The ribosome and RNase P are cellular ribonucleoprotein complexes that perform peptide bond synthesis and phosphodiester bond cleavage, respectively. Both are ancient biological assemblies that were already present in the last universal common ancestor of all life. The large subunit rRNA in the ribosome and the RNA subunit of RNase P are the ribozyme components required for catalysis. Here, we explore the idea that these two large ribozymes may have begun their evolutionary odyssey as an assemblage of RNA “fragments” smaller than the contemporary full-length versions and that they transitioned through distinct stages along a pathway that may also be relevant for the evolution of other non-coding RNAs.

The ribosome and RNase P stand out among all cellular ribonucleoprotein (RNP) complexes. These two RNPs, which play central roles in translation and tRNA processing, respectively, are ancient biological entities that were already present in the last universal common ancestor (LUCA) of all life (1–6), emerging prior to the divergence of living organisms into the primary domains: Archaea, Bacteria, and Eucarya (7, 8). In eukaryotes, they function in the nucleus/cytoplasm and in protein-synthesizing organelles (mitochondria and plastids). This early emergence and long evolutionary history emphasize the fundamental and essential biological roles played by these two pervasive RNPs. Notably, peptide bond synthesis and phosphodiester bond cleavage, respectively, are catalyzed by the large subunit ribosomal RNA (rRNA) and the RNA subunit of RNase P (RPR) in the corresponding RNPs. The recognition of RNA-mediated catalysis in the self-splicing group I intron and RNase P (RP), in the compact three-dimensional, modular structure of the functional core of its covalently continuous counterpart. This early emergence and long evolutionary history emphasize the fundamental and essential biological roles played by these two perversive RNPs. Notably, peptide bond synthesis and phosphodiester bond cleavage, respectively, are catalyzed by the large subunit ribosomal RNA (rRNA) and the RNA subunit of RNase P (RPR) in the corresponding RNPs. The recognition of RNA-mediated catalysis in the self-splicing group I intron and RNase P, as well as the ribosome, constituted a true paradigm shift in our understanding of the cellular catalytic repertoire (9–11).

An intriguing unsolved puzzle relates to the evolution of the two large ribozymes at the heart of the ribosome and RNase P. These catalytic RNAs may represent relics of a primordial RNA world (12), the implication being that these RNAs were functional before their transition into contemporary, multisubunit RNP forms. Indeed, Crick (13) stated explicitly that “the primitive ribosome could have been made entirely of RNA,” an early view also championed by Woese (14, 15). In this review, we explore the premise that large ribozymes may have begun their evolutionary odyssey as an assemblage of RNA “fragments” smaller than the existing, full-length versions, a likely first step for the domain accretion that followed. Trajectories of extant macromolecules, deduced from the footprints of evolution, have loose ends not unlike any complicated tapestry. Notwithstanding these potential gaps, the extant diversity of biogenesis routes and structures of rRNA and RPR has shaped our narrative on the evolution of these two ribozymes.

Evolution of large ribozymes may have begun with smaller, isolated fragments

rRNA and RPR are long, covalently continuous RNA molecules whose secondary structures are characterized by an interspersed pattern of conserved and variable regions, as well as long-range base-pairing interactions that bring distant parts of the RNA molecule close together (3, 16–20). Tertiary contacts, mediated by noncovalent interactions, further act as braces to tie remote parts of the secondary structure together and to form the compact three-dimensional, modular structure of the functional small subunit (SSU) and large subunit (LSU) rRNAs, as well as the RPR (21–28). It is hardly conceivable that this structural and functional complexity was present at the dawn of translation and tRNA 5′-processing. So, what were the ancestral forms of the RNA components of the primordial ribosome and RNase P, and how did they evolve into their contemporary counterparts? An underlying premise that guides the evolutionary models discussed here is that a ribozyme (or a noncoding RNA) does not have to be covalently continuous to function: it may be composed of a collection of smaller “fragments” that are able to interact noncovalently to generate a three-dimensional structure that contains the functional core of its covalently continuous counterpart. We enumerate several recent findings, including the high-resolution structures of the ribosome and RNase P, that provide support for this notion and integrate insights from these observations to extend previously elaborated models of rRNA and RNase P RNA evolution (29–32).
Ribosomal RNA

Based on comparative structural studies of the ribosome and its constituent RNAs in many different protein-synthesizing systems, various inferences have been drawn about the origin and evolution of the ribosome and of its different functional domains (5, 33–37). These studies have provided important insights into and have stimulated imaginative ideas about rRNA/ribosome evolution. Among the conclusions that are particularly germane here is the notion of modularity and domain accretion: that RNA molecules comprise a set of discrete modules/domains that have been acquired sequentially over the evolutionary history of the ribosome (5, 34, 35, 37). Paramount among these domains, and almost certainly the oldest, is the peptidyl transferase center (PTC), located in the LSU rRNA. The accretion model (37) envisions the ribosome and translation beginning with small, interacting RNAs, with the primitive rRNA growing in complexity via the incorporation of new modules. However, the evolutionary mechanism whereby contemporary SSU and LSU rRNA molecules—covalently continuous and long—might have arisen from small, noncovalently interacting RNA species has been little considered. Here, we specifically address this question.

The discovery of ribosomes that contain fragmented rRNAs (documented in Refs. 29–31) inspired the idea that contemporary long, covalently continuous rRNA molecules may have evolved from a collection of much smaller, noncovalently interacting RNA species that comprised the primordial ribosome (38, 39). The existence of such naturally fragmented rRNAs and the demonstration that the fragmentation is inherent and not the result of degradation during sample preparation (40, 41) indicate that covalent continuity, as seen in conventional 16S-18S SSU and 23S–28S LSU rRNAs, is not absolutely essential for function. Indeed, in protein-synthesizing cell lysates that have been treated with nuclease to remove endogenous mRNA, ribosomes contain fragmented (degraded) rRNAs but are nevertheless active in translation (42). Of course, whether a particular rRNA cleavage is deleterious depends on where these scissions occur within the rRNA structure. A single phosphodiester bond cleavage within the decoding site near the 3′-end of 16S rRNA, mediated by the bacterial cytotoxin colicin E3, is sufficient to inactivate the bacterial ribosome (43, 44). Likewise, a single incision within the α-sarcin loop, a binding site for elongation factors (45–47) that is universally conserved near the 3′-end of LSU rRNAs, is sufficient to inactivate the ribosome (48, 49). On the other hand, nonconserved regions of rRNA have been shown to be tolerant toward genetic insertions (50), so it is hardly surprising that cleavages within naturally fragmented rRNAs are confined to these variable regions (31). (It is important to emphasize that although variable regions may have initially been devoid of function, the possibility remains that some may have acquired function later during ribosome evolution. For example, in some eukaryotic rRNAs, certain variable regions have become greatly enlarged into so-called expansion segments, some of which now have a regulatory function in protein synthesis (51).)

