Diversity and roles of (t)RNA ligases

Johannes Popow · Alexander Schleiffer · Javier Martinez

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Abstract The discovery of discontiguous tRNA genes triggered studies dissecting the process of tRNA splicing. As a result, we have gained detailed mechanistic knowledge on enzymatic removal of tRNA introns catalyzed by endonuclease and ligase proteins. In addition to the elucidation of tRNA processing, these studies facilitated the discovery of additional functions of RNA ligases such as RNA repair and non-conventional mRNA splicing events. Recently, the identification of a new type of RNA ligases in bacteria, archaea, and humans closed a long-standing gap in the field of tRNA processing. This review summarizes past and recent findings in the field of tRNA splicing with a focus on RNA ligation as it preferentially occurs in archaea and humans. In addition to providing an integrated view of the types and phyletic distribution of RNA ligase proteins known to date, this survey also aims at highlighting known and potential accessory biological functions of RNA ligases.

Keywords RNA ligase · tRNA · 2',3'-cyclic phosphate · ScTRL1 · Rnl1 · Rnl2 · HSPC117 · DDX1 · CGI-99 · FAM98 · ASW · RtcA · RtcB

Introns within tRNA genes

Transfer RNAs (tRNA) are transcribed as precursor transcripts and are subjected to a series of posttranscriptional processing events before they are matured to fulfill their biological functions [1, 2]. Sequence analysis of tRNA genes in yeast [3, 4], and later in archaea [5], plants [6], and mammals [7], revealed the existence of tRNA genes disrupted by intervening sequences. Intron harboring tRNA genes are now known to occur in the genomes of organisms from all three domains of life [8]. After the discovery of intron-containing tRNAs [3–7], the mechanistic features of tRNA splicing were extensively studied [2, 9–11]. This review aims at providing an overview of the mechanistic aspects and enzymes involved in tRNA splicing across the three major lines of descent considering in particular a novel type of RNA ligases recently identified in bacteria, archaea, and humans.

tRNA introns in bacteria, archaea, and eukaryotes

In contrast to bacterial pre-tRNAs—which are disrupted by self-splicing group I introns [12, 13]—archaeal and eukaryal pre-tRNA transcripts undergo enzymatic splicing. The latter achieves intron removal by endoribonucleolytic cleavage and subsequent ligation rather than by two consecutive transesterification events as employed by self-splicing introns or the spliceosome.

In archaea, as many as 70% of genetically encoded pre-tRNAs can be disrupted by introns of 16–44 nucleotides in length, which are characterized by the presence of a highly conserved “bulge-helix-bulge” motif [14, 15]. In some archaeal species, pre-tRNAs harbor up to three introns inserted at various sites [16] (Fig. 1A, schemes a and b).
More recently, split tRNA (Fig. 1A, scheme c) and tri-split archaeal tRNA genes, which encode for parts of the mature domain, have been discovered [17, 18]. This type of tRNA arises from distinct transcription units that are joined by trans-splicing to yield a functional tRNA.

Eukaryal tRNA introns are relatively short and range from 12 to 104 nucleotides in e.g., humans [8]. In eukarya, tRNA introns are less abundant than in archaeae—e.g., only 6% of human tRNA genes and 20% of yeast tRNA genes are disrupted by introns [8]—and do not display any conserved structural motifs. Rather, the position of the intron is highly invariable in almost all known eukaryotic pre-tRNAs. An exception to this rule is exemplified by the non-canonical introns found in the circularly permuted tRNA genes of the red alga Cyanidioschyzon merolae (see scheme accompanying Fig. 1B) [19].

**Origin and function of introns within tRNA**

Several hypotheses for evolutionary origins of tRNA introns have been put forward. An “intron-early” scenario proposes the existence of discontinuous primitive tRNA genes interrupted by introns harboring, for example, genes encoding splicing enzymes or aminoacyl transferases. Throughout evolution, these introns could have lost their initial functions and gradually acquired their current features [20]. The related “split early” hypothesis speculates that tRNA introns derive from flanking sequences of a priori split tRNA genes [21, 22]. The contrasting “intron late” or “split late” scenarios assume that tRNA introns arose later in evolution and propagated themselves by gene conversion or transposition [23]. Recent analyses of archaeal genome sequences suggest that tRNA introns in at least some archaeal species have been inserted into contiguous tRNA genes, e.g., by transposition [24]. A following separation of the sequences encoding the mature domains could have given rise to today’s split tRNA genes [25, 26].

As their phylogenetic origins, the functions of tRNA introns are still being explored. The presence of introns in some particular pre-tRNAs has been demonstrated to be required for enzymatic modification of nucleotides such as methylation [27] and pseudouridylation [28–30]. Only recently has the function of tRNA introns in vivo begun to be addressed in genetic experiments carried out with Saccharomyces cerevisiae. Here, removal of all introns of a particular tRNA isodecoder family did not affect growth or translation of the obtained mutants at laboratory conditions.

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**Fig. 1** Position and conserved features of introns within end-matured archaeal and eukaryal pre-tRNAs [11]. A Secondary structure diagram of end-matured, intron-containing archaeal tRNAs. Schemes a and b represent end-matured tRNAs with introns in the D-arm or the T-arm, respectively. Scheme c depicts an end-matured split pre-tRNA assembled from two separate primary transcripts [25]. B Secondary structure diagram of end-matured, intron-containing eukaryotic pre-tRNAs. The accompanying scheme represents non-canonical introns and end processing sites of a permuted pre-tRNA transcript in the red alga C. merolae. The non-canonical intron in the acceptor arm is assumed to be excised by tRNA end processing enzymes rather than pre-tRNA splicing factors [19]. A, adenosine; C, cytosine; G, guanosine; U, uridine; W, pseudouridine; Y, pyrimidine; R, purine; asterisks indicate positions of additional introns, 5'-exonic regions are depicted in blue, 3'-exonic regions in red and intronic regions in green. Full grey circles indicate nonconserved nucleotides in regions of variable length, full blue circles indicate the position of the anticodon.

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Endonucleolytic excision of tRNA introns

Yeast mutants accumulating pre-tRNAs [32] initially provided access to substrates suitable for studying tRNA processing in biochemical experiments [33, 34]. These studies led to the conclusion that splicing of pre-tRNAs occurs in two steps. During an endonuclease reaction, the intron is first excised and the resulting tRNA exon halves are then ligated to form a mature tRNA (Fig. 2A) [35]. The biochemical fractionation of extracts catalyzing pre-tRNA cleavage has led to the identification of tRNA endonuclease proteins in archaea [36] and yeast [37], facilitating the characterization of the homologous human proteins [38].

In both archaea and eukarya, the tRNA endonuclease catalyses the cleavage of pre-tRNA at the two exon–intron boundaries and generates tRNA exon halves displaying a 2′,3′-cyclic phosphate at the 3′-end of the 5′-exon and a 5′-hydroxyl at the 5′-end of the 3′-exon (Fig. 2B, box at the top). In addition, the reaction yields a linear intron with 2′,3′-cyclic phosphate and 5′-hydroxyl termini (Fig. 2A). Although being mechanistically and evolutionarily related [39], it has been shown that archaeal and eukaryal tRNA splicing endonucleases differ with respect to substrate recognition [9].

