorporation of exogenous γ-phosphate from ATP upon ligation of yeast $tRNA^{Phe}$, a hallmark of the yeast tRNA ligase reaction mechanism. Furthermore, they demonstrated the presence of a 2′-phosphate at the splice junction. A 2′-phosphotransferase activity was detected in both HeLa extracts (Zillmann et al. 1992) and is implicated in the dephosphorylation of ligated tRNA in microinjected *Xenopus* oocytes (Culver et al. 1993). However, early studies implicated a different ligase in tRNA splicing in HeLa extracts and *Xenopus* oocytes. It was demonstrated that the product of the tRNA splicing reactions catalyzed by these extracts did not contain a 2′-phosphate at the splice junction. This novel ligase uses the cyclic phosphate at the end of the 5′ half of tRNA to generate the 3′,5′-phosphodiester bond in the mature tRNA, as there is no incorporation of exogenous phosphate at the splice junction (Nishikura and De Robertis

1981; Filipowicz et al. 1983; Laski et al. 1983). Thus, there appear to be redundant pathways for ligation of tRNA molecules in metazoans. The further characterization of the alternate eukaryotic ligase activity could conceivably shed light on the nature of the archaeal ligase mechanism.

RECOGNITON OF PRE-tRNA BY THE tRNA SPLICING ENDONUCLEASE

Eukaryotic Endonuclease: The Ruler Mechanism

Early speculation concerning the ability of the tRNA endonuclease to recognize and cleave tRNA substrates relied on comparison of the primary and tertiary sequences of the precursor molecules. As previously mentioned, there is no sequence conservation around the splice sites and the precursors all fold into the same tertiary structure. The introns are of variable sequence and can be altered by both addition and deletion of nucleotides as well as changes in the sequence with little effect on removal by endonuclease (Johnson et al. 1980; Strobel and Abelson 1986; Reyes and Abelson 1988). The exception to this generalization is the A-I base pair necessary for accurate cleavage at the 3′ splice site. Mutations that abolish this base pair are not substrates for the splicing endonuclease (Willis et al. 1984; Baldi et al. 1992). This led to the hypothesis that recognition involved specific interactions with the conserved tertiary structure present in the mature domain of the pre-tRNA. Experimental support for this hypothesis involved the engineering of mutant pre-tRNA, which altered distinct features of the primary, secondary, or tertiary structures of the molecule (for review, see Culbertson and Winey 1989). This suggested a ruler model for recognition and cleavage by the endonuclease in which the enzyme recognizes the mature domain and measures the conserved distance to the splice sites (Fig. 4) (Greer et al. 1987; Reyes and Abelson 1988). To test this model, tRNA molecules were constructed in which the conserved distance was altered by insertion and deletion of base pairs in the anticodon stem. Addition of a single base pair increased the length of the intron by 2 nucleotides, one at either end (Reyes and Abelson 1988) as predicted by the model.

Archaeal Endonuclease: The Bulge-helix-bulge Motif

In contrast to the yeast results, it was demonstrated that for the archaeal endonuclease, the pre-tRNA substrate could be drastically altered and

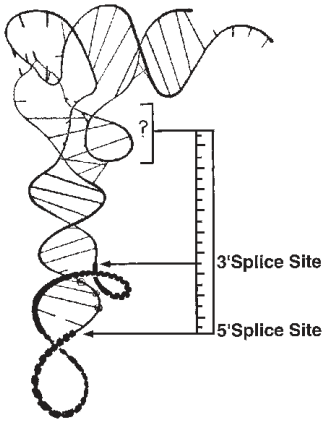

Figure 4 Ruler model for interaction of the yeast endonuclease with pre-tRNA (see text). (Reprinted, with permission, from Reyes and Abelson 1988 [copyright Cell Press].)

cleavage would still occur. Synthetic tRNA molecules where almost the entire tRNA mature domain was removed were accurately cleaved by the endonuclease (Thompson and Daniels 1988). As in the case of the eukaryotic endonuclease, the intron plays a mostly passive role, as large deletions can be tolerated by the enzyme. There is, however, a strict requirement for sequence and structure present at the exon–intron boundaries (Thompson and Daniels 1988). This bulge-helix-bulge (BHB) motif is the only requirement for intron recognition and removal by the archaeal tRNA splicing endonuclease.

GENES FOR THE ENDONUCLEASE: A BRIDGE BETWEEN KINGDOMS

Once the genes for the eukaryotic and archaeal endonuclease were in hand, a comparison between the yeast subunits and the single *H. volcanii* endonuclease revealed that these proteins were related. A domain of 130 amino acids is found in the carboxyl terminus of the SEN2 and SEN34 genes of the yeast endonuclease and in the *H. volcanii* endonuclease. Furthermore, homologs of this domain were discovered in the Archaea *M. jannaschii* and *P. aerophilum*, where the domain represented the entirety of the protein (Fig. 5). This homology led to the suggestion that the two yeast subunits each contain an active site for the cleavage reaction. It had been demonstrated that a mutant in the SEN2 gene, *sen2-3*, was defective in 5′ splice-site cleavage (Ho et al. 1990). This mutation, Gly-292 to glu-