A model of rRNA evolution based on the idea of fragmented rRNAs has been presented and elaborated in detail elsewhere (29–31) and is briefly described here. The model envisages a stepwise evolutionary pathway (Fig. 1), with four main stages, exemplified by current examples of diverse rRNA structure. In Stage I (Fig. 1), physically separate rRNA coding modules, corresponding to discrete portions of the conserved structural core of contemporary rRNAs, are separately transcribed to generate precursor transcripts that undergo processing to generate mature 5′ and 3′ termini. The final transcripts interact noncovalently to form a primitive functional core able to catalyze peptide bond formation. Additional functional domains may have been acquired during this stage, again via noncovalent interactions. Contemporary ribosomes that exemplify features of this stage include the bipartite SSU rRNA in the mitochondrion ribosome (mitoribosome) of Euglena gracilis (52) and, in particular, the multipartite SSU and LSU rRNAs in the mitoribosome of Plasmodium falciparum, the malaria parasite (53), and Clathrina clathrus, a calcareous sponge (54). In the 6-kb Plasmodium mitochondrial DNA (mtDNA), numerous short subgenic LSU rRNA coding modules are interspersed with one another and with protein-coding and tRNA genes and are encoded on both strands of the mitochondrial genome. In Clathrina, reminiscent of the situation in Plasmodium, subgenic SSU and LSU rRNA coding modules are found on three of the six linear chromosomes that comprise the mitochondrial genome and are interspersed with one another and with both protein-coding and tRNA genes. In Clathrina and Plasmodium, it is clear that the rRNA subgene sets on complementary strands or separate chromosomes must be independently transcribed, with subsequent processing of primary transcripts to release the mature small rRNA pieces for mitoribosome assembly.

In Stage II (Fig. 1), rRNA-coding modules are physically and transcriptionally linked and might be interspersed with non-rRNA-coding sequences, as in the example of the P. falciparum mtDNA. However, at this stage, all rRNA modules are co-transcribed, thus ensuring equivalent steady-state levels of the mature, noncovalently interacting products and thereby diminishing the likelihood of formation of nonfunctional, partial assemblies (a possibility in Stage I). This decisive advantage may have been the selective pressure that brought the different modules together under a single promoter. At this stage, physically linked subgenic modules are not ordered in the same 5′-to-3′ transcriptional direction as the corresponding sequences in a conventional rRNA gene; the resulting long co-transcripts, however, are processed to give small rRNA pieces, which then associate noncovalently akin to Stage I. Contemporary examples of Stage II are the bipartite mitochondrial LSU rRNA in the ciliate protozoan Tetrahymena pyriformis, where the 5′ ~ 280 nt (α species) is encoded downstream of the rest of the LSU rRNA gene (β species); the α and β species are separated by a tRNA1-leu gene (55). An even more extreme example is highlighted in the mtDNA of the chlorophyte alga, Chlamydomonas reinhardtii, where subgenic SSU and LSU rRNA modules are scrambled and interspersed with one another and with protein-coding and tRNA genes, all co-transcribed, with post-transcriptional processing of the resulting long transcript (Fig. 2) (39, 56).
In Stage III (Fig. 1), rearrangements at the DNA level culminated in the standard 5′-to-3′ order of SSU and LSU rRNA sequence observed in contemporary, conventional SSU and LSU rRNAs, with SSU-coding modules physically linked to and upstream of LSU modules in the direction of transcription. The selective pressure for such rearrangement may have been the emergence of co-transcriptional folding of longer transcripts such that generation of the mature tertiary structure was facilitated and enhanced, compared with post-transcriptional assembly of separate transcripts. At this stage, rRNA transcripts retain processing sites that liberate mature rRNA segments from larger primary transcripts, so that coding modules are effectively separated by internal transcribed spacer sequences (ITSs) that are removed either co- or post-transcriptionally. The result is a ribosome in which constituent rRNAs are fragmented to an extent dependent on the number of ITSs in the primary transcript, with different parts of SSU and LSU rRNAs held together via noncovalent interactions (as in Stages I and II). Examples of Stage III are the fragmented LSU rRNAs that are found in the cytoplasmic ribosomes of kinetoplastid protozoa, such as *Crithidia fasciculata* (40, 57) and *Trypanosoma brucei* (58), and especially the alga *E. gracilis*, whose cyto-
plasmic LSU rRNA exists and functions as a complex of 14 separate pieces (41, 59).

Stage IV (Fig. 1) envisages progressive loss of processing sites, which effectively converts ITSs (removed during processing) into variable regions that are now retained in the mature rRNA. Effectively, subgenic rRNA modules are “pasted together,” ultimately resulting in a collection of relatively short rRNA segments being converted over evolutionary time into a few long SSU and LSU rRNAs. We emphasize that because the Stage IV pattern of rRNA gene organization, transcription, and processing is common to all three domains of life, the evolutionary transitions we propose here must have been complete before the divergence of these domains from the LUCA. (Incidentally, this idea of converting ITSs to variable regions may be applicable in other cases of ncRNA evolution. For example, yeast U2 snRNA is 6 times larger than the mammalian counterpart due to the addition of 945 nucleotides (nt), whose deletion has no effect on growth, in sharp contrast to the two smaller domains that flank it (60). We also refer below to similar instances with the RPR.)

Considering that the above model of rRNA evolution was initially elaborated more than two decades ago, it is instructive to place it within the extensive body of work on ribosome structure, function, and evolution that has appeared subsequently (4, 5, 21–26, 33–37, 61, 62). By general agreement, the most ancient part of the ribosome, and its functional heart, is the PTC, a self-folding domain in the large subunit (34, 36). Importantly, the PTC is effectively devoid of ribosomal proteins (4, 22, 26), leading to the conclusion that the ribosome is a ribozyme (10, 63). Instead of proteins, Mg$^{2+}$ interactions appear to constitute the major stabilizing force within the PTC (4, 64).

Another striking and universally conserved feature of the PTC is that it comprises two subregions that display 2-fold pseudo-symmetry (relating to the backbone and the orientation of the subregions but not the nucleotide sequence per se) (36, 65–67). These subregions provide the LSU A-site and P-site docking platforms for aminoacyl-tRNA and peptidyl-tRNA, respectively, orienting them appropriately for peptide bond formation. This primitive RNA machine may have been further stabilized initially by interactions with short RNAs complementary to the extremities of the PTC. The inference is that a primordial PTC alone could have functioned as an incipient ribosome capable of peptide bond synthesis, although it is likely that peptides or proteins must also have emerged and co-evolved with their rRNA partners very early in ribosome evolution.