Archaeal tRNA endonucleases

Studies modifying the substrates used for in vitro processing reactions revealed that splice site recognition by archaeal tRNA splicing endonucleases relies on the presence of the bulge-helix-bulge motif [14]. Archaeal tRNA endonucleases consist of two dimers, each composed of a catalytic and a structural subunit. These dimers combine in an antiparallel fashion and interact with the pseudosymmetric substrate. Each exon–intron boundary is cleaved by one of the diagonally juxtaposed catalytic subunits, which together form the two composite active sites [40]. Despite the conservation of the recognition motif, four types of oligomeric organization of archaeal splicing endonucleases have co-evolved with minor changes of their substrates [41] giving rise to z4 (homotetramers, found in Euryarchaeota), z2 (homodimers, found in Euryarchaeota), (zβ)2 (dimers of heterodimers found in some Crenarchaeota such as e.g., Nanoarchaeum equitans), and zβγδ (heterotetramers, also found in Crenarchaeota) enzymes [39, 42–44]. In the case of the homotetrameric endonucleases, two of the four identical chains function as structural subunits whereas the two remaining polypeptides serve as catalytic subunits [45]. The monomers of the homodimeric splicing endonucleases might have arisen from an in-frame gene duplication and thus encode for a fusion of a structural and a catalytic domain [42].

Eukaryal tRNA endonucleases

The eukaryal tRNA endonucleases are phylogenetically related to their archaeal counterparts [39]. The yeast endonuclease is of the zβγδ type and consists of two catalytic subunits, Sc SEN2 and Sc SEN34, and two structural subunits, Sc SEN15 and Sc SEN54 [37]. All four yeast subunits have homologous human proteins termed Hs TSEN2, Hs TSEN34, Hs TSEN15, and Hs TSEN54 [38]. Although it has been shown that at least one eukaryal tRNA endonuclease retained the ability to process archaeal pre-tRNA substrates [46], eukaryal endonucleases seem to have a different mode of splice site recognition: Eukaryal tRNA endonucleases cleave pre-tRNA substrates at a conserved distance from a structural feature located in the mature domain [47]. Apart from a strictly conserved A:I base pair (Fig. 1B) [48], little sequence constraint seems to exist for the intron itself, which can be extensively mutated without disturbing its proper recognition by the endonuclease [49].

RNA ligation

Proteins catalyzing the final step of tRNA splicing—ligation of tRNA exon halves [35]—have first been identified by biochemical fractionation approaches in yeast [50, 51] and plants [52]. Initial biochemical experiments already indicated that the ligation step is mechanistically not as conserved among the different archaeal and eukaryal organisms as the endonuclease reaction [53–56]. Interestingly, although not requiring tRNA splicing enzymes, Escherichia coli has been shown to catalyze ligation of tRNA exon halves [57]. Even earlier, an RNA ligase activity had been identified in bacteriophage T4-infected E. coli [58]. These experiments led to the conclusion that, based on the type of phosphodiester bond established, distinct types of RNA ligase activity exist [10] (Fig. 2B). Bacteriophages on one hand, and fungi and plants on the other, utilize two related but mechanistically distinct multistep reactions to prepare the 2′,3′-cyclic phosphate and 5′-hydroxyl termini produced by the upstream endonuclease reaction for ligation (Fig. 2B, left box). Both mechanisms require the hydrolysis of the 2′,3′-cyclic phosphate and phosphorylation of the 5′-hydroxyl prior to ligation and as a result the phosphate at the newly formed phosphodiester linkage originates from the nucleotide triphosphate cofactor used for the kinase reaction [10]. To indicate that a 5′-phosphate is joined to a 3′-hydroxyl
moiety, these two pathways are hereinafter referred to as 5′–3′ RNA ligase mechanisms. In addition, two mechanisms exist that incorporate the cleavage site-derived phosphate into the splice junction (Fig. 2B, central and right boxes). 3′–5′ RNA ligation converts 2′,3′-cyclic phosphate and 5′-hydroxyl termini into a 3′,5′ phosphodiester and can

**Fig. 2** Enzymatic splicing of pre-tRNA. **A** Splicing of introns within pre-tRNA transcripts in archaea and eukaryotes is accomplished by separate cleavage and ligation reactions catalyzed by an endonuclease and a ligase enzyme, respectively. 5′-exons are depicted in blue, 3′-exons in red, and introns in green. **B** RNA ligation mechanisms. Brackets in gray indicate the names of enzymes catalyzing the indicated reactions. **Pase** phosphatase; **CPD** cyclic phosphodiesterase; **Ap** adenosine 5′-monophosphate; **Gpp** guanosine 5′-diphosphate; **Nppp** unspecified nucleoside 5′-triphosphate; **Ap-Lig** adenylated ligase protein; **NAD** nicotinamide adenine dinucleotide; **Appr-1,2-cyclic phosphate**
be detected in archaean and vertebrate cell extracts. During 2'–5' RNA ligation, predominantly found in euabacteria and archaean, 2',3'-cyclic phosphate and 5'-hydroxyl moieties are joined to give rise to a 2',5'-phosphodiester bond [59, 60].

5'–3' RNA ligases in bacteriophages, bacteria, archaea, and kinetoplastids

Several bacterial species have evolved to sacrifice individual members of their populations upon phage infection by activating various suicide response pathways [61]. One of these suicide response mechanisms entails the activation of the latent anticodon nuclease PrrC in phage-infected E. coli CTr5x. The nuclease—kept in an inactive state in the E. coli of the latent anticodon nuclease PrrC in phage-infected of these suicide response mechanisms entails the activation in wheat germ extracts [55, 72] by using a nucleolytic piece of evidence for the existence of a eukaryotic tRNA and an ATP-dependent ligase reaction constituted the first step reaction, an ATP-independent endonuclease reaction, the finding that tRNA splicing in yeast occurs as a two-related protein families sharing characteristic features T4 Rnl1 and T4 Rnl2 constitute distinct and only distantly joined to give rise to a 2',5'-phosphodiester bond [59, 60].

5'–3' RNA ligases in yeast and plants

The finding that tRNA splicing in yeast occurs as a two-step reaction, an ATP-independent endonuclease reaction, and an ATP-dependent ligase reaction constituted the first piece of evidence for the existence of a eukaryotic tRNA ligase [35]. Later, an RNA ligase activity was characterized in wheat germ extracts [55, 72] by using a nucleolytic fragment—obtained by RNase T1 digestion of tobacco mosaic virus RNA [73]—as an artificial ligase substrate.

Fig. 3 Domain and polypeptide organization of archetypical RNA ligase systems from bacteriophage T4 (T4 Pnkp, T4 Rnl2, and T4 Rnl1) [70, 77, 79, 80], S. cerevisiae (ScTRL1) [74], A. thaliana (AtRNl) [52, 75], and B. florda (BrNL and B/Kinase/CPD) [87]. Nucleotidyl transferase domains are depicted in light blue, kinase domains in green, the DxDT Pase (aspartic acid-based phosphatase) domain in white and 2H-CPD (two conserved histidine based phosphoesterase) domains in yellow. No published domain boundaries are currently available for the A. thaliana and B. florda Kinase/CPD modules, which is indicated by a gradual transition between the two respective colors. The positions of key motifs are drawn to scale. Pnkp polynucleotide kinase/phosphatase; Pase phosphatase; CPD cyclic phosphodiesterase.