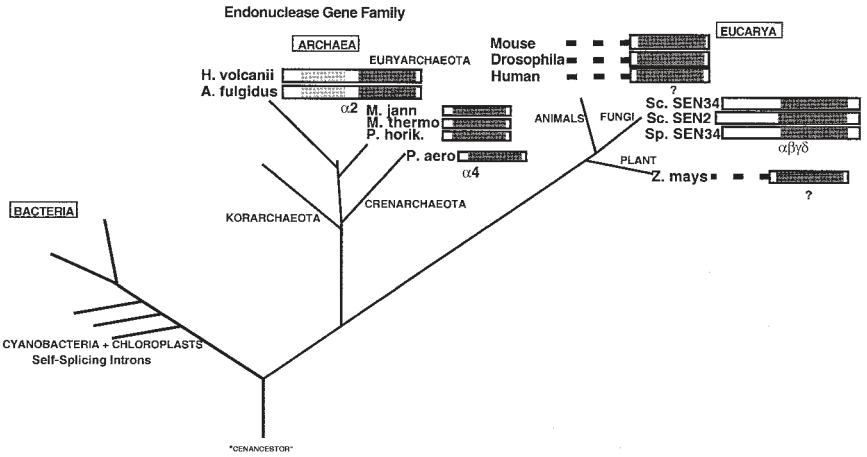

Figure 5 Graphic representation of archaeal and eukaryotic endonuclease proteins. The sizes of the various proteins (*open rectangle*) and location of the conserved sequence regions (*black box*) are indicated. Light gray blocks represent a degenerative domain repeat present in several archaeal endonucleases (see text). Proteins are placed on the Woesnian evolutionary tree. Endonuclease configuration is noted (see text for details): ($\alpha 4$) tetramer; ($\alpha 2$) dimer; ($\alpha\beta\gamma\delta$) heterotetramer; (?) configuration unknown. Protein designations: (H. volcanii) *H. volcanii* EndA (AF001578); (A. fulgidus) *Archaeoglobus fulgidus* (AE001041); (M. jann) *M. jannaschii* (MJ1424); (M. thermo) *Methanobacterium thermoautotrophicum* (AF001577); (P. horik) *Pyrococcus horikoshii* (AB009474); (Sc Sen2) *Saccharomyces cerevisiae* Sen2 (P16658); (Sc Sen34) *S. cerevisiae* Sen34 (P39707); (Sp Sen34) *Schizosaccharomyces pombe* Sen34; (Z. mays) *Zea mays* open reading frame contained in the intron of HMG (X72692); Mouse, Drosophila, and Human represent homologs of the endonuclease domain that have been detected in a partially sequenced genomic database submission (C.R. Trotta, unpubl.).

tamate, lies within the conserved domain, suggesting that SEN2 contains the active site for 5′ splice-site cleavage and, by extension, that SEN34 carries the active site for 3′ splice-site cleavage. A mutant in SEN34 changing a conserved histidine at position 242 to alanine resulted in a marked decrease in cleavage at the 3′ splice site, whereas cleavage at the 5′ splice site was normal, strongly supporting the two-active-site model (Trotta et al. 1997).

In the dimeric *H. volcanii* endonuclease, identical subunits cleave the symmetrically disposed splice sites in BHB substrate. Thus, the arrangement of active sites in eukaryotic and archaeal endonucleases must be

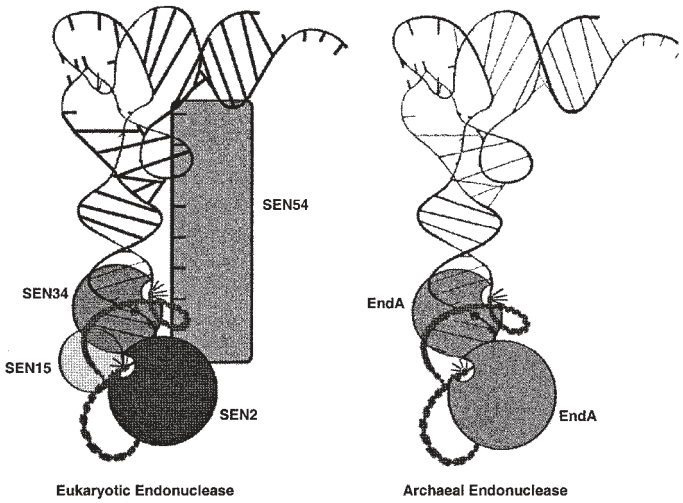

Figure 6 Comparison of the models proposed for the eukaryotic (yeast) and archaeal (*H.volcanii*) endonucleases (see text for details). (Reprinted, with permission, from Li et al. 1998 [copyright American Association for the Advancement of Science].)

similar (as depicted in Fig. 6).

tRNA SPLICING AT THE ATOMIC LEVEL

Given the relatedness of the tRNA splicing endonuclease from the two kingdoms, it seemed expedient to choose the archaeal system for structural work. The endonuclease of the archaeon *M. jannnaschii* was chosen, because this enzyme consists of only the homologous domain implicated by the endonuclease sequence alignment. It is small and proved easy to express and purify.

Extensive biochemical characterization of this enzyme by Lykke-Andersen and Garrett (1997) showed it to be a homotetramer in solution, an observation confirmed by the crystal structure (Fig. 7B) (Li et al. 1998). Each monomer consists of two domains: the amino-terminal domain (residues 9–84), which is composed of three α helices and a mixed antiparallel/parallel β-pleated sheet of four strands, and the carboxy-terminal domain (residues 85–179), which contains two α helices flanking a five-stranded mixed β-sheet (Fig. 7A). The manner in which four of these monomers are brought together provides insight into the architecture and evolution of other members of the endonuclease family of both Archaea and eukaryotes.