The modular construction of the ribosome is further emphasized by studies that seek to delineate a timeline for the incorporation of different functional parts into the final structure we see today. Fox (5) has summarized cogent arguments for inferring the relative ages of different parts of the translation machinery and LSU rRNA structure as well as the temporal appearance of individual ribosomal proteins (RPs). Strategies based on RNA-RNA, RNA-protein, and protein-protein connectivity (35, 68) have been particularly powerful in identifying the oldest regions of the LSU rRNA, whose secondary structure is characterized by six discrete domains (I to VI). Domain V (containing the PTC) is considered to be the oldest, with Domains II and IV also being very old and likely added in that order. Notably, because Domain IV has major contacts with the SSU, the appearance of this domain likely signaled the beginning of the formation of the SSU. Remarkably, the oldest regions of the LSU rRNA identified by these approaches are largely coincident with the universally conserved LSU rRNA core identified by comparative secondary structure mapping (29–31, 33, 69).

The evolutionary model summarized in Fig. 1 readily accommodates the notion of a timeline in the incorporation of the various structural domains in rRNA. Initially, interactions between older and newly acquired domains could have involved noncovalent bonding, principally complementary base pairing. Depending on the number of domains in question, the evolving LSU rRNA could have functioned as a collection of small, noncovalently interacting RNAs (Fig. 1). However, the model has latitude in that the conversion to a larger, covalently continuous LSU rRNA could have occurred incrementally rather than all at once. For example, localization of a Domain IV coding module upstream in and the same transcriptional orientation as a Domain V module could ultimately have led to their co-transcription, generating a Domain IV/Domain V product, with coding modules effectively separated by an ITS. This IV-ITS-V co-transcript might have been processed to excise the ITS, thereby generating the individual Domain IV and Domain V RNAs (Stage III, Fig. 1), or the ITS might have been retained, to become a variable region separating two conserved regions (Stage IV, Fig. 1). New rRNA domains could have been added in such a stepwise fashion, effectively converting initially noncovalently interacting small RNAs into a progressively larger covalently continuous species.

One aspect of ribosome evolution to which the original model (29–31) only alluded is the necessity of co-evolution of rRNA and RPs. Although the examples cited earlier of ribosomes containing naturally fragmented rRNAs serve to underscore the idea that rRNAs do not have to be covalently continuous to function, and interacting small rRNA pieces have been demonstrated experimentally (70), RPs were likely important in the assembly and stabilization of ribosomes having fragmented rRNAs. As noted above, it is possible that a primordial PTC-like RNA, stabilized by interactions with Mg$^{2+}$, might have been able to catalyze peptide bond synthesis in the absence of proteins; however, incorporation of additional rRNA domains likely coincided with recruitment of RPs, as well as other non-ribosomal proteins, such as helicases and chaperones required in the assembly of ribosomal subunits. As with rRNA domains, a timeline in the appearance of RPs seems likely, with some ribosomal proteins arguably older than others (5).

Formation of the eukaryotic spliceosome is an example of how an assemblage of noncovalently interacting RNPs can be formed, emphasizing the role of various assembly proteins in the process (71, 72). Pathways of ribosome assembly, including the order of the addition of RPs to rRNA, have been well worked out in a number of instances (63, 73–78). In the specific mitochondrial cases cited earlier (P. falciparum (53) and C. reinhardtii (39)), co-transcriptional assembly of the organellar ribosome is precluded by the fact that rRNA subgenic coding modules are scrambled in the mitochondrial genome and inter-
spersed with one another and with non-rRNA genes. In these instances, long primary transcripts undergo post-transcriptional processing to yield the mature small rRNA pieces, which must then be assembled to generate the long-range noncovalent base-pairing interactions that constitute the universally conserved SSU and LSU rRNA cores. Mitochondrial RPs must participate in and help to mediate this unusual post-transcriptional assembly process. Despite the keen interest in elucidating how a functional mitoribosome containing fragmented rRNAs is cobbled together, these studies are technically challenging: pure mitochondria are often difficult to come by in large quantities, RNAs of interest are usually present in very low amounts, and the inventory of proteins (in some cases even RPs) involved in the processing/assembly pathway is incomplete. Nevertheless, the recent success in mapping the assembly path of the mitochondrial SSU in trypanosomes (79) should inspire similar studies in other mitochondrial systems, given the prospects for new insights.

**RNase P**

RNase P is a Mg$^{2+}$-dependent endonuclease that functions primarily in tRNA 5′ maturation in all three domains of life, with additional long noncoding RNA biogenesis-related functions in metazoans (1, 3, 80, 81). The RNase P RNP employs the RPR as the catalyst to perform RNA processing (9). It is instructive to consider some parallels between the LSU rRNA and RPR. First, akin to the rRNA, covalent continuity is not essential for RPR function (82), and even termini can be shifted, as evidenced by the near-native function of many circularly permuted RPRs (83). Second, long-distance, molecular struts staple independent domains in the extant RPR tertiary structures (27, 28, 84, 85). Last, there is dramatic variation in RPR size with insertions of “variable” segments interspersed with conserved regions (3, 86). Relying on these and many other recent findings from various biochemical and structural studies of RNase P, we postulate an evolutionary trajectory of RNase P that extends a model proposed two decades ago (32). We first provide a structural and functional context within which to appreciate how the current RPR might have emerged in four different stages (Fig. 3). In parallel with our view of rRNA evolution, the pathway we propose allows for the addition of further functional domains to RPR throughout its evolution, again initially through noncovalent interactions.

The bacterial RPR, like protein enzymes, was shown to be modular and made up of two independently folding domains: the catalytic (C) domain that performs the canonical RNase P cleavage and the specificity (S) domain that aids in substrate recognition but is incapable of cleavage in the absence of the C domain (82, 87, 88). There are 13 universally conserved nt in RPRs from all three domains of life (1–3). These conserved nt are dispersed across five conserved regions (CR-I to CR-V) and positioned in nearly identical secondary structure locations even in RPRs of varying sizes (∼200–450 nt). Phylogenetic analyses suggest that the C domain, which contains the active site as well as CR-I, -IV, and -V, may have been the earliest embodiment of the RPR based on the available sequence data (1, 89, 90). However, the observation that a 31-nt RNA derived...
from Escherichia coli RPR is competent to bind and cleave pre-tRNAs (albeit inefficiently) supports the idea that the C domain may have emerged from an amalgamation of smaller modules (91). Moreover, in vitro evolution experiments have yielded ~40-nt ribozymes, indicating that RNAs of this size may suffice for ligand binding and catalysis (92). Such mini-RNAs have been postulated as the predecessors to macro-RNAs like the RPR (93). (We point out that the 31-nt RNA segment derived from E. coli RPR is absent in many archaeal and all eukaryotic RPRs.)