A thorough characterization of the splice junction revealed that a 2'-phosphomonoester-3',5'-phosphodiester linkage is established in this system [55, 72]. The same has been shown to be true for the yeast tRNA ligase [50]. Yeast (ScTRL1) and plant (exemplified by AtRNl from Arabidopsis thaliana) tRNA ligases—both identified by activity-guided chromatographic purification [50–52]—are multifunctional enzymes harboring all the activities required to join the 2',3'-cyclic phosphate and 5'-hydroxyl termini in three functionally independent domains [74, 75] (Fig. 3). First, the 2',3'-cyclic phosphate is hydrolyzed by a cyclic phosphodiesterase (CPD) activity to yield a 2'-phosphate-3'-hydroxyl terminated 5'-exon [50, 76]. In a second step, the 5'-hydroxyl at the 3'-exon is phosphorylated by a kinase activity [50]. The actual ligation is preceded by ATP-dependent adenylation of a lysine located within a KxxG consensus motif in the active site of the nucleotidyl transferase domain [50, 76]. Next, the enzyme transfers the adenyl moiety to the 5'-phosphate at the 3'-exon [50]. The adenylated RNA is then joined to the 2'-phosphate-3'-hydroxyl group at the 5'-exon with the concomitant release of AMP [51, 52] (Fig. 2B, left box, right branch and Fig. 3). Although RNA ligases of bacteriophage T4, fungi, and plants share important mechanistic features and key residues required for catalysis (Fig. 2B, left box and Fig. 3), their overall sequence similarity is low [52]. However, the presence of the conserved nucleotidyl transferase domain with its ligase motifs in bacteriophage T4, fungal and plant RNA ligases suggests a common evolutionary origin for these.

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enzymes [69]. Despite their relatedness, several differences exist between yeast, plant, and bacteriophage T4 ligases. For example, ligase- and kinase/phosphatase activities reside on separate polypeptides in bacteriophage T4 whereas yeast and plant tRNA ligases are multifunctional proteins harboring all activities required for ligation (Fig. 2B, left box, right branch and Fig. 3). Hydrolysis of 2',3'-cyclic phosphates by yeast and plant CPD domains yields 2'-phosphate-3'-hydroxyl products rather than the 2',3'-cis diol termini generated by T4 Pnkp (Fig. 2B, left box) [50, 55, 72, 76]. This arises from the distinct phosphoesterase domains employed by the bacteriophage T4 (aspartic acid based DxDxT phosphoesterase domain [77]) and yeast/plant (2H phosphoesterase domain, characterized by two conserved histidines [78]) ligation pathways [77, 79, 80] (Fig. 3). In contrast to bacteriophage T4 ligases, the yeast and plant enzymes do not accept 3'-phosphate RNA substrates and ligation typically depends on the presence of a 2'-phosphate at the substrate termini [79, 81]. Yeast and plant RNA ligases generate splice junctions bearing a 2'-phosphate [72]. To convert this non-canonical 2'-phosphomonoester-3',5'-phosphodiester linkage into a 3',5'-phosphodiester, an NAD+-dependent phosphotransferase, termed ScTPT1 in yeast, removes the 2'-phosphate generating ADP-ribose-1''',2'''-cyclic phosphate (Fig. 2B, left box, right branch) [82–85]. Yeast and plant tRNA ligases also subtly differ with respect to substrate selection. While yeast tRNA ligase prefers tRNA exon halves over artificial substrates such as, e.g., linear introns [51], the plant enzyme ligates circular introns as efficiently as tRNA exon halves in direct competition experiments [52].

### 5′–3′ RNA ligases in higher eukaryotes

Although a 5′–3′ RNA ligase activity has been detected in biochemical experiments carried out with HeLa cell extracts [86], no homologues of known 5′–3′ RNA ligase proteins could be identified in animals [52]. It therefore seems likely that the 5′–3′ RNA ligase in animals has diverged too far from the RNA ligases already known in order to be identified by existing algorithms or represents a completely novel type of enzyme. This view is supported by the recent identification of a 5′–3′ RNA ligase in the cephalochordate Branchiostoma floridae (BfRNL) featuring a stand-alone ligase protein with weak homology to yeast and plant RNA ligases [87]. In this system, the cyclic phosphodiesterase and kinase modules (BfKinase/CPD or BfCLP1) do not reside on the ligase polypeptide (Fig. 2B, left box, right branch and Fig. 3). Enzymes catalyzing kinase (HsCLP1) [88], cyclic phosphodiesterase (HsCPD) [89–91], and phosphotransferase (HsTRPT1) [92] activities have been identified in humans. The fact that these enzymes can function in tRNA splicing pathways in vivo [93–95] has triggered speculation that a yet unidentified 5′–3′ RNA ligase protein might also exist in humans [87]. In support of this assumption, the RNA kinase, HsCLP1, is an integral component of the tRNA endonuclease complex, suggesting the occurrence of coupled endonuclease and kinase reactions in humans [38, 88]. In contrast, tRNA exon half phosphorylation is associated with ligation in S. cerevisiae, where ligase and kinase activities both reside on ScTRL1. However, the relevance of the 5′–3′ RNA ligase mechanism for tRNA splicing in vertebrates is under debate since the 2′-phosphotransferase is not essential in mice [96].

To account for the substantial differences in sequence and domain organization, it has been suggested to group 5′–3′ RNA ligases in bacteriophages, yeast, plants, and animals into classes [66, 87]; however, no uniform nomenclature has become generally accepted to date.

### 3′–5′ RNA ligases in bacteria, archaea, and animals

Biochemical studies revealed that ligation of tRNA exon halves in vertebrates and archaea is mainly achieved by an alternative mechanism. A 3′–5′ RNA ligase activity resulting in incorporation of the precursor-derived 2′,3′-cyclic phosphate into the splice junction as a 3′,5′-phosphodiester was for the first time detected in HeLa cell extracts (Fig. 2B, central box) [53, 56, 97]. The same reaction has later been shown to occur in archael cell extracts [54, 98, 99]. Intensive attempts to identify the human tRNA ligase proteins by chromatographic purification were triggered by these initial findings [100, 101]. Recently, chromatographic purification led to the identification of RtcB/HSPC117 proteins as 3′–5′ RNA ligases in the crenarchaeon Pyrobaculum aerophilum and in humans [102, 103]. Concurrently, a candidate approach revealed RtcB in Escherichia coli as a 3′–5′ RNA ligase [104]. The high degree of conservation of HSPC117/RtcB proteins suggested a shared role for this protein family in many organisms already during their initial characterization [102–104].

### Archaeal and bacterial RtcB proteins

RNA ligase activity of the recombinant RtcB proteins from *P. aerophilum* and *E. coli* strictly depends on the presence of bivalent metal ions [102, 104]. Studies probing the active sites of archael and bacterial RtcB by mutagenesis [102, 104, 105] could in part confirm early predictions concerning essential residues based on structural analyses of homologous RtcB proteins (pdb files 1UC2 and 2EPG) [106, 107]. However, all crystal structures of RtcB proteins available to date represent apo forms of the enzymes and
thus the exact functions of individual amino acids lining the presumed active site cannot be assigned unambiguously. Extended structural studies of RtcB proteins in complex with bivalent metal ions, additional cofactors, and RNA substrates will be required to clarify the active site geometry and the distinct metal ion specificity observed for enzymes from different species [102, 104].