Two sets of interactions are crucial to formation of the tetramer. The

first interaction involves the formation of an isologous dimer between two monomers. Formation of this dimer is mediated by the interaction of β9 from one monomer and β9′ from another monomer. This tail-to-tail interaction is mediated by main-chain hydrogen bonds between

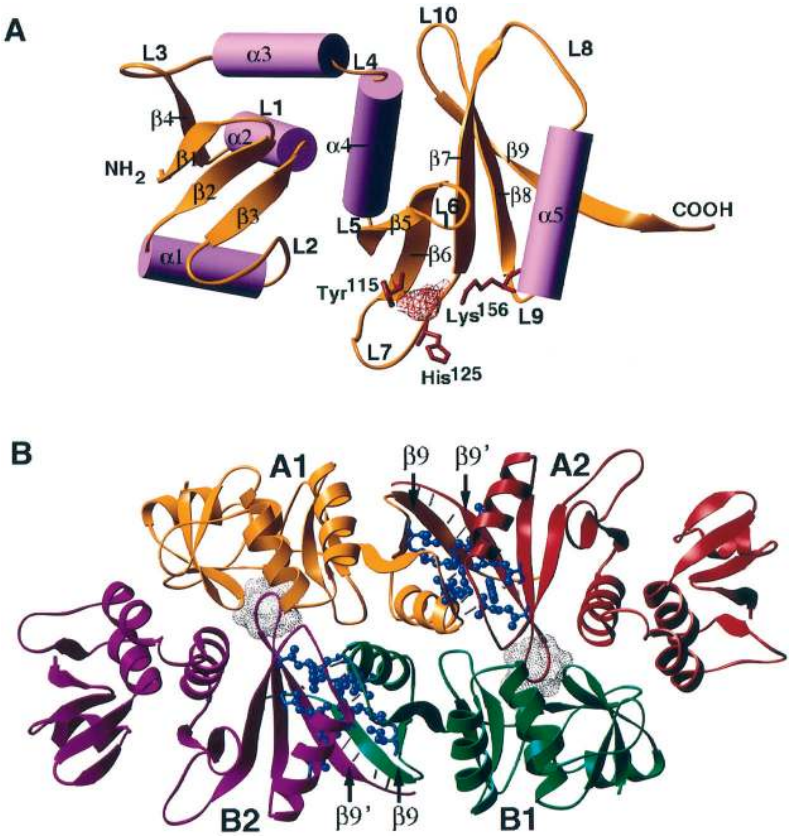

Figure 7 Crystal structure of the *M. jannaschii* tRNA splicing endonuclease. (*A*) Ribbon representation of the endonuclease monomer. The proposed catalytic triad residues are within 7 Å of one another and are shown in red ball-and-stick. Also shown is electron density detected near the putative catalytic triad, where the scissile phosphate is proposed to be located for cleavage. (*B*) Endonuclease tetramer. Each subunit is represented by a distinct label and color. The main-chain hydrogen bonds formed between β9 and β9′ and between loops L8 and L8′ for the formation of isologous dimers are shown as thin lines. Side chains of the hydrophobic residues enclosed at the dimer interface are shown as blue ball-and-stick models. The heterologous interaction that forms the tetramer is mediated by interaction between subunits A1 and B2 (or B1 and A2) through the acidic loops L10 and L8 and is highlighted by dotted surfaces. (Reprinted, with permission, from Li et al. 1998 [copyright American Association for the Advancement of Science].)

monomers and leads to a two-stranded β-sheet spanning the subunit boundary. Loop L8 from both monomers interacts to form more hydrogen bonds, and together these interactions enclose a hydrophobic core at the subunit interface. This leads to an extremely stable dimeric unit that Li et al. (1998) have suggested is conserved in endonucleases from other Archaea and eukaryotes (see below).

The second interaction involves the heterologous interaction between the two dimers that compose the tetramer. The main interaction is an insertion of acidic residues in loop L10 into a polar groove formed between the amino- and carboxy-terminal domains of the endonuclease monomer (Fig. 7B). This causes the two dimers to be translated relative to each other by about 20 Å and brings two subunits, A1 and B1, much closer together than the other two subunits, A2 and B2. This interaction is essential to the configuration of the two symmetrically disposed active sites and is also proposed to be conserved in the endonucleases from other organisms (see below).

Active Site of the tRNA Splicing Endonuclease

As discussed earlier, the product of the endonuclease cleavage reaction is a 2′,3′ cyclic phosphate and a 5-hydroxyl, a product identical to that of other ribonucleases such as RNase A. In a well-characterized acid–base-catalyzed reaction, RNase A utilizes a histidine, His-12, to abstract a proton from the 2′-hydroxyl of the ribose, leading to an in-line attack on the adjacent phosphodiester bond and the formation of a pentacovalent reaction intermediate stabilized by Lys-41. The general acid, His-119, then protonates the 5′-leaving group leading to the 2′,3′ cyclic phosphate and 5′-hydroxyl product. In a second step, a proton is abstracted from a water molecule, OH^- attacks, and the 2′,3′ cyclic phosphate is hydrolyzed to the 3′-phosphate (Walsh 1979; Thompson and Raines 1994). Inspection of the sequence alignment between the conserved members of the endonuclease family showed a single histidine residue that is absolutely conserved. Recall that the histidine-to-alanine mutation in Sen34 causes a marked reduction in the catalytic efficiency of 3′ splice-site cleavage. Similar mutants have been created in this conserved histidine of *M. jannaschii* and *H. volcanii* with a similar reduction in the catalytic efficiency of the cleavage reaction (Lykke-Andersen and Garrett 1997; C.J. Daniels, pers. comm.). Thus, it would appear that the tRNA splicing endonucleases catalyze the cleavage of RNA by a general acid–base catalysis.