In Stage I (an early RNA world setting), a primitive RPR variant was likely embedded within a larger, self-replicating genomic RNA (Fig. 3) (32). One model posits that an RNA akin to the tRNA acceptor–T-stem coaxial stack (including the 3′-CCA) was a tag that marked the 3′-ends and guided replication of an ancient RNA genome by ribozymes (94). If genome replication is to be uncoupled from catalysis, then all ribozymes embedded within the larger genomic RNA would have to be liberated from the larger transcript (94). By removing the genomic tag (Fig. 3A), the RPR could have played a crucial role in this regard. Such a cis-cleaving capability of the RPR is well-supported by experimental evidence. Bacterial and archaeal RPRs, either in their entirety or only the C domain, cleave covalently tethered pre-tRNAs and model stem-loop substrates (95–98). In one instance, the rate of self-cleavage by an archaeal RPR C-domain–pre–tRNA conjugate was only 12-fold slower compared with the same reaction with the full-length RPR (C + S domains) (98).

In Stage II, the need to contribute to small RNA biogenesis in trans possibly resulted in excision of the C domain from the RNA genome. If site-specific, self-cleavage of the genomic RNA resulted in smaller RNAs that needed additional 5′-processing, an efficient trans-acting RPR would have been subject to positive selection. Many studies have documented the ability of the C domain alone to catalyze 5′-processing of pre-tRNAs and model substrates in trans; the activity is weak, however, without protein cofactors (82, 99–102). Interestingly, these archaeal/bacterial RPR C-domain deletion derivatives foreshadowed the discovery of a shorter, naturally occurring doppelgänger: an archaeal RPR variant, lacking most of the S domain, was discovered in Thermoproteacae (103). This variant, as exemplified by the 208-nt version in Pyrobaculum aerophilum, supports weak tRNA 5′-maturation in vitro. Even though protein cofactors in vivo are likely obligatory for functioning of this abbreviated RPR, that it exists at all in members of the Thermoproteacae family within the crenarchaeal phylum (103, 104) attests to its early origins and supports the suggestion that the RPR progenitor could have been just the stand-alone C domain. The ancestral nature of the C domain is also showcased by its presence in the minimal consensus structure of the bacterial (~210-nt) and eukaryotic (~160-nt) RPRs that was determined by extensive phylogenetic analyses (1, 89, 105). (Although there is no major difference between Stages I and II with respect to RPR function, the free-standing existence of the RPR C domain and other ribozymes would have facilitated their conversion into corresponding DNAs by reverse transcription, a key requisite for the transition from the RNA to the DNA world.)

In Stage III (perhaps coinciding with the LUCA), adventitious interactions between the RPR C domain and another RNA likely engendered substrate-recognition payoffs. One such trans-acting RNA must have been the forerunner to the extant S domain, which has now been demonstrated in various RPRs to be critical for tRNA recognition (27, 28, 84, 85, 99, 101, 106). However, there are no reports of these two RPR domains being transcribed from two independent genes and then assembled together (either with or without protein subunits). Even in cases where only short C domain-like RPRs have been reported (e.g. fungal mitochondria, Crenarchaeata) (103, 104, 107), there is no evidence for the presence of a free-standing RPR S domain. Combining for short, conserved stretches in S domains whose sizes range from 50 to 700 nt is difficult. We list three reasons, however, to support the likelihood of Stage III.

First, a majority of extant RPRs have long-range, tertiary interactions that bridge the two domains and contribute to the ribozyme’s stability and activity (27, 28, 84, 85). These interdomain interactions, which entail tetraloop-tetraloop receptor contacts, confer weak activity on an assembly of S and C domain versions, each of which is inactive on its own (82, 88).

Second, high-resolution structures of bacterial and eukaryotic RNase P have shown that the S domains in these two RPRs (separated by billions of years in evolution) recognize the “elbow” structure that is unique to the L-shaped tRNA structure (28, 84, 85). All S domains house CR-II and CR-III, each of which forms a T-loop that consists of five consecutive nt that form a U-turn structure (108). Nucleotides at positions 1 and 5 form a closing base pair, whereas position 2 stacks with position 1 and pairs with the base at position 4. The unstacked nucleobase at position 3 is used to facilitate tertiary/intermolecular contacts through either base pairing or base stacking (108). The two T-loops in the RPR interdigitate and offer two stacked bases to directly interact with the D (dihydrouridine)- and TψC-loops (28, 84). The early presence of isolated S domain–like RNAs that could specifically recognize the elbow structure is supported by the salient observation that the head-to-tail interdigitated T-loop motif observed in RPRs is also used by 23S rRNA and T-box riboswitches to accomplish tRNA recognition (109).

Last, the bifurcated recognition of the tRNA substrate by RNase P has a thematic parallel in tRNA synthetases (32). The noncovalent interaction between the C and S domains would have been beneficial, especially if it co-evolved with the emergence of anticodon-bearing tRNAs from simple minihelices. Various arguments have been advanced to support the idea that the anticodon-D-stem stack, which allowed decoding of mRNAs, was probably added to the more ancient acceptor helix stacked on the TψC-stem loop (110, 111). This adaptation was the edifice necessary to build the theater of protein synthesis. RPRs position the cleavage site by exploiting specific contacts between the C domain and the tRNA acceptor helix and 3′-CCA (when present) and between the S domain and the elbow structure (Fig. 4, A and B). Aminoacyl-tRNA synthetases have an ancient catalytic domain that recognizes the acceptor helix and an anticodon recognition domain (that was likely added later) to read the anticodon (Fig. 4C) (110, 111). Thus, the transformation of mini-helices to the L-shaped tRNAs must...
have led to the co-evolution of enzymes wherein the newer tRNA structural elements were harnessed to build specificity in recognition. Although there are no reports of independently encoded RPR S and C domains, there is an example of a split alanyl-tRNA synthetase in *Nanoarchaeum equitans*, where the catalytic and anticodon recognition domains are expressed from separate genes. When the two individual domains were reconstituted in vitro, the noncovalent assembly was functional (112).

In Stage IV, rearrangements at the DNA level must have finally led to the fused arrangement (Fig. 3). Akin to the rRNA situation, various benefits likely provided the driving force for insertion of the S domain—coding sequence within that of the C domain gene. Foremost, the higher stability of the bimodular ribozyme that is assembled from a single RNA. Second, there were likely payoffs for RNA folding especially with appropriate in vivo transcriptional pause sites that allow attainment of the native fold (113). Third, a single gene ensures that there are no disparities in the stoichiometric amounts of the two domains synthesized. Last, the catalytic process is simplified, as the overall binding reaction is reduced from a three- to a two-component system with payoffs resulting from cooperative binding of the two domains to the substrate. A similar argument has been put forth for effective DNA recognition by two DNA-binding domains connected by a linker (114).