Initially, HSPC117/RtcB proteins were assumed to catalyze the direct nucleophilic attack of the 2',3'-cyclic phosphate by a 5'-hydroxyl group, which seemed likely for two main reasons. First, 2',3'-cyclic phosphates are energy-rich substrates with a favorable leaving group [108] and second, HSPC117/RtcB-catalyzed ligation reactions did not seem to be strictly dependent on the addition of nucleotide triphosphate cofactors [102–104, 108]. However, only enzyme preparations that were rigorously purified in the presence of chelating agents proved to yield RtcB preparations sufficiently pure to demonstrate the real cofactor requirements of RtcB-catalyzed 3'–5' RNA ligation [108]. Thus, 3'–5' RNA ligation by RtcB proteins occurs as a sequential reaction involving the stoichiometric hydrolysis of nucleotide triphosphates rather than direct nucleophilic hydrolysis of the 2',3'-cyclic phosphate by a 5'-hydroxyl group [108]. One advantage of this somewhat counterintuitive strategy might lie in the suppression of the backward reaction—the cleavage of the newly formed phosphodiester—as it is catalyzed by bacterial 2'–5'-ligases (see below) [59].

The human tRNA ligase complex

3'–5' RNA ligation appears to be the prevalent human tRNA splicing pathway [53, 56] and relies on HSPC117 as the essential ligase component as supported by two experimental observations: RNAi-mediated depletion of HSPC117 severely impairs tRNA maturation and mutation of a strictly conserved cysteine residue abolishes ligase activity of the affinity purified protein [103]. Human HSPC117, together with the proteins DDX1, CGI-99, FAM98B, and ASW, forms a stable complex of about 200 kDa (Fig. 4) [103]. Even earlier, the observed co-selection of HSPC117, DDX1, and CGI-99 with cruciform DNA duplexes hinted that these three proteins might interact [109]. In contrast to depletion of HSPC117, RNAi-mediated silencing of the associated proteins does not severely affect RNA ligase activity in HeLa cell extracts, suggesting further functions of the interacting proteins that remain to be explored [103]. Potential roles of these accessory proteins may include targeting of the complex to appropriate cellular compartments, stabilization of the essential subunit, interaction with substrates or adapter proteins and prevention of illegitimate, promiscuous ligation. Moreover, individual complex components may mediate a transient association of the ligase complex with the tRNA endonuclease, as it has been suggested for the yeast tRNA endonuclease and ligase [110]. In contrast to its archaeal and bacterial orthologues, recombinant HSPC117 did not act as an RNA ligase in vitro. In addition, HSPC117 per se was incapable of replacing the yeast tRNA ligase TRL1 in complementation experiments (J. Popow, unpublished results).

The human tRNA ligase complex components seem to be constitutively and widely expressed in all human and mouse tissues analyzed so far, indicating that these proteins cooperate in a great variety of cellular contexts [103, 111, 112]. DDX1 is a member of the DEAD-box family of putative RNA helicases characterized by the presence of nine conserved sequence motifs [113]. The DEAD-box helicase domain of DDX1 is interrupted by the insertion of a SPRY domain (Fig. 4) detectable in numerous proteins and presumably acting as a protein interaction platform [114, 115]. DDX1 has been associated with many molecular functions ranging from mRNA processing [116, 117] to recognition of DNA double-strand breaks [118] and has been demonstrated to exhibit 3'–5' RNA unwinding activity [117]. The association of DDX1 with HSPC117 suggests an involvement in tRNA splicing. On the other hand, RNAi-mediated depletion of DDX1 only mildly impaired tRNA maturation activity, suggesting that its function might be dispensable for RNA ligation by HSPC117 in HeLa cell extracts [103]. Alternatively, a lack of DDX1 might also have been compensated for by other proteins present in the assayed extracts. Further experiments assessing the effect of mutations within conserved motifs of DDX1 on ligase activity—carried out with purified RNA ligase complex—might successfully address the function of this protein in tRNA splicing. Co-localization studies and extended analysis of proteins associating
with DDX1 could answer the question whether DDX1 mediates its functions in conjunction with HSPC117 or whether it is a shared component of multiple protein complexes acting in distinct biological processes. Furthermore, it might be rewarding to test whether the amplification of DDX1 observed in cancerous cell lines [111, 119, 120], its function in viral replication [121–123], and dsRNA recognition in dendritic cells [124] are linked to RNA ligase activity or tRNA processing.

Published information concerning the function of CGI-99 is scarce. Although the protein has been found to interact with human [112, 125] and viral [125] proteins in yeast two-hybrid assays, these studies provided little insight into its molecular function. It remains to be determined whether the interaction of CGI-99 with the PA subunit of influenza virus polymerase [125] or its modulation of transcription by RNA polymerase II [126] is in any respect related to RNA ligase activity.

The identification of FAM98B as a human tRNA ligase complex component is the first piece of information published concerning its molecular function. Within the context of tRNA splicing, the exact role of FAM98B needs to be established. Since its RNAi-mediated depletion has almost no impact on RNA ligase activity in HeLa cell extracts [103], it is likely that FAM98B acts as an interaction platform recruiting accessory proteins or RNA substrates. On the other hand, it might also be the case that other cellular proteins compensated for a lack of FAM98B in these experiments. Alternatively, FAM98B may mediate completely unrelated, yet-to-be-discovered functions of HSPC117 complexes.

ASW has first been characterized in a genetic screen searching for genes differentially expressed in early neural specification in Xenopus laevis [127]. Although this study could demonstrate that alteration of ASW levels in X. laevis leads to severe developmental defects, it provided limited insights into mechanistic aspects of ASW function. ASW has been speculated to be specific for vertebrates as no homologous genes were detectable in Drosophila melanogaster or Caenorhabditis elegans [127]. Nonetheless, homologues of ASW are detectable in the genomes of at least some arthropods and nematodes (Fig. 5A). Future studies might answer the question of whether the link between ASW and HSPC117 explains any of the developmental defects observed upon manipulation of ASW levels in X. laevis.

Taken together, apart from HSPC117, which appears to be conserved among all domains of life, the additional components of the HSPC117 complex do not show a comparably broad taxonomic coverage. Orthologues of DDX1, FAM98B, and CGI-99 are spread more widely than ASW, which seems to be restricted to fewer species (Fig. 5A).

2′−5′ RNA ligases in archaea and bacteria

Another RNA ligase activity leading to the unusual 2′,5′-phosphodiester bond has been shown to act in extracts prepared from various bacterial species (Fig. 2B, right box) [57]. Its biochemical purification led to the identification of the ligase gene ligT, conserved not only in bacteria but also in euryarchaeota and crenarchaeota [59, 78]. The apparent dependence of its activity on the presence of modifications in its tRNA substrates suggests that it is involved in repair or processing of tRNA in its host [10, 59]. LigT from E. coli catalyses an ATP-independent equilibrium reaction between 2′,3′-cyclic phosphate and 5′-hydroxyl termini and 2′,5′-phosphodiester bonds [59]. More recently, a homologous archaeal 2′−5′ RNA ligase has been characterized in the euryarchaeon Pyrococcus furiosus confirming the broad phyletic occurrence of LigT proteins [60]. Although the high degree of conservation suggests an evolutionarily important role for 2′−5′ ligases, the exact biological function of LigT proteins and 2′,5′-phosphodiester bond formation are still unknown [59, 60, 78]. Interestingly, a few eukaryotic genomes also encode homologs of bacterial and archaeal LigT proteins (Figs. 5B, 6). The activities and biological functions of these proteins have not yet been addressed.