With the crystal structure for the *M. jannaschii* endonuclease, this histidine was localized at the atomic level. Figure 7A shows the environment surrounding His-125, which is found in a cluster with the absolutely con-

served residues Tyr-115 (from loop L7) and Lys-156 (from α5), forming a pocket into which the scissile phosphate is proposed to fit. The three residues can be spatially superimposed with the catalytic triad of Rnase A (Li et al. 1998), leading to the prediction that His-125 of the *M. jannaschii* enzyme is equivalent to the general base, Tyr-115 is equivalent to the general acid, and Lys-156 stabilizes the transition state. Preliminary experiments indicate a role for both His-125 and Lys-156, but the role of Tyr-115 has yet to be established (C.R. Trotta, unpubl.).

Because the *M. jannaschii* enzyme is a homotetramer, each monomer is expected to contain a separate active site for cleavage. However, it is expected that only two of these active sites function in cleavage of the symmetric substrate. Recently, Diener and Moore (1998) have solved the NMR solution structure of a true archaeal substrate molecule. This molecule and a similar modeled substrate based on the TAR RNA structure (Li et al. 1998) dock in plausible fashion with the enzyme (Fig. 8). The scissile phosphates are shown to fit nicely into the A1 and B1 active sites of the tetramer. The A2 and B2 active sites are too far apart to allow for interaction with the substrate.

ON THE EVOLUTION OF THE ENDONUCLEASES

The Archaeal Endonucleases

The archaeal endonuclease of *H. volcanii* appears to be configured in a different manner from the *M. jannaschii* endonuclease. The protein behaves as a homodimer, not a homotetramer, but must be arrayed in a similar manner due to the identical substrate specificity. Based on the observation that the *H. volcanii* endonuclease is actually an in-frame duplication of the endonuclease domain, Li et al. (1998) proposed a model to describe how this enzyme is configured (Fig. 9B). The *H. volcanii* enzyme can be thought of as a pseudo-dimer with the amino-terminal endonuclease domain repeat comprising one pseudo-monomer and the carboxy-terminal repeat comprising the other. The repeats are connected by a stretch of polypeptide and serve to form the pseudo-dimer by the β9-β9′ hydrophobic interaction that occurs between monomers in the *M. jannaschii* enzyme. Loop L10 present in the amino-terminal repeat allows dimerization of the pseudo-dimers to form the active enzyme. Careful examination of the sequence of the amino-terminal repeat shows the absence of the amino acids for the catalytic triad, thus, the dimeric enzyme contains only two active sites present in the carboxy-terminal repeat.

The occurrence of an in-frame gene duplication event explains the configuration of *H. volcanii* enzyme (Lykke-Andersen and Garrett 1997).

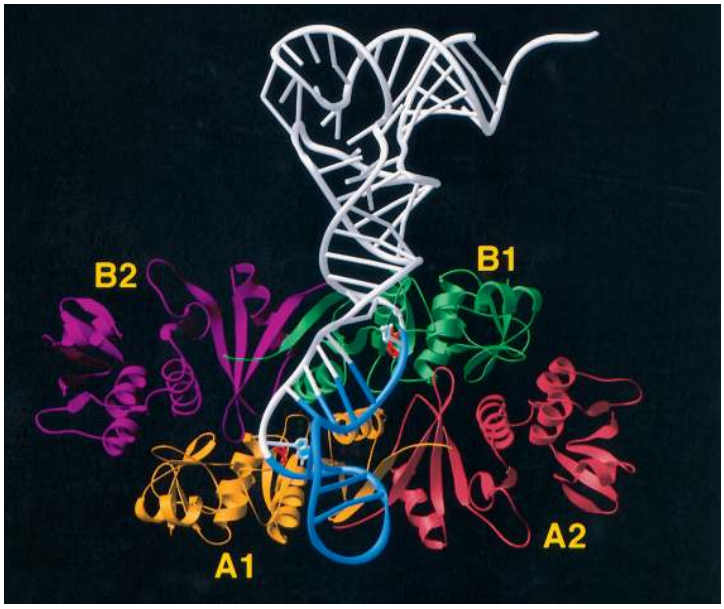

Figure 8 Endonuclease docked with a hypothetical pre-tRNA substrate. The hypothetical tRNA substrate was constructed from joining the known crystal structure of yeast $tRNA^{Phe}$ with the archaeal substrate solved by Diener and Moore (1998). This represents the pre-$tRNA^{Arch\text{-}Euk}$ demonstrated to be a substrate for both the eukaryotic and archaeal endonucleases (Fabbri et al. 1998) (see text). The scissile phosphates are shown to dock directly in the active sites present on subunits A1 and B1 of the endonuclease.

This event has been localized to a set of closely related organisms of the Euryarchaeota branch of the Archaea (Fig. 5), which includes the genus *Haloferax*, *Archaeoglobus*, and *Methanosarcina.*

The Yeast Endonuclease

Like the *M. jannaschii* endonuclease, the yeast tRNA splicing endonuclease is a tetramer. The two active-site-containing subunits, Sen2 and Sen34, must be configured in a similar manner to the A1 and B1 subunits of *M. jannaschii.* This arrangement is proposed to be facilitated by interactions with Sen54 and Sen15. Sen54 and Sen15 both contain a stretch of amino acids at their carboxyl terminus that is homologous to the *M. jannaschii* endonuclease carboxyl terminus (Lykke-Andersen and Garrett 1997). This homology includes the crucial regions involved in the two sets of interactions seen in formation of the tetramer: the β9-β9′ hydro-