**Conclusions**

Here, we have argued that long ncRNAs such as LSU rRNA and RPR had their evolutionary origins in smaller RNAs whose genomic coding regions were subsequently “stitched together” during evolution. This concept, which thematically mirrors the genesis of multidomain proteins, likely has parallels in shaping the evolution of other ncRNAs. For example, tRNAs are generally considered to be composed of two domains, each comprising one-half of the tRNA molecule (94, 115–117). As described above, one domain contains the 3′-terminal CCA to which amino acids are attached; the other contains the anticodon loop that interacts with mRNA. The two domains are considered to be of different ages, with the CCA domain the older (5). If this is the case, the two domains could well have been melded together evolutionarily by a pathway resembling the one described here for rRNA and RPR. There are other instances where this theme is not as well-defined but that merit a closer look. For example, the signal recognition particle (SRP) RNA comprises two structurally and functionally separable modules: a smaller (Alu I) domain that is believed to be involved in translational arrest and a larger domain that is essential for recognizing the nascent sequence of secretory proteins emerging from the ribosome (118, 119). The SRP RNA appears to have been forged from two distinct structural modules, thereby engendering functional gains, although the presence of bacterial variants that have the larger domain either without or with the smaller (Alu) domain complicates tracing the thread of evolution. (While there are examples of split transfer-mRNAs (tmRNAs) (120), we excluded them from consideration as these fragments arose from gene permutation events and differ thematically from the examples described above.)

We have presented a portrait of ncRNAs to support the seemingly inescapable “consolidation” of domains (i.e., transition from small to large) perspective, but the counterview of “fragmentation” (large to small) also warrants consideration. For example, the evolutionary transition of ancient group II introns to the spliceosome is believed to have entailed fragmentation of the group II intron into smaller RNAs that eventually became the snRNAs capable of reassembling in *trans* to form the present-day splicing machinery (121, 122). One can argue that fragmentation was likely advantageous in the case of the spliceosome. An early genome riddled with *cis*(self)-splicing introns of varying efficiency may have favored a more capable *trans*-splicing system, which eventually afforded other significant payoffs, including alternative splicing and the versatility afforded by fabrication of spliceosome variants with shared and distinctive components. Such flexibility would have been impossible with a single-subunit RNA, such as the group II intron from which spliceosomal snRNAs are thought to have evolved (121, 122). In fact, at first glance, the existence of contemporary systems, described earlier, in which ribosomes contain naturally fragmented SSU and/or LSU rRNA species might seem to present a parallel to the spliceosome. These fragmented rRNAs undoubtedly evolved from ancestors having covalently continuous rRNAs (i.e., the fragmentation does not represent a retained ancestral trait but rather is a derived characteristic).

This contention is supported by the patchy distribution of fragmented rRNAs throughout the eukaryotic or mitochondrial phylogenetic tree, in which species having fragmented rRNAs
are embedded among species that have covalently continuous counterparts, which are in the majority (29–31). For both eukaryotic and mitochondrial ribosomes, we infer from the respective eukaryotic and mitochondrial phylogenetic trees that the last eukaryotic common ancestor had covalently continuous cytosolic and mitochondrial rRNAs (29–31). While the benefits (if any) of rRNA fragmentation are not immediately evident, these examples serve to emphasize the structural flexibility that is inherent in the ribosome’s evolutionary history and help us to deduce the rRNA evolutionary origins. However, the selective pressures at different hierarchical levels, which balance the choice between fragmentation and consolidation, confound attempts to unambiguously map the evolutionary trajectories of the rRNA and RPR.

While we have highlighted some unifying themes from a comparison of ribosomes and RNase P, we recognize that the molecular paleontology of these two catalytic RNPs does not permit the fashioning of a simple, linear evolutionary chronology of their catalytic RNA components. Nevertheless, the smorgasbord of ribosome and RNase P variants—wrought by chance and necessity—is arresting for its striking plurality and for highlighting the value of studying ancient and fundamental macromolecular machines in diverse organisms to better appreciate the dynamic nature of their evolution.

Acknowledgments—We thank Mike Ibba and Karin Musier-Forsyth (Ohio State University) for valuable input, and Hong-Duc Phan (Ohio State University) for assistance with illustrations.