Phyletic association of RNA ligases

RNA ligase proteins are detectable within all major lines of descent. Considering the different ligation mechanisms and classes of RNA ligase polypeptides, it seems, however, that several species abandoned one or the other mechanism of RNA ligation (Figs. 5B, 6). Most representatives of the Basidiomycota and Spermatophyta lineages might have lost RtcB/HSPC117 homologues during the course of evolution. However, there seem to be rare instances (such as the basidiomycote Piriformospora indica, see supplemental information for details) where an RtcB/HSPC117 homologue has been retained [52, 102, 103, 128] (Figs. 5B, 6). None of the 5′−3′ RNA ligases known to date could be allocated to several higher eukaryotes, such as e.g., humans (Fig. 5B). Nevertheless, this picture might change upon the identification of a currently unknown type of 5′−3′ RNA ligase. The detection of 5′−3′ RNA ligase activity in humans [86] together with the recent discovery of the B. floridiae 5′−3′ RNA ligase BfRNL [87] argue in favor of this view.

Subcellular localization of tRNA splicing in eukaryotes

The subcellular localization of tRNA processing events does not seem to be conserved among all eukaryotes. Many localization studies were carried out in the yeast
S. cerevisiae which, however, seems to differ in this respect from many other eukaryotes [129]. tRNA splicing is assumed to occur in the cytoplasm in S. cerevisiae, based on the localization of yeast tRNA endonuclease subunits to the outer mitochondrial membrane [130]. Controversial data have been reported concerning the localization of homologues. Homologues of proteins (right-hand side labels) were identified within the NCBI non-redundant protein database by BLAST or Hidden Markov Model searches (see supplemental material for further details).
tRNA splicing in plants. On the one hand, some plant tRNA splicing enzymes at least partly localize to the nucleus [131]. On the other hand, impaired nuclear export of tRNA results in accumulation of unspliced pre-tRNA in Arabidopsis thaliana [132] arguing in favor of cytoplasmic tRNA splicing in plants in analogy to the coupling of nuclear export and cytoplasmic tRNA splicing in yeast rna1 mutants [32, 133, 134]. In the vertebrate X. laevis, tRNA splicing—preceded by 3′-end formation including CCA addition—has been shown to occur in the nucleus by micro-dissection and micro-injection studies carried out with oocytes [135–137]. In accord with these observations, subunits of the human tRNA endonuclease have been reported to be nuclear proteins [38].

Functions of RNA ligases unrelated to tRNA splicing

The biochemical characterization of identified tRNA ligase enzymes revealed that they accept not only tRNA exon halves but also a broad range of artificial substrates such as nucleolytic RNA fragments, synthetic RNA duplexes, and linear introns generated by tRNA endonucleases [52, 53, 55, 97, 102–105]. In addition, human cell extracts have been shown to not only ligate 2′,3′-cyclic phosphate bearing but also 3′-phosphate-terminated RNA substrates [97, 138]. Biochemical fractionation of HeLa extracts revealed that RNA 3′-phosphate terminal cyclase RTCD1 can convert RNA 3′-phosphates into 2′,3′-cyclic phosphates in an ATP-dependent reaction [139, 140]. Despite the thorough characterization of bacterial and human RNA 3′-phosphate terminal cyclase proteins [140, 141], the physiological function of this enzyme class is still unknown. The recent mechanistic characterization of RtcB from E. coli suggests that 3′-phosphate-terminated RNA is per se a substrate for this enzyme [108], raising questions about the genuine function of RNA terminal cyclase RtcA in E. coli. The potential to prepare a variety of RNA termini for ligation and the relaxed substrate specificity of the ligase itself in many organisms suggests that tRNA splicing enzymes might also act in other biological contexts. As an example of such a case, ScTRL1 has been shown to be involved in stress-induced, non-canonical splicing of the HAC1 transcript in S. cerevisiae [142, 143]. A related pathway acts in human cells, however, the involved RNA ligase has to date not been identified [144–147]. Furthermore, RNA ligases have been proposed as host factors for the propagation of viruses, viroids, and viroid-like satellite RNAs in humans and plants [148]. In fact, HSPC117 and DDX1 are involved in RNA processing during replication of the hepatitis delta virus [121], presumably acting as the host RNA ligase factors assumed to be required for cyclization of the viral RNA genome as previously proposed [149]. Furthermore, components of the HSPC117-complex together with the RNA cyclase, RTCD1, have been shown to interact with kinesin-associated RNA transport granules in mouse brain extracts [150]. The exact functions of these RNA metabolic enzymes in the context of RNA transport await further experiments. In addition to processing of RNA transcripts of various origins, RNA ligases are assumed to be involved in RNA repair pathways. Apart from the well-studied example of tRNA repair by bacteriophage T4 RNA ligase [62], further examples of RNA repair have been revealed [67, 79, 151]. These studies showed an amazing potential of RNA ligases to function as safeguards against the deleterious effects of cytotoxic nucleases in yeast and bacteria. Similar efforts might unveil examples of RNA repair in unanticipated physiological contexts not only in bacteria and yeast but also in higher organisms. The high degree of conservation of HSPC117/RtcB proteins and preliminary data indicating that HSPC117 seems to be encoded by an essential gene in mice [152] are highly suggestive of universal and important roles for this protein family. Assigning a biological function to the operon harboring the ligase, rtcB, and the cyclase, rtcA, in some bacteria may help to uncover functions of this type of ligase unrelated to tRNA splicing since E. coli does not encode for intron harboring tRNAs. Finally, the identification of genuine substrates interacting with RNA ligase proteins in vivo may yield fascinating insights into the various processes potentially requiring RNA ligases.

Fig. 6 Schematic illustration of the phyletic distribution of identified RNA ligase polypeptide sequences