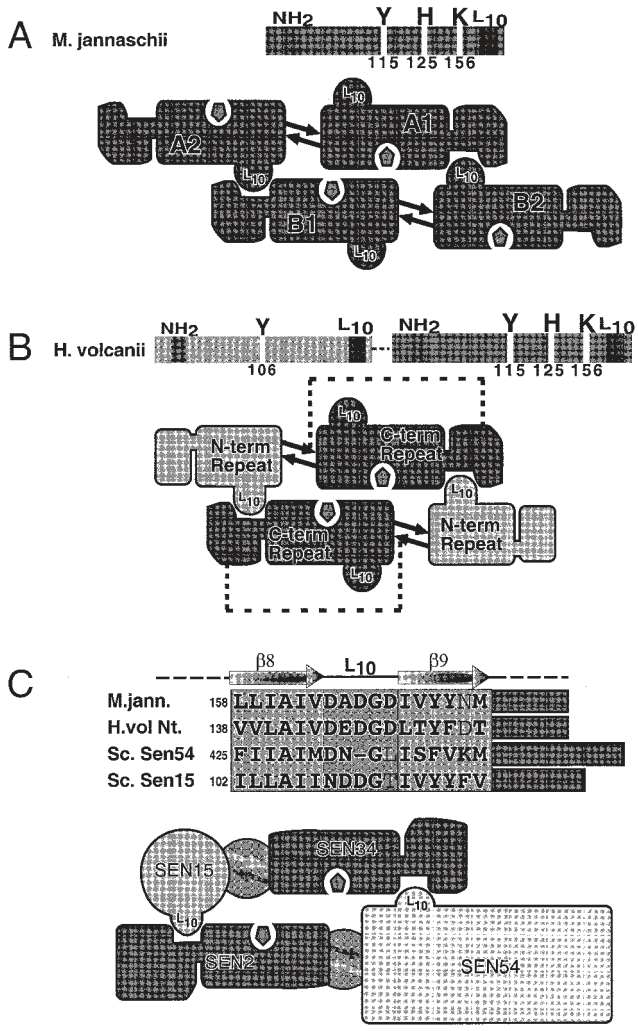

Figure 9 Model of the tRNA splicing endonucleases of *M. jannaschii*, *H. volcanii*, and *S. cerevisiae.* (*A*) The *M. jannaschii* endonuclease is graphically depicted. Several important features are shown in the primary sequence and by a model: loop L10 for tetramerization, the carboxy-terminal β9 strands (*arrows*) for dimerization, and the conserved catalytic residue His-125 (*pentagon*). (*B*) The *H. volcanii* endonuclease consists of a tandem repeat of the endonuclease domain. The amino-terminal repeat has degenerated to possess only the loop L10 and carboxy-terminal interaction domains present in the *M. jannaschii.* The carboxy-terminal repeat contains the groove for loop L10 insertion, as well as the catalytic triad including the conserved histidine (*pentagon*). The dashed line represents the polypeptide chain connecting the amino-terminal repeat and the carboxy-terminal

phobic tail interaction and the loop L10 insertion sequence. Thus, it is proposed (Fig. 9C) that the Sen54-Sen2 and Sen34-Sen15 interactions detected in the two-hybrid assay are mediated by the β9-β9′ interaction. The loop L10 interactions are proposed as in the *M. jannaschii* structure to mediate dimer-dimer interactions.

Interestingly, it has recently been shown that the eukaryotic endonuclease can recognize and cleave an archaeal bulge-helix-bulge containing tRNA substrate (Fabbri et al. 1998). This tRNA substrate was constructed by creating a hybrid tRNA containing an archaeal intron fused to yeast pre-tRNA^{Phe}. The eukaryotic enzyme can recognize and correctly cleave the 5′ and 3′ splice sites in this substrate. In doing so, the enzyme dispenses with the ruler mechanism. There is no change in the size or location of the intron released from the pre-tRNA upon addition or deletion of base pairs in the anticodon stem as was seen for endonucleolytic cleavage of eukaryotic substrates. This result proves that the disposition of the active sites is identical in the archaeal and eukaryotic endonucleases and that this architecture has been conserved since the divergence from a common ancestor. It must be through specialization of the subunits of the yeast enzyme that the ruler mechanism has evolved. The SEN2 gene has acquired a transmembrane sequence, which likely anchors the endonuclease in the inner nuclear membrane, perhaps near nuclear pore structures. This has been shown to be a primary localization of the tRNA splicing ligase (Clark and Abelson 1987), and the two enzymes likely act in concert at this site (Greer 1986).

ORIGIN OF tRNA INTRONS

The relatedness of the eukaryotic and archaeal enzymes almost certainly proves that the endonuclease gene was present in their last common ancestor. The simplest hypothesis is that its function then was to splice tRNA precursors, but it could also be that it had a different function and has been independently recruited in both lines to a tRNA splicing function.

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repeat. (*C*) Proposed structural model of the yeast endonuclease. (*Upper*) The sequences of the carboxyl terminus of the (M. jann.) *M. jannaschii*, (H vol. Nt.) *H. volcanii* amino-terminal repeat, and Sc. Sen54 and Sc. Sen15 are shown to be homologous. (*Lower*) These important interaction elements are modeled in the yeast endonuclease. The two interaction elements are modeled as loop L10 conserved in Sen54 and Sen15 and the carboxy-terminal interaction (*circled arrows*) (see text for details). (Adapted with permission, from Li et al. 1998 [copyright American Association for the Advancement of Science].)