References

1. Evans, D., Marquez, S. M., and Pace, N. R. (2006) RNase P: interface of the RNA and protein worlds. Trends Biochem. Sci. 31, 333–341 CrossRef Medline
2. Gopalvan, V. (2007) Uniformity amid diversity in RNase P. Proc. Natl. Acad. Sci. U.S.A. 104, 2031–2032 CrossRef Medline
3. Ellis, J. C., and Brown, J. W. (2009) The RNase P family. RNA Biol. 6, 362–369 CrossRef Medline
4. Hsiao, C., Mohan, S., Kalahar, B. K., and Williams, L. D. (2009) Peeling the onion: ribosomes are ancient molecular fossils. Mol. Biol. Evol. 26, 2415–2425 CrossRef Medline
5. Fox, G. E. (2010) Origin and evolution of the ribosome. Cold Spring Harb. Perspect. Biol. 2, a003483 CrossRef Medline
6. Daniels, C. J., Lai, L. B., Chen, T.-H., and Gopalvan, V. (2019) Both kinds of RNase P in all domains of life: surprises galore. RNA 25, 286–291 CrossRef Medline
7. Woese, C. R., and Fox, G. E. (1977) Phylogenetic structure of the prokaryotic domain: the primary kingdoms. Proc. Natl. Acad. Sci. U.S.A. 74, 5088–5090 CrossRef Medline
8. Woese, C. R., Kandler, O., and Wheelis, M. L. (1990) Towards a natural system of organisms: proposal for the domains Archaea, Bacteria, and Eucarya. Proc. Natl. Acad. Sci. U.S.A. 87, 4576–4579 CrossRef Medline
9. Guerrier-Takada, C., Gardiner, K., Marsh, T., Pace, N., and Altman, S. (1983) The RNA moiety of ribonuclease P is the catalytic subunit of the enzyme. Cell 35, 849–857 CrossRef Medline
10. Cech, T. R. (2000) The ribosome is a ribozyme. Science 289, 878–879 CrossRef Medline
11. Steitz, T. A., and Moore, P. B. (2003) RNA: the first macromolecular catalyst: the ribosome is a ribozyme. Trends Biochem. Sci. 28, 411–418 CrossRef Medline
12. Robertson, M. P., and Joyce, G. F. (2012) The origins of the RNA world. Cold Spring Harb. Perspect. Biol. 4, a003608 CrossRef Medline
13. Crick, F. H. C. (1968) The origin of the genetic code. J. Mol. Biol. 38, 367–379 CrossRef Medline
14. Woese, C. R. (1972) The emergence of genetic organization. in Exobiology (Ponnampерuma, C., ed) pp. 301–341, North-Holland Publishing Co., Amsterdam
15. Woese, C. R. (1980) Just So stories and Rube Goldberg machines: speculations on the origin of the protein synthetic machinery. in Ribosomes: Structure, Function, and Genetics (Chamblish, G., Craven, G. R., Davies, J., Davis, K., Kahan, L., and Nomura, M., eds) pp. 357–373, University Park Press, Baltimore
16. James, B. D., Olsen, G. J., Liu, J. S., and Pace, N. R. (1988) The secondary structure of ribonuclease P RNA, the catalytic element of a ribonuclease-protein enzyme. Cell 52, 19–26 CrossRef Medline
17. Gutell, R. R. (1994) Collection of small subunit (16S- and 16S-like) ribosomal RNA structures: 1994. Nucleic Acids Res. 22, 3502–3507 CrossRef Medline
18. Gutell, R. R., Larsen, N., and Woese, C. R. (1994) Lessons from an evolving rRNA: 16S and 23S rRNA structures from a comparative perspective. Microbiol. Rev. 58, 10–26 CrossRef Medline
19. Schnare, M. N., Damberger, S. H., Gray, M. W., and Gutell, R. R. (1996) Comprehensive comparison of structural characteristics in eukaryotic cytoplasmic large subunit (23 S-like) ribosomal RNA. J. Mol. Biol. 256, 701–719 CrossRef Medline
20. Seif, E. R., Forget, L., Martin, N. C., and Lang, B. F. (2003) Mitochondrial RNA P RNAs in ascomycete fungi: lineage-specific variations in RNA secondary structure. RNA 9, 1073–1083 CrossRef Medline
21. Wimberly, B. T., Brodersen, D. E., Clemens, W. M., Jr., Morgan-Warren, R. J., Carter, A. P., Vonrhein, C., Hartsch, T., and Ramakrishnan, V. (2000) Structure of the 30S ribosomal subunit. Nature 407, 327–339 CrossRef Medline
22. Ban, N., Nissen, P., Hansen, J., Moore, P. B., and Steitz, T. A. (2000) The complete atomic structure of the large ribosomal subunit at 2.4 Å resolution. Science 289, 905–920 CrossRef Medline
23. Harms, J., Schluenzen, F., Zarivach, R., Bashan, A., Gat, S., Agmon, I., Bartels, H., Franceschi, F., and Yonath, A. (2001) High resolution structure of the large ribosomal subunit from a mesophilic eubacteria. Cell 107, 679–688 CrossRef Medline
24. Yusupov, M. M., Yusupova, G. Z., Baoucom, A., Lieberman, K., Earnest, T. N., Cate, J. H. D., and Noller, H. F. (2001) Crystal structure of the ribosome at 5.5 Å resolution. Science 292, 883–896 CrossRef Medline
25. Schuwirth, B. S., Borovinskaya, M. A., Hau, C. W., Zhang, W., Vila-Sanjurjo, A., Holton, J. M., and Cate, J. H. D. (2005) Structures of the bacterial ribosome at 3.5 Å resolution. Science 310, 827–834 CrossRef Medline
26. Selmer, M., Dunham, C. M., Murphy, F. V., 4th, Weixbaumer, A., Petry, S., Kelley, A. C., Weir, J. R., and Ramakrishnan, V. (2006) Structure of the 70S ribosome complexed with mRNA and tRNA. Science 313, 1935–1942 CrossRef Medline
27. Torres-Larios, A., Singer, K. K., Krassilnikov, A. S., Pan, T., and Mondragon, A. (2005) Crystal structure of the RNA component of bacterial ribonuclease P. Nature 437, 584–587 CrossRef Medline
28. Reiter, N. J., Osterman, A., Torres-Larios, A., Singer, K. K., Pan, T., and Mondragon, A. (2010) Structure of a bacterial ribonuclease P holoenzyme in complex with tRNA. Nature 468, 784–789 CrossRef Medline
29. Gray, M. W., and Schnare, M. N. (1990) Evolution of the modular structure of ribosomal RNA. in The Ribosome: Structure, Function and Evolution (Hill, W. E., Dahlberg, A., Garret, R., Moore, P. B., Schlessinger, D., and Warner, J. R., eds) pp. 589–597, American Society for Microbiology, Washington, D. C.
30. Gray, M. W., Greenwood, S. J., Smallman, D. S., Spencer, D. F., and Schnare, M. N. (1995) Ribosomal RNA in pieces: a modern paradigm of the primordial ribosome. in Tracing Biological Evolution in Protein and Gene Structures (Gö, M., and Schimmel, P., eds) pp. 65–76, Elsevier Science B.V., Amsterdam
31. Gray, M. W., and Schnare, M. N. (1996) Evolution of rRNA gene organization. in Ribosomal RNA: Structure, Evolution, Processing, and Function in Protein Biosynthesis (Zimmermann, R. A., and Dahlberg, A. E., eds) pp. 49–69, CRC Press, Inc., Boca Raton, FL
42. Kennedy, T. D., Hanley-Bowdoin, L. K., and Lane, B. G. (1981) Structural
40. Gray, M. W. (1981) Unusual pattern of ribonucleic acid components in
39. Boer, P. H., and Gray, M. W. (1988) Scrambled ribosomal RNA gene
38. Clark, C. (1987) On the evolution of ribosomal RNA.
37. Petrov, A. S., Gulen, B., Norris, A. M., Kovacs, N. A., Bernier, C. R.,
36. Altman, S., and Kirsebom, L. A. (1999) Ribonuclease P.
35. Feagin, J. E., Harrell, M. I., Lee, J. C., Coe, K. J., Sands, B. H., Cannone, J. J.,
34. Smith, T. F., Lee, J. C., Gutell, R. R., and Hartman, H. (2008) The origin and evolution of the ribosome.