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References

1. Hartmann RK, Gossinger M, Spath B, Fischer S, Marchfelder A (2009) The making of tRNAs and more—RNase P and tRNase Z. Prog Mol Biol Transl Sci 85:319–368
2. Phizicky EM, Hopper AK (2010) tRNA biology charges to the front. Genes Dev 24:1832–1860
3. Goodman HM, Olson MV, Hall BD (1977) Nucleotide sequence of a mutant eukaryotic gene: the yeast tyrosine-inserting ochre suppressor SUP4-0. Proc Natl Acad Sci USA 74:5453–5457
4. Valenzuela P, Venegas A, Weinberg F, Bishop R, trumpet WJ (1978) Structure of yeast phenylalanine-tRNA genes: an intervening DNA segment within the region coding for the tRNA. Proc Natl Acad Sci USA 75:190–194
5. Kaine BP, Gupta R, Woese CR (1983) Putative introns in tRNA genes of prokaryotes. Proc Natl Acad Sci USA 80:3309–3312
6. Stange N, Beier H (1986) A gene for the major cytoplasmic tRNA from Nicotiana rustica contains a 13-nucleotides-long intron. Nucleic Acids Res 14:8691
7. van Tol H, Stange N, Gross HJ, Beier H (1987) A human and a plant intron-containing tRNA gene are both transcribed in a HeLa cell extract but spliced along different pathways. EMBO J 6:35–41
8. Chan PP, Lowe TM (2009) GtRNAdb: a database of transfer RNA genes detected in genomic sequence. Nucleic Acids Res 37:D93–D97
9. Abelson J, Trotta CR, Li H (1998) tRNA splicing. J Biol Chem 273:12685–12688
10. Arndt EA, Abelson J (1998) RNA ligases: function, mechanism, and sequence conservation. In: Simons RW, Gronberg-Manago M (eds) RNA structure and function. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, pp 695–726
11. Trotta CR, Abelson J (1999) tRNA splicing: an RNA world addition or an ancient reaction? In: Gesteland RF, Cech TR, Atkins JF (eds) The RNA World, 2nd edn. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, pp 561–584
12. Haugen P, Simon DM, Bhattacharya D (2005) The natural history of group I introns. Trends Genet 21:111–119
13. Reinhold-Hurek B, Shub DA (1992) Self-splicing introns in tRNA genes of widely divergent bacteria. Nature 357:173–176
14. Thompson LD, Daniels CJ (1988) A tRNA(Trp) intron endonuclease from Halobacterium volcanii. Unique substrate recognition properties. J Biol Chem 263:17951–17959
15. Marck C, Grosjean H (2003) Identification of BH4 splicing motifs in intron-containing tRNAs from 18 archaea: evolutionary implications. RNA 9:1516–1531
16. Sugahara J, Yachie N, Arakawa K, Tomita M (2007) In silico screening of archaeal tRNA-encoding genes having multiple introns with bulge-helix-bulge splicing motifs. RNA 13:671–681
17. Fujishima K, Sugahara J, Kikutake K, Hirano R, Sato A, Tomita M, Kanai A (2009) Tri-split tRNA is a transfer RNA made from 3 transcripts that provides insight into the evolution of fragmented tRNAs in archaea. Proc Natl Acad Sci USA 106:2683–2687
18. Randau L, Munch R, Hohn MJ, Jahn D, Soll D (2005) Nanoarchaeum equitans creates functional tRNAs from separate genes for their 5’- and 3’-halves. Nature 433:537–541
19. Soma A, Onodera A, Sugahara J, Kanai A, Yachie N, Tomita M, Kawamura F, Sekine Y (2007) Permuted tRNA genes expressed via a circular RNA intermediate in Cyanidioschyzon merolae. Science 318:450–453
20. Ho CK (1988) Primitive ancestry of transfer RNA. Nature 333:24
21. Di Giulio M (2006) The non-monophyletic origin of the tRNA molecule and the origin of genes only after the evolutionary stage of the last universal common ancestor (LUCA). J Theor Biol 240:343–352
22. Di Giulio M (2008) Transfer RNA genes in pieces are an ancestral character. EMBO Rep 9:820 (author reply 820-1)
23. Amstutz H, Munz P, Heyer WD, Leupoid U, Kohli J (1985) Concerted evolution of tRNA genes: intergenic conversion among three unlinked serine tRNA genes in S. pombe. Cell 40:879–886
24. Fujishima K, Sugahara J, Tomita M, Kanai A (2010) Large-scale tRNA intron transposition in the archaeal order Thermoproteales represents a novel mechanism of intron gain. Mol Biol Evol 27:2233–2243
25. Heinemann IU, Soll D, Randau L (2009) Transfer RNA processing in archaea: unusual pathways and enzymes. FEBS Lett 584:303–309
26. Randau L, Soll D (2008) Transfer RNA genes in pieces. EMBO Rep 9:623–628
27. Strobel MC, 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
28. Choffat Y, Suter B, Behra R, Kubli E (1988) Pseudouridine modification in the tRNA(Tyr) anticodon is dependent on the presence, but independent of the size and sequence, of the intron in eucaryotic tRNA(Tyr) genes. Mol Cell Biol 8:3332–3337
29. Johnson PF, Abelson J (1978) The yeast tRNA(Tyr) gene intron is essential for correct modification of its tRNA product. Nature 302:681–687
30. Szweykowska-Kulinska Z, Senger B, Keith G, Fasulo F, Grosjean H (1994) Intron-dependent formation of pseudouridines in the anticodon of Saccharomyces cerevisiae minor tRNA(Thr). EMBO J 13:4636–4644
31. Mori S, Kajita T, Endo T, Yoshihisa T (2011) The intron of Saccharomyces cerevisiae SUP4-o. Proc Natl Acad Sci USA 74:5453–5457
32. Stange N, Abelson J (1986) A yeast mutant which accumulates tRNA genes containing intervening sequences. Nature 274:438–445
33. Abelson J, Trotta CR, Li H (1998) tRNA splicing: an RNA world addition or an ancient reaction? In: Gesteland RF, Cech TR, Atkins JF (eds) The RNA World, 2nd edn. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, pp 561–584
34. Haugen P, Simon DM, Bhattacharya D (2005) The natural history of group I introns. Trends Genet 21:111–119
38. 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
39. Lykke-Andersen J, Garrett RA (1997) RNA-protein interactions of an archaeal homotrimetric splicing endonuclease with an exceptional evolutionary history. EMBO J 16:6290–6300
40. Trotta CR, Paushkin SV, Patel M, Li H, Peltz SW (2006) Cleavage of pre-tRNAs by the splicing endonuclease requires a composite active site. Nature 441:375–377
41. Tocchini-Valentini GD, Fruscoloni P, Tocchini-Valentini GP (2005) Coevolution of tRNA intron motifs and tRNA endonuclease architecture in Archaea. Proc Natl Acad Sci USA 102:15418–15422
42. Li H, Abelson J (2000) Crystal structure of a dimeric archaeal splicing endonuclease. J Mol Biol 302:639–648
43. Mitchell M, Xue S, Erdman R, Randau L, Soll D, Li H (2009) Crystal structure and assembly of the functional Nanoarchaeum equitans tRNA splicing endonuclease. Nucleic Acids Res 37:5793–5802
44. Tocchini-Valentini GD, Fruscoloni P, Tocchini-Valentini GP (2005) Structure, function, and evolution of the tRNA endonuclases of Archaea: an example of subfunctionalization. Proc Natl Acad Sci USA 102:8933–8938
45. Li H, Trotta CR, Abelson J (1998) Crystal structure and evolution of a transfer RNA splicing enzyme. Science 280:279–284
46. Fabbri S, Fruscoloni P, Bufardeci E, Di Nicola Negri E, Baldi MI, Attardi DG, Mattoccia E, Tocchini-Valentini GP (1998) Conservation of substrate recognition mechanisms by tRNA splicing endonucleases. Science 280:284–286
47. Reyes VM, Abelson J (1988) Substrate recognition and splice site determination in yeast tRNA splicing. Cell 55:719–730
48. Baldi MI, Mattoccia E, Bufardeci E, Fabbri S, Tocchini-Valentini GP (1992) Participation of the intron in the reaction catalyzed by the Xenopus tRNA splicing endonuclease. Science 255:1404–1408
49. Johnson JD, Ogden R, Johnson P, Abelson J, Dembeck P, Itakura K (1980) Transcription and processing of a yeast tRNA gene containing a modified intervening sequence. Proc Natl Acad Sci USA 77:2564–2568
50. Greer CL, Peebles CL, Gegenheimer P, Abelson J (1983) Mechanism of action of a yeast RNA ligase in tRNA splicing. Cell 32:537–546
51. 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
52. Englert M, Beier H (2005) Plant RNA 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
53. Filipowicz W, Shatkin AJ (1983) Origin of splice junction phosphate in tRNAs processed by HeLa cell extract. Cell 32:547–557
54. Kjems J, Garrett RA (1988) Novel splicing mechanism for the ribosomal RNA intron in the archaeabacterium Desulforococcus mobilis. Cell 54:693–703
55. Konarska M, Filipowicz W, Domdey H, Gross HJ (1981) Formation of a 2'-phosphomonoester, 3',5'-phosphodiester linkage by a novel RNA ligase in wheat germ. Nature 293:112–116
56. Laski FA, Fire AZ, RajBhandary UL, Sharp PA (1983) Characterization of tRNA precursor splicing in mammalian extracts. J Biol Chem 258:11974–11980
57. Greer CL, Javor B, Abelson J (1983) RNA ligase in bacteria: formation of a 2',5' linkage by an E. coli extract. Cell 33:899–906
58. Silber R, Malathi VG, Hurwitz J (1972) Purification and properties of bacteriophage T4-induced RNA ligase. Proc Natl Acad Sci USA 69:3009–3013
59. Arn EA, Abelson JN (1996) The 2'-5' RNA ligase of Escherichia coli. J Biol Chem 271:31145–31153
60. Kanaï A et al (2009) Characterization of a heat-stable enzyme possessing GTP-dependent RNA ligase activity from a hyperthermophilic archaeon, Pyrococcus furiosus. RNA 15:420–431
61. Kaufmann G (2000) Anticodon nucleases. Trends Biochem Sci 25:70–74
62. Amitur SR, Levitz R, Kaufmann G (1987) Bacteriophage T4 anticodon nuclease, polynucleotide kinase and RNA ligase reprocess the host lysine tRNA. EMBO J 6:2499–2503
63. Cameron V, Uhlenbeck OC (1977) 3'-Phosphatase activity in T4 polynucleotide kinase. Biochemistry 16:5120–5126
64. Richardson CC (1965) Phosphorylation of nucleic acid by an enzyme from T4 bacteriophage-infected Escherichia coli. Proc Natl Acad Sci USA 54:158–165
65. Snopik TJ, Wood WB, Conley MP, Chen P, Cozzarelli NR (1977) Bacteriophage T4 RNA ligase is gene 63 product, the protein that promotes tail fiber attachment to the baseplate. Proc Natl Acad Sci USA 74:3355–3359
66. Ho CK, Shuman S (2002) Bacteriophage T4 RNA ligase 2 (gp24.1) exemplifies a family of RNA ligases found in all phylogenetic domains. Proc Natl Acad Sci USA 99:12709–12714
67. Chan CM, Zhou C, Huang RH (2009) Reconstituting bacterial RNA repair and modification in vitro. Science 326:247
68. Martins A, Shuman S (2004) An RNA ligase from Deinococcus radiodurans. J Biol Chem 279:50654–50661
69. Shuman S, Lima CD (2004) The polynucleotide ligase and RNA capping enzyme superfamily of covalent nucleotidyltransferases. Curr Opin Struct Biol 14:757–764
70. Yin S, Ho CK, Shuman S (2003) Structure–function analysis of T4 RNA ligase 2. J Biol Chem 278:17601–17608
71. Stuart KD, Schnaufer A, Ernst NL, Panigrahi AK (2005) Complex management: RNA editing in trypanosomes. Trends Biochem Sci 30:97–105
72. Konarska M, Filipowicz W, Gross HJ (1982) RNA ligation via 2'-phosphomonoester, 3',5'-phosphodiester linkage: requirement of 2',5'-cyclic phosphate termini and involvement of a 5'-hydroxyl polynucleotide kinase. Proc Natl Acad Sci USA 79:1474–1478
73. Konarska M, Filipowicz W, Domdey H, Gross HJ (1981) Binding of ribosomes to linear and circular forms of the 5'-terminal leader fragment of tobacco-mosaic-virus RNA. Eur J Biochem 114:221–227
74. Sawaya R, Schwer B, Shuman S (2003) Genetic and biochemical analysis of the functional domains of yeast tRNA ligase. J Biol Chem 278:43928–43938
75. Wang LK, Schwer B, Englert M, Beier H, 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
76. Xu Q, Teplow DB, Lee TD, Abelson J (1990) Domain structure in yeast tRNA ligase. Biochemistry 29:6132–6138
77. Galburt EA, Pelletier J, Wilson G, Stoddard BL (2002) Structure of a tRNA repair enzyme and molecular biology workhorse: T4 polynucleotide kinase. Structure 10:1249–1260
78. Mazumder R, Iyer LM, Vasudevan S, Aravind L (2002) Detection of novel members, structure-function analysis and evolutionary classification of the 2H phosphoesterase superfamily. Nucleic Acids Res 30:5229–5243
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79. Schwer B, Sawaya R, Ho CK, Shuman S (2004) Portability and fidelity of RNA-repair systems. Proc Natl Acad Sci USA 101:2788–2793