Certainly recruitment is a theme that is a feature of the enzymes in this system. It has recently been shown that tRNA ligase functions in the ligation of an unusually spliced yeast messenger RNA (Cox and Walter 1996; Sidrauski et al. 1996). The mRNA encodes a transcription factor, HAC1, which up-regulates transcription of genes involved in the maintenance of unfolded protein in the endoplasmic reticulum (ER). The presence of unfolded proteins appears to be sensed by a receptor tyrosine kinase-like protein, IRE1, which spans the membrane of the ER. The Ire1 protein contains a nuclease domain capable of cleaving the Hac1 mRNA and releasing an intron (Sidrauski and Walter 1997). Ligation of the two Hac1 exons is dependent on the function of tRNA ligase both in vivo and in vitro (Sidrauski et al. 1996; Sidrauski and Walter 1997).

Two hypotheses have been postulated for the origin of tRNA introns. The first (Cavalier-Smith 1991) posits that existing proteins were recruited to splice introns resulting from the partial deletion of preexisting group I or group II self-splicing introns in tRNA or rRNA genes. Support for such a hypothesis is derived from the presence of group I and group II introns in the tRNA genes of a handful of bacterial species. In particular, a group I intron found in the $tRNA^{Leu}$ gene of cyanobacteria and bacteria interrupts the anticodon loop at the same position as a protein-dependent intron found in some Archaea (Wich et al. 1987). A second hypothesis proposes the expansion of loops of the tRNA molecule. In both instances, the preexistence of compatible splicing machinery in the cell would ensure against lethality of the insertion (Belfort and Weiner 1997). It is unlikely that agreement on a time for the origin of tRNA introns is possible; however, the new addition to the ongoing dialogue is that at least the splicing endonuclease is ancient.

CONCLUDING REMARKS

Whatever the origin of tRNA introns, it is clear that they are here to stay. Both Archaea and eukaryotes have failed to displace all introns from tRNA genes. This would suggest that the introns serve some selective advantage for the organism and that maintenance of the splicing system is necessary for survival. It is clear from studies in yeast that one function of introns is to aid in the modification of tRNAs (for review, see Grosjean et al. 1997). Thus, the modification enzymes have evolved to depend on the presence of the intron for correct substrate recognition and modification.

By studying the enzymology of the removal process, we have begun

to understand just how introns have evolved. It will be interesting to fully delineate the splicing pathway of the archaeal system through discovery of the ligase that must function to ligate the products of the endonuclease reaction. Perhaps we will again be afforded a glimpse into the RNA World that existed before the invention of protein enzymes.

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REFERENCES:

Abelson J., Trotta C.R., and Li H. 1998. tRNA splicing. *J. Biol. Chem.* **273:** 12685–12688.

Apostol B.L., Westaway S.K., Abelson J., and Greer C.L. 1991. Deletion analysis of a multifunctional yeast tRNA ligase polypeptide: Identification of essential and dispensable functional domains. *J. Biol. Chem.* **266:** 7445–7455.

Arn E.A. and Abelson J.N. 1996. The 2′-5′ ligase from *Escherichia coli*: Purification, cloning and genomic disruption. *J. Biol. Chem.* **271:** 31145–31153.

———. 1998. RNA ligases: Function, mechanism and sequence conservation. In *RNA structure and function* (ed. R.W. Simons and M. Grunberg-Manago), pp. 695–726. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York.

Baldi M.I., Mattoccia E., Bufardeci E., Fabbri S., and Tocchini-Valentini G.P. 1992. Participation of the intron in the reaction catalyzed by the *Xenopus* tRNA splicing endonuclease. *Science* **255:** 1404–1408.

Belford H.G., Westaway S.K., Abelson J., and Greer C.L. 1993. Multiple nucleotide cofactor use by yeast ligase in tRNA splicing: Evidence for independent ATP- and GTP-binding sites. *J. Biol. Chem.* **268:** 2444–2450.

Belfort M. and Weiner A. 1997. Another bridge between kingdoms: tRNA splicing in Archaea and eukaryotes. *Cell* **89:** 1003–1006.

Biniszkiewicz D., Cesnaviciene E., and Shub D.A. 1994. Self-splicing group-I intron in cyanobacterial initiator methionine tRNA: Evidence for lateral transfer of introns in bacteria. *EMBO J* **13:** 4629–4635.

Cavalier-Smith T. 1991. Intron phylogeny: A new hypothesis. *Trends Genet.* **7:** 145–148.

Clark M.W. and Abelson J. 1987. The subnuclear localization of tRNA ligase in yeast. *J. Cell Biol.* **105:** 1515–1526.

Cox J.S. and Walter P. 1996. A novel mechanism for regulating activity of a transcription factor that controls the unfolded protein response. *Cell* **86:** 391–404.

Culbertson M.R. and Winey M. 1989. Split tRNA genes and their products: A paradigm for the study of cell-function and evolution. *Yeast* **5:** 405–427.

Culver G.M., McCraith S.M., Consaul S.A., Stanford D.R., and Phizicky E.M. 1997. A 2′-phosphotransferase implicated in tRNA splicing is essential in *Saccharomyces cerevisiae. J. Biol. Chem.* **272:** 13203–13210.

Culver G.M., McCraith S.M., Zillmann M., Kierzek R., Michaud N., Lareau R.D., Turner D.H., and Phizicky E.M. 1993. An NAD derivative produced during tRNA splicing: ADP-ribose 1″-2″ cyclic phosphate. *Science* **261:** 206–208.

Daniels C.J., Gupta R., and Doolittle W.F. 1985. Transcription and excision of a large intron in the $tRNA^{Trp}$ gene of an Archaebacterium, *Halobacterium volcanii. J. Biol. Chem.* **260:** 3132–3134.