33. Mears, J. A., Cannone, J. J., Stagg, S. M., Gutell, R. R., Agrawal, R. K., and
32. Terada, T., Shirouzu, M., Rost, M., Schüler, M., Giesebricht, J., Dabrowski, M., Mielke, T., Fucini, P., Yokoyama, S., and Spahn, C. M. T. (2007) Structural basis for interaction of the ribosome with the switch regions of GTP-bound elongation factors. Mol. Cell 25, 751–764
31. Fujii, K., Susanto, T. T., Saurabh, S., and Barna, M. (2018) Decoding the function of expansion segments in ribosomes. Mol. Cell 72, 1013–1020.e6
30. Spencer, D. F., and Gray, M. W. (2011) Ribosomal RNA genes in Chlamydomonas reinhardtii mitochondria. Curr. Genet. 14, 583–590 CrossRef Medline
29. Spencer, D. F., and Gray, M. W. (1988) Transfer RNA genes and the genetic code in Chlamydomonas reinhardtii mitochondria. J. Biol. Chem. 262, 2879–2887 Medline
28. Heineon, T. Y. K., Schnare, M. N., Young, P. G., and Gray, M. W. (1987) Rearranged coding segments, separated by a transfer RNA gene, specify the two parts of a discontinuous large subunit ribosomal RNA in Tetrahymena pyriformis mitochondria. J. Biol. Chem. 262, 865–880 CrossRef Medline
27. Spencer, D. F., Collins, J. C., Schnare, M. N., and Gray, M. W. (1987) Multiple spacer sequences in the nuclear large subunit ribosomal RNA gene of Crithidia fasciculata. EMBO J. 6, 1063–1071 CrossRef Medline
26. Campbell, D. A., Kubo, K., Clark, C. G., and Boothroyd, J. C. (1987) Precise identification of cleavage sites involved in the unusual processing of trypanosome ribosomal RNA. J. Biol. Chem. 196, 113–124 CrossRef Medline
25. Schnare, M. N., Collings, J. C., Schnare, M. N., and Gray, M. W. (1990) Fourteen internal transcribed spacers in the circular ribosomal DNA of Euglena gracilis. J. Biol. Chem. 215, 85–91 CrossRef Medline
24. Connell, S. R., Takemoto, C., Wilson, D. N., Wang, H., Murayama, K., Terada, T., Shirouzu, M., Rost, M., Schüler, M., Giesebricht, J., Dabrowski, M., Mielke, T., Fucini, P., Yokoyama, S., and Spahn, C. M. T. (2007) Structural basis for interaction of the ribosome with the switch regions of GTP-bound elongation factors. Mol. Cell 25, 751–764 CrossRef Medline
23. Shuster, E. O., and Guthrie, C. (1988) Two conserved domains of yeast 23S ribosomal RNA: structure, function, and evolution of the ribosome. J. Mol. Biol. 215, 19–31 CrossRef Medline
22. Shi, Y. (2017) The spliceosome: a protein-directed metalloribozyme. J. Mol. Biol. 429, 2640–2653 CrossRef Medline
21. Fox, G. E., and Naik, A. K. (2004) The evolutionary history of the trans-spliceosome. JBC Reviews: Ribozyme evolution
20.控股股东，影响细胞分裂。Cold Spring Harb. Perspect. Biol. 3, a003707 CrossRef Medline
19. Will, C. L., and Lührmann, R. (2011) Spliceosome structure and function. Cold Spring Harb. Perspect. Biol. 3, a003707 CrossRef Medline
18. Shi, Y. (2017) The spliceosome: a protein-directed metalloribozyme. J. Mol. Biol. 429, 2640–2653 CrossRef Medline
17. Fox, G. E., and Naik, A. K. (2004) The evolutionary history of the translation machinery. In The Genetic Code and the Origin of Life (de Poulain, L. R., ed) pp. 92–105, Springer, Boston
16. Smallman, D. S., Schnare, M. N., and Gray, M. W. (1996) RNA: RNA interactions in the large subunit ribosomal RNA of Euglena gracilis. Biochim. Biophys. Acta 1305, 1–6 CrossRef Medline
15. Gutell, R. R. (1992) Evolutionary characteristics of 16S and 23S rRNA differentially affects EF-G and EF-Tu binding. J. Mol. Biol. 257, 9054–9060 Medline
14. Garcia-Ortega, L., Álvarez-Garcia, E., Gavilanes, J. G., Martínez-del-Pozo Á., and Joseph, S. (2010) Cleavage of the sarcin–ricin loop of 23S rRNA differentially affects EF-G and EF-Tu binding. Nucleic Acids Res. 38, 4108–4119 CrossRef Medline
13. Yokoyama, T., and Suzuki, T. (2008) Ribosomal RNAs are tolerant toward genetic insertions: evolutionary origin of the expansion segments. Nucleic Acids Res. 36, 3539–3551 CrossRef Medline
12. Fujii, K., Susanto, T. T., Saurabh, S., and Barna, M. (2018) Decoding the function of expansion segments in ribosomes. Mol. Cell 72, 1013–1020.e6 CrossRef Medline
11. Spencer, D. F., and Gray, M. W. (2011) Ribosomal RNA genes in Euglena gracilis mitochondrial DNA: fragmented genes in a seemingly fragmented genome. Mol. Genet. Genomics 285, 19–31 CrossRef Medline
10. Feagin, J. E., Harrell, M. I., Lee, J. C., Coe, K. J., Sands, B. H., Cannone, J. J., Tani, G., Schnare, M. N., and Gutell, R. R. (2012) The fragmented mitochondrial ribosomal RNAs of Plasmodium falciparum. PLoS ONE 7, e38320 CrossRef Medline
9. Fujii, K., Susanto, T. T., Saurabh, S., and Barna, M. (2018) Decoding the function of expansion segments in ribosomes. Mol. Cell 72, 1013–1020.e6 CrossRef Medline
8. Spencer, D. F., and Gray, M. W. (2011) Ribosomal RNA genes in Euglena gracilis mitochondrial DNA: fragmented genes in a seemingly fragmented genome. Mol. Genet. Genomics 285, 19–31 CrossRef Medline
7. Feagin, J. E., Harrell, M. I., Lee, J. C., Coe, K. J., Sands, B. H., Cannone, J. J., Tani, G., Schnare, M. N., and Gutell, R. R. (2012) The fragmented mitochondrial ribosomal RNAs of Plasmodium falciparum. PLoS ONE 7, e38320 CrossRef Medline
6. Lavrov, D. V., Pett, W., Voigt, O., Würheide, G., Forget, L., Lang, B. F., and Kayal, E. (2013) Mitochondrial DNA of Clathrina clathrus (Calcarea, Calcinae): six linear chromosomes, fragmented tRNAs, tRNA editing, and a novel genetic code. Mol. Biol. Evol. 30, 865–880 CrossRef Medline
5. Heineon, T. Y. K., Schnare, M. N., Young, P. G., and Gray, M. W. (1987) Rearranged coding segments, separated by a transfer RNA gene, specify the two parts of a discontinuous large subunit ribosomal RNA in Tetrahymena pyriformis mitochondria. J. Biol. Chem. 262, 865–880 Medline
4. Endo, Y., and Wool, I. G. (1982) The site of action of α-sarcin on eukaryotic ribosomes: the sequence at the α-sarcin cleavage site in 28 S ribosomal ribonucleic acid. J. Biol. Chem. 257, 9054–9060 Medline
3. Garcia-Ortega, L., Álvarez-Garcia, E., Gavilanes, J. G., Martínez-del-Pozo Á., and Joseph, S. (2010) Cleavage of the sarcin–ricin loop of 23S rRNA differentially affects EF-G and EF-Tu binding. Nucleic Acids Res. 38, 4108–4119 CrossRef Medline
2. Yokoyama, T., and Suzuki, T. (2008) Ribosomal RNAs are tolerant toward genetic insertions: evolutionary origin of the expansion segments. Nucleic Acids Res. 36, 3539–3551 CrossRef Medline
1. Fuji, K., Susanto, T. T., Saurabh, S., and Barna, M. (2018) Decoding the function of expansion segments in ribosomes. Mol. Cell 72, 1013–1020.e6 CrossRef Medline
JBC REVIEWS: Ribozyme evolution