80. Wang LK, Lima CD, Shuman S (2002) Structure and mechanism of T4 polynucleotide kinase: an RNA repair enzyme. EMBO J 21:3873–3880

81. Schütz K, Hesselberth JR, Fields S (2010) Capture and sequence analysis of RNAs with terminal 2',3'-cyclic phosphates. RNA 16:621–631

82. Culver GM, Consaul SA, Tycowski KT, Filipowicz W, Phizicky EM (1994) tRNA splicing in yeast and wheat germ. A cyclic phosphodiesterase implicated in the metabolism of ADP-ribosyl 1',2'-cyclic phosphate. J Biol Chem 269:24926–24934

83. Culver GM, McCraith SM, Consaul SA, Stanford DR, Phizicky EM (1997) A 2'-phosphotransferase implicated in tRNA splicing is essential in Saccharomyces cerevisiae. J Biol Chem 272:13203–13210

84. 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-ribosyl 1',2'-cyclic phosphate. Science 261:206–208

85. McCraith SM, Phizicky EM (1990) A highly specific phosphodiesterase from Saccharomyces cerevisiae implicated in tRNA splicing. Mol Cell Biol 10:1049–1055

86. Zillmann M, Gorovsky MA, Phizicky EM (1997) Conserved mechanism of tRNA splicing in eukaryotes. Mol Cell Biol 11:5410–5416

87. Englert M, Sheppard K, Gundlapalli S, Beier H, Soll D, Aslanian A, Yates JR 3rd, Soll D (2011) RtcB, a novel RNA ligase, can catalyze tRNA splicing and HAC1 mRNA splicing in vivo. J Biol Chem 286:30253–30257

88. 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

89. Kurihara T, Fowler AV, Takahashi Y (1987) cDNA cloning and amino acid sequence of bovine brain 2',3'-cyclic-nucleotide 3'-phosphodiesterase. J Biol Chem 262:3256–3261

90. Kurihara T, Takahashi Y, Nishiyama A, Kumanishi T (1988) cDNA cloning and amino acid sequence of human brain 2',3'-cyclic-nucleotide 3'-phosphodiesterase. Biochem Biophys Res Commun 152:837–842

91. Vogel US, Thompson RJ (1987) Molecular cloning of the myelin splicing enzyme 2',3'-cyclic-nucleotide 3'-phosphohydrolase. FEBS Lett 218:261–265

92. Spinelli SL, Malik HS, Consaul SA, Phizicky EM (1998) A functional homolog of a yeast tRNA splicing enzyme is conserved in higher eukaryotes and in Escherichia coli. Proc Natl Acad Sci USA 95:14136–14141

93. Hu QD, Lu H, Hui F, Ying K, Li J, Xie Y, Mao Y, Li YY, Schwer B, Sawaya R, Ho CK, Shuman S (2004) A human homolog of the yeast gene encoding tRNA ligase (hClp1) can function as a tRNA splicing enzyme in vivo. RNA 10:1737–1745