Diener J. and Moore P. 1998. Solution structure of a substrate for the archaeal pre-tRNA splicing endonucleases: The bulge-helix-bulge motif. *Mol. Cell* **1:** 883–894.

Fabbri S., Fruscoloni P., Bufardeci E., Di Nicola Negri E., Baldi M.I., Gandini Attardi D., Mattoccia E., and Tocchini-Valentini G.P. 1998. Conservation of substrate recognition mechanisms by tRNA splicing endonucleases. *Science* **280:** 284–286.

Filipowicz W., Konarska M., Gross H., and Shatkin A.. 1983. RNA 3′-terminal phosphate cyclase activity and RNA ligation in HeLa cell extract. *Nucleic Acids Res.* **11:** 1405–1418.

Gomes I. and Gupta R. 1997. RNA splicing ligase activity in the archaeon *Haloferax volcanii. Biochem. Biophys. Rev.* **237:** 588–594.

Goodman H.M., Olson M.V., and Hall B.D. 1977. Nucleotide sequences of a mutant eukaryotic gene: The yeast tyrosine inserting suppressor, SUP4-0. *Proc. Natl. Acad. Sci.* **74:** 5453–5457.

Green C.J., Sohel I., and Vold B.S. 1990. The discovery of new intron-containing human tRNA genes using the polymerase chain reaction. *J. Biol. Chem.* **265:** 12139–12142.

Greer C.L. 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 C.L., Soll D., and Willis I. 1987. Substrate recognition and identification of splice sites by the tRNA-splicing endonuclease and ligase from *Saccharomyces cerevisiae. Mol. Cell. Biol.* **7:** 76–84.

Grosjean H., Szweykowska-Kulinska Z., Motorin Y., Fasiolo F., and Simos G. 1997. Intron-dependent enzymatic formation of modified nucleosides in eukaryotic tRNAs: A review. *Biochimie* **79:** 293–302.

Ho C.K., Rauhut R., Vijayraghavan U., and Abelson J. 1990. Accumulation of pre-tRNA splicing 2/3 intermediates in a *Saccharomyces cerevisiae* mutant. *EMBO J* **9:** 1245–1252.

Hopper A.K., Banks F., and Evangelidis V. 1978. A yeast mutant which accumulates precursor tRNAs. *Cell* **14:** 211–219.

Johnson J., Ogden R., Johnson P., Abelson J., Dembeck P., and Itakura K. 1980. Transcription and processing of a yeast tRNA gene containing a modified intervening sequence. *Proc. Natl. Acad. Sci.* **77:** 2564–2568.

Kjems J. and Garrett R.A. 1985. An intron in the 23S rRNA gene of the Archaebacterium *Desulfurococcus mobilis. Nature* **318:** 675–677.

Kjems J., Jensen J., Olesen T., and Garrett R.A. 1989. Comparison of tRNA and rRNA intron splicing in the extreme thermophile and Archaebacterium *Desulfurococcus mobilis. Can. J. Microbiol.* **35:** 210–214.

Kleman-Leyer K., Armbruster D.A., and Daniels C.J. 1997. Properties of *H. volcanii* tRNA intron endonuclease reveal a relationship between the archaeal and eucaryal tRNA intron processing systems. *Cell* **89:** 839–848.

Klenk H.P., Clayton R.A., Tomb J.F., White O., Nelson K.E., Ketchumm K.A., Dodson R.J., Gwinn M., Hickey E.K., Peterson J.D. 1998. The complete genome sequence of

the hyperthermophilic, sulphate-reducing archaeon *Archaeoglobus fulgidus. Nature* **390:** 364–370.

Knapp G., Beckmann J.S., Johnson P.F., Fuhrman S.A., and Abelson J.N. 1978. Transcription and processing of intervening sequences in yeast tRNA genes. *Cell* **14:** 221–236.

Konarska M., Filipowicz W., Domdey H., and Gross H. 1981. Formation of a 2′-phosphomonoester, 3′,5′-phosphodiester linkage by a novel RNA ligase in wheat germ. *Nature* **293:** 112–116.

Kuhsel M.G., Strickland R., and Palmer J.D. 1990. An ancient group I intron shared by eubacteria and chloroplasts. *Science* **250:** 1570–1573.

Laski F., Fire A., RajBhandary U., and Sharp P. 1983. Characterization of tRNA precursor splicing in mammalian extracts. *J. Biol. Chem.* **258:** 11974–11980.

Lee M.C. and Knapp G. 1985. tRNA splicing in *Saccharomyces cerevisiae*: Secondary and tertiary structures of the substrates. *J. Biol. Chem.* **260:** 3108–3115.

Li H., Trotta C.R., and Abelson J.N. 1998. Crystal structure and evolution of a tRNA splicing enzyme. *Science* **280:** 279–284.

Lykke-Andersen J. and Garrett R.A. 1997. RNA-protein interactions of an archaeal homotetrameric splicing endoribonuclease with an exceptional evolutionary history. *EMBO J.* **16:** 6290–6300.

McCraith S.M. and Phizicky E.M. 1990. A highly specific phosphatase from *Saccharomyces cerevisiae* implicated in tRNA splicing. *Mol. Cell. Biol.* **10:** 1049–1055.

———. 1991. An enzyme from *Saccharomyces cerevisiae* uses NAD^+ to transfer the splice junction 2′-phosphate from ligated tRNA to an acceptor molecule. *J. Biol. Chem.* **266:** 11986–11992.