74. Klein, D. J., Moore, P. B., and Steitz, T. A. (2004) The roles of ribosomal proteins in the structure assembly, and evolution of the large ribosomal subunit. J. Mol. Biol. 340, 141–177 CrossRef Medline

75. Shajani, Z., Sykes, M. T., and Williamson, J. R. (2011) Assembly of bacterial ribosomes. Annu. Rev. Biochem. 80, 501–526 CrossRef Medline

76. Davis, J. H., and Williamson, J. R. (2017) Structure and dynamics of bacterial ribosome biogenesis. Philos. Trans. R. Soc. Lond. B Biol. Sci. 372, 20160181 CrossRef Medline

77. Klinge, S., and Woolford, J. L. (2019) Ribosome assembly coming into focus. Nat. Rev. Mol. Cell Biol. 20, 116–131 CrossRef Medline

78. Peña, C., Hurt, E., and Panse, V. G. (2017) Eukaryotic ribosome assembly, transport and quality control. Nat. Struct. Mol. Biol. 24, 689–699 CrossRef Medline

79. Saurer, M., Ramrath, D. J. F., Niemann, M., Calderaro, S., Prange, C., Mattei, S., Scialla, A., Leitner, A., Bieri, P., Horn, E. K., Leubundgut, M., Boehringer, D., Schneider, A., and Ban, N. (2019) Mitochondrial ribosomal subunit biogenesis in trypanosomes involves an extensive assembly machinery. Science 365, 1144–1149 CrossRef Medline

80. Jarrous, N., and Gopalan, V. (2010) Archaeal/eukaryal RNase P: subunits, functions and RNA diversification. Nucleic Acids Res. 38, 7885–7894 CrossRef Medline

81. Harris, M. E., Kazantsev, A. V., Chen, J. L., and Pace, N. R. (1997) Analysis of the tertiary structure of the ribonuclease P ribozyme-substrate complex by site-specific photoaffinity crosslinking. RNA 3, 561–576 Medline

82. Lan, P., Tan, M., Zhang, Y., Niu, S., Chen, J., Shi, S., Qiu, S., Wang, X., Peng, X., Cai, G., Cheng, H., Wu, J., Li, G., and Lei, M. (2018) Structural insight into precursor tRNA processing by yeast ribonuclease P. Science 362, eaat6678 CrossRef Medline

83. Wu, J., Niu, S., Tan, M., Huang, C., Li, M., Song, Y., Wang, Q., Chen, J., Shi, S., Lan, P., and Lei, M. (2018) Cryo-EM structure of the human ribonuclease P ribozyme-subunit. Cell 175, 1393–1404.e11 CrossRef Medline

84. Kachouri, R., Stribinskis, V., Zhu, Y., Ramos, K. S., Westhof, E., and Li, Y. (2005) A surprisingly large RNase P RNA in Candida glabrata. RNA 11, 1064–1072 CrossRef Medline

85. Lai, L. B., Chan, P. P., Cozen, A. E., Bernick, D. L., Brown, J. W., Gopalan, V., and Lowe, T. M. (2010) Discovery of a minimal form of RNase P in Pyrobaculum. Proc. Natl. Acad. Sci. U.S.A. 107, 22493–22498 CrossRef Medline

86. Chan, P. B., Brown, J. W., and Lowe, T. M. (2012) Modeling the Thermoproteaceae RNase P RNA. RNA Biol. 9, 1155–1160 CrossRef Medline

87. Marquez, S. M., Harris, J. K., Kelley, S. T., Brown, J. W., Dawson, S. C., Roberts, E. C., and Pace, N. R. (2005) Structural implications of novel diversity in eucaryal RNase P RNA. RNA 11, 739–751 CrossRef Medline

88. Loria, A., and Pan, T. (2017) Recognition of the T stem-loop of a pre-tRNA substrate by the ribozyme from Bacillus subtilis ribonuclease P. Biochemistry 36, 6317–6325 CrossRef Medline

89. Wisely, C. A., and Martin, N. C. (1991) Dramatic size variation of yeast mitochondrial RNAs suggests that RNase P RNAs can be quite small. J. Biol. Chem. 266, 19154–19157 Medline

90. Chan, C. W., Chetnani, B., and Mondragón, A. (2013) Structure and function of the T-loop structural motif in noncoding RNAs. Wiley Interdiscip. Rev. RNA 4, 507–522 CrossRef Medline

91. Water, E., Hohn, M. J., Ahel, I., Graham, D. E., Adams, M. D., Barnstead, M., Beeson, K. Y., Bibbs, L., Bolanos, R., Keller, M., Kretz, K., Lin, X., Mathur, E., Ni, J., Podar, M., and Farrier, C. O. (2005) The genomic of Nanoarchaeum equitans: insights into early archaeal evolution and derived parasitism. Proc. Natl. Acad. Sci. U.S.A. 102, 12984–12988 CrossRef Medline

92. Wong, T. N., Sonnichsen, T. R., and Pan, T. (2007) Folding of noncoding RNAs during transcription facilitated by paused-induced nonnative structures. Proc. Natl. Acad. Sci. U.S.A. 104, 17995–18000 CrossRef Medline

93. Klemm, J. D., and Pabo, C. O. (1996) Oct-1 POU domain-DNA interaction: cooperative binding of isolated subdomains and effects of covalent linkage. Genes Dev. 10, 27–36 CrossRef Medline

94. Noller, H. F. (1993) On the origin of the ribosome: co-evolution of subdomains of tRNA and rRNA. In The RNA World (Gesteland, R. F., and Atkins, J. F., eds) pp. 137–156, Cold Spring Harbor Laboratory Press, Plainview, NY

95. Schimmel, P., Giegé, R., Moras, D., and Yokoyama, S. (1993) An operational RNA code for amino acids and possible relationship to genetic code. Proc. Natl. Acad. Sci. U.S.A. 90, 8763–8768 CrossRef Medline
117. Schimmel, P., and Henderson, B. (1994) Possible role of aminoacyl-RNA complexes in noncoded peptide synthesis and origin of coded synthesis. Proc. Natl. Acad. Sci. U.S.A. 91, 11283–11286 CrossRef Medline

118. Nagai, K., Oubridge, C., Kuglstatter, A., Menichelli, E., Isel, C., and Jovine, L. (2003) Structure, function and evolution of the signal recognition particle. EMBO J. 22, 3479–3485 CrossRef Medline

119. Zwieb, C., and Bhuiyan, S. (2010) Archaea signal recognition particle shows the way. Archaea 2010, 485051 CrossRef Medline

120. Mao, C., Bhardwaj, K., Sharkady, S. M., Fish, R. I., Driscoll, T., Wower, J., Zwieb, C., Sobral, B. W., and Williams, K. P. (2009) Variations on the tmRNA gene. RNA Biol. 6, 355–361 CrossRef Medline

121. Sharp, P. A. (1991) "Five easy pieces". Science 254, 663 CrossRef Medline

122. Fica, S. M., Tuttle, N., Novak, T., Li, N.-S., Lu, J., Koodathingal, P., Dai, Q., Staley, J. P., and Piccirilli, J. A. (2013) RNA catalyses nuclear pre-mRNA splicing. Nature 503, 229–234 CrossRef Medline