94. Swer B, Aronova A, Ramirez A, Braun P, Shuman S (2008) Mammalian 2',3' cyclic nucleotide phosphodiesterase (CNP) can function as a tRNA splicing enzyme in vivo. RNA 14:204–210

95. Harding HP, Lackey JG, Hsu HC, Zhang Y, Deng J, Xu RM, Damha MJ, Ron D (2008) An intact unfolded protein response in Trp1 knockout mice reveals phylogenic divergence in pathways for RNA ligation. RNA 14:225–232

96. Filipowicz W, Konarska M, Gross HJ, Shatkin AJ (1983) RNA 3'-terminal phosphate cyclase activity and RNA ligation in HeLa cell extract. Nucleic Acids Res 11:1405–1418

97. Gomes I, Gupta R (1997) RNA splicing ligase activity in the archaeon Haloferax volcanii. Biochem Biophys Res Commun 237:588–594

98. Zofallova L, Guo Y, 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

99. Englert M (2005) Mechanisms des pre-tRNA-Spleitens: Struktur und Funktion plazenzlicher und animaler RNA-Ligasen. Diss. Universität Würzburg, Würzburg

100. Perkins KK, Furneaux H, Hurwitz J (1985) Isolation and characterization of an RNA ligase from HeLa cells. Proc Natl Acad Sci USA 82:684–688

101. Engler M, Sheppard K, Aslanian A, Yates JR 3rd, Soll D (2011) Archaeal 3'-phosphate RNA splicing ligase characterization identifies the missing component in tRNA maturation. Proc Natl Acad Sci USA 108:1290–1295

102. Popow J et al (2011) HSPC117 is the essential subunit of a human tRNA splicing ligase complex. Science 331:760–764

103. Tanaka N, Shuman S (2011) RtcB is the RNA ligase component of an Escherichia coli RNA repair operon. J Biol Chem 286:7727–7731

104. Okada C, Maegawa Y, Yao M, Tanaka I (2006) Crystal structure of an RtcB homolog protein (PH1602-extein protein) from Pyrococcus horikoshii reveals a novel fold. Proteins 63:1119–1122

105. Tanaka N, Meineke B, Shuman S (2011) A novel mechanism of RNA repair by RtcB via sequential 2',3'-cyclic phosphodiesterase and 3'-phosphate/5'-hydroxyl ligation reactions. J Biol Chem 286:43134–43143

106. Drewett V, Molina H, Millar A, Muller S, von Hesler F, Shaw PE (2001) DNA-bound transcription factor complexes analysed by mass-spectrometry: binding of novel proteins to the human c-fos SRE and related sequences. Nucleic Acids Res 29:479–487

107. 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

108. Godbout R, Squire J (1993) Amplification of a DEAD box helicase. FEBS Lett 312:37–41

109. Godbout R, Squire J (1993) Association of human DEAD box protein DDX1 with a cleavage stimulation factor involved in 3'-end processing of pre-mRNA. Mol Cell Biol 13:3046–3059

110. Chen HC, Lin WC, Tsay YG, Lee SC, Chang CJ (2002) An RNA helicase, DDX11, interacting with poly(A) RNA and heterogeneous nuclear ribonucleoprotein K. J Biol Chem 277:40403–40409
118. Li L, Monckton EA, Godbout R (2008) A role for DEAD box 1 at DNA double-strand breaks. Mol Cell Biol 28:6413–6425
119. Godbout R, Packer M, Bie W (1998) Overexpression of a DEAD box protein (DDX1) in neuroblastoma and retinoblastoma cell lines. J Biol Chem 273:21161–21168
120. Squire JA, Thorner PS, Weitzman S, Maggi JD, Dirks P, Doyle J, Hale M, Godbout R (1995) Co-amplification of MYCN and a DEAD box gene (DDX1) in primary neuroblastoma. Oncogene 10:1417–1427
121. Cao D, Haussecker D, Huang Y, Kay MA (2009) Combined proteomic-RNAi screen for host factors involved in human hepatitis delta virus replication. RNA 15:1971–1979
122. Xu L, Khadijah S, Fang S, Wang L, Tay FP, Liu DX (2010) The cellular RNA helicase DDX1 interacts with coronavirus non-structural protein 14 and enhances viral replication. J Virol 84:8571–8583
123. Edgcomb SP, Carmel AB, Naji S, Ambrus-Aikelin G, Reyes JR, Godbout R, Packer M, Bie W (1998) Overexpression of a novel gene ashwin functions in cell survival Xenopus laevis. Biochem Biophys Res Commun 257:475–483
124. Zhang Z et al (2011) DDX1, DDX21, and DHX36 helicases are functional activators. J Virol 75:8597–8604
125. Park MY, Wu G, Gonzalez-Sulser A, Vaucheret H, Poethig RS (2005) Nuclear processing and export of microRNAs in Arabidopsis. Proc Natl Acad Sci USA 102:3691–3696
126. Hopper AK, Pai DA, Engelke DR (2010) Cellular dynamics of tRNA ligase mutants are nonviable and accumulate tRNA splicing intermediates. J Biol Chem 265:4577–4582
127. Phizicky EM, Consaul SA, Nehrke KW, Abelson J (1992) Yeast RNA-dependent ATPase Involved in HIV-1 Rev Function and Virus Replication. J Mol Biol 415:61–74
128. Zhang Z et al (2011) DDX1, DDX21, and DHX36 helicases form a complex with the adaptor molecule TRIF to sense dsRNA in dendritic cells. Immunity 34:866–878
129. Huarte M, Sanz-Esquerrro JJ, Roncal F, Ortin J, Nieto A (2001) PA subunit from influenza virus polymerase complex interacts with a cellular protein with homology to a family of transcriptional activators. J Virol 75:8857–8864
130. Perez-Gonzalez A, Rodriguez A, Huarte M, Salanueva IJ, Nieto A (2006) Novel gene ashwin functions in cell survival Xenopus laevis. Biochem Biophys Res Commun 257:475–483
131. Patil SS, Alexander TB, Uzman JA, Lou CH, Gohil H, Sater AK, Perez-Gonzalez A, Rodriguez A, Huarte M, Salanueva IJ, Nieto A (2006) hCLE/CGI-99, a human protein that interacts with the PA subunit from influenza virus polymerase, is a mRNA transcription modulator. Biochimie 89:1351–1365
132. Iwawaki T, Tokuda M (2011) Function of yeast and amphioxus tRNA ligase in IRE1alpha-dependent XBP1 mRNA splicing. Biochem Biophys Res Commun 413:527–531
133. Yoshida H, Matsui T, Yamamoto A, Okada T, Mori K (2001) Single-stranded antisense siRNAs guide target RNA cleavage in RNAi. Cell 107:881–891
134. Chin K, Shatkin AJ (1985) Proofreading and aminoacylation characterizations of RAT1: an essential gene of Saccharomyces cerevisiae required for the efficient nucleocytoplasmic trafficking of mRNA. Genes Dev 6:1173–1189
135. Corbett AH, Koepp DM, Schlenstedt G, Lee MS, Hopper AK, Silver PA (1995) Rna1p, a Ran/TC4 GTPase activating protein, is required for nuclear import. J Cell Biol 130:1017–1026
136. Nishikura K, De Robertis EM (1981) Intranuclear location of the tRNA splicing enzymes. Cell 23:89–93
137. Iwawaki T, Tokuda M (2011) Function of yeast and amphioxus tRNA ligase in IRE1alpha-dependent XBP1 mRNA splicing. Biochem Biophys Res Commun 413:527–531