Nishikura K. and De Robertis E. 1981. RNA processing in microinjected *Xenopus* oocytes. Sequential addition of base modification in the spliced transfer RNA. *J. Mol. Biol.* **145:** 405–420.

Ogden R.C., Lee M.C., and Knapp G. 1984. Transfer RNA splicing in *Saccharomyces cerevisiae*: Defining the substrates. *Nucleic Acids Res.* **12:** 9367–9382.

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.

Phizicky E.M., Schwartz R.C., and Abelson J. 1986. *Saccharomyces cerevisiae* tRNA ligase: Purification of the protein and isolation of the structural gene. *J. Biol. Chem.* **261:** 2978–2986.

Phizicky E.M., Consaul S.A., Nehrke K.W., and Abelson J. 1992. Yeast tRNA ligase mutants are nonviable and accumulate tRNA splicing intermediates. *J. Biol. Chem.* **267:** 4577–4582.

Rauhut R., Green P.R., and Abelson J. 1990. Yeast tRNA-splicing endonuclease is a heterotrimeric enzyme. *J Biol. Chem.* **265:** 18180–18184.

Reinhold-Hurek B. and Shub D.A. 1992. Self-splicing introns in tRNA genes of widely divergent bacteria. *Nature* **357:** 173–176.

Reyes V.M. and Abelson J. 1988. Substrate recognition and splice site determination in yeast tRNA splicing. *Cell* **55:** 719–730.

Schneider A., Perry-McNally K., and Agabian N. 1993. Splicing and 3′-processing of the tyrosine tRNA of *Trypanosoma brucei. J. Biol. Chem.* **268:** 21868–21874.

Schwartz R., Greer C., Gegenheimer P., and Abelson J. 1983. Enzymatic mechanism of an RNA ligase from wheat germ. *J. Biol. Chem.* **258:** 8374–8383.

Sidrauski C. and Walter P. 1997. The transmembrane kinase Ire1p is a site-specific endonuclease that initiates mRNA splicing in the unfolded protein response. *Cell* **90:** 1031–1039.

Sidrauski C., Cox J.S., and Walter P. 1996. tRNA ligase is required for regulated mRNA splicing in the unfolded protein response. *Cell* **86:** 405–413.

Spinelli S., Consaul S., and Phizicky E. 1997. A conditional lethal yeast phophotransferase (tpt1) mutant accumulates tRNAs with a 2′-phosphate and an undermodified base at the splice junction. *RNA* **3:** 1388–1400.

Stange N. and Beier H. 1986. A gene for the major cytoplasmic $tRNA^{Tyr}$ from *Nicotiana rustica* contains a 13 nucleotides long intron. *Nucleic Acids Res.* **14:** 8691.

Strobel M. and Abelson J. 1986. Effect of intron mutations on processing and function of *Saccharomyces cerevisiae* SUP53 tRNA in vitro and in vivo. *Mol. Cell. Biol.* **6:** 2663–2673.

Swerdlow H. and Guthrie C. 1984. Structure of intron-containing tRNA precursors: analysis of solution conformation using chemical and enzymatic probes. *J. Biol. Chem.* **259:** 5197–5207.

Thompson J.E. and Raines R.T. 1994. Value of general acid-base catalysis to ribonucleaseA. *J. Am. Chem. Soc.* **116:** 5467–5468.

Thompson L.D. and Daniels C.J. 1988. A $tRNA^{trp}$ intron endonuclease from *Halobacterium volcanii*: Unique substrate recognition properties. *J. Biol. Chem.* **263:** 17951–17959.

Thompson L.D., Brandon L.D., Nieuwlandt D.T., and Daniels C.J. 1989. Transfer RNA intron processing in the halophilic archaebacteria. *Can. J. Microbiol.* **35:** 36–42.

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.

Valenzuela P., Venegas A., Weinberg F., Bishop R., and Rutter W.J.. 1978. Structure of yeast phenylalanine-tRNA genes: An intervening DNA segment within the coding region for the tRNA. *Proc. Natl. Acad. Sci.* **75:** 190–194.

Walker G., Uhlenbeck O.C., Bedows E., and Gumport R.I. 1975. T4-induced RNA ligase joins single-stranded oligoribonucleotides. *Proc. Natl. Acad. Sci.* **72:** 122–126.

Walsh C. 1979. *Enzymatic reaction mechanisms.* W.H. Freeman, San Francisco, California.

Wich G., Leinfelder W., and Bock A. 1987. Genes for stable RNA in the extreme thermophile *Thermoproteus-tenax* introns and transcription signals. *EMBO J.* **6:** 523–528.

Willis I., Hottinger H., Pearson D., Chisholm V., Leupold U., and Soll D. 1984. Mutations affecting excision of the intron from a eukaryotic dimeric tRNA precursor. *EMBO J.* **3:** 1573–1580.

Winey M. and Culbertson M.R. 1988. Mutations affecting the tRNA splicing endonuclease activity of *Saccharomyces cerevisiae. Genetics* **118:** 49–63.

Xu Q., Teplow D., Lee T.D., and Abelson J. 1990. Domain structure in yeast tRNA ligase. *Biochemistry* **29:** 6132–6138.

Zillmann M., Gorovsky M.A., and Phizicky E.M. 1991. Conserved mechanism of tRNA splicing in Eukaryotes. *Mol. Cell. Biol.* **11:** 5410–5416.

———. 1992. Hela cells contain a 2′-phosphate-specific phosphotransferase similar to a yeast enzyme implicated in tRNA splicing. *J. Biol. Chem.* **267:** 10289–10294.