

Ribosomal RNA

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All proteins are synthesized by ribosomes, large RNA–protein complexes that function as ribozymes and are the targets of several clinically relevant antibiotics. Ribosomes contain highly conserved rRNA species, which catalyse the key steps in protein synthesis, together with 70–80 proteins that play important roles in the correct folding and packaging of the rRNAs.

Introduction

The ribosomal RNAs (rRNAs) lie at the core of the protein synthesis machinery. These RNAs were long regarded as mere scaffolds for the ribosomal proteins (r-proteins) but recent work has shown that the rRNAs in fact carry out the key reactions in translation. A major function of the r-proteins is ensuring the correct structure of the rRNA, allowing its tight packing around the active centre of the ribosome.

In all organisms the ribosome consists of two subunits. These are designated the 40S and 60S subunits in eukaryotes and the 30S and 50S subunit in Bacteria, Archaea and the cytoplasmic organelles of eukaryotes, mitochondria and chloroplasts. In almost all organisms the small ribosomal subunit contains a single RNA species (the 18S rRNA in eukaryotes and the 16S rRNA elsewhere). In Bacteria and Archaea, the large subunit contains two rRNA species (the 5S and 23S rRNAs); in most eukaryotes the large subunit contains three RNA species (the 5S, 5.8S and 25S/28S rRNAs). Sequence analysis shows that the 5.8S rRNA corresponds to the 5' end of the bacterial and archaeal 23S rRNAs, and was presumably generated early in eukaryotic evolution by insertion of a spacer sequence. Chloroplast large ribosomal subunits also contain three RNAs; in this case the 4.5S rRNA is derived from the 3' terminus of the bacterial 23S rRNA. Finally, in mitochondria the large subunit rRNA is smaller in size and is designated the 21S rRNA.

Organization of the Ribosomal RNA Genes

In almost all organisms the rRNAs are not synthesized as simple transcripts, but are generated from large precursors (pre-rRNAs) by posttranscriptional processing. In Bacteria and Archaea the primary transcript generally includes the 16S, 23S and 5S rRNAs (see **Figure 1a**). These are flanked by the 5' and 3' external transcribed spacers (5'-ETS and 3'-ETS) and separated by the internal transcribed

Introductory article

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spacer (ITS) regions. A transfer RNA (tRNA) gene is generally located in the ITS between the 16S and 23S rRNA genes, and one or more may also be located 3' to the 5S gene. In eukaryotes, the 18S, 5.8S and 25/28S rRNAs are cotranscribed by RNA polymerase I, while the 5S gene is independently transcribed by RNA polymerase III (**Figure 1b,c**).

Most Bacteria and Archaea contain either a single rDNA operon or multiple copies of the operon dispersed in the genome (for example, *Escherichia coli* has seven). In contrast, eukaryotes generally have many copies of the rDNA organized in tandem repeats; in humans approximately 300–400 rDNA repeats are present in five clusters (on chromosomes 13, 14, 15, 21 and 22). These sites are often referred to as nucleolar organizer regions, reflecting the fact that nucleoli were observed to assemble at these locations in newly formed interphase nuclei. In the majority of eukaryotes the 5S rRNA genes are present in separate repeat arrays (**Figure 1c**). Unusually, in the yeast *Saccharomyces cerevisiae*, a 5S rRNA gene is present in each of the 100–200 tandemly repeated rDNA repeats (on chromosome XII) (**Figure 1b**).

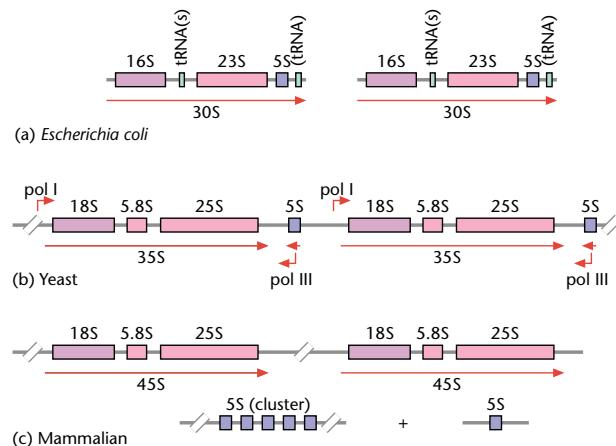


Figure 1 Ribosomal DNA (rDNA) organization in different species.

Pre-rRNA Processing

In all organisms, the mature rRNAs are generated by posttranscriptional processing reactions. In Bacteria and Archaea, the endonuclease RNAase III cleaves stem structures formed by complementary sequences that flank each of the mature rRNA sequences (see **Figure 2a**). The separated pre-rRNAs are 3' processed by the 3' to 5' exoribonuclease RNAase T and 5' processed by the endonuclease RNAase E. Processing occurs cotranscriptionally, but the requirement for the stem structures means that each mature rRNA must be fully synthesized before its processing can commence. RNAase III also processes other RNA substrates, messenger RNAs (mRNAs) and phage and plasmid transcripts, while RNAase T participates in the processing of tRNAs and other stable RNAs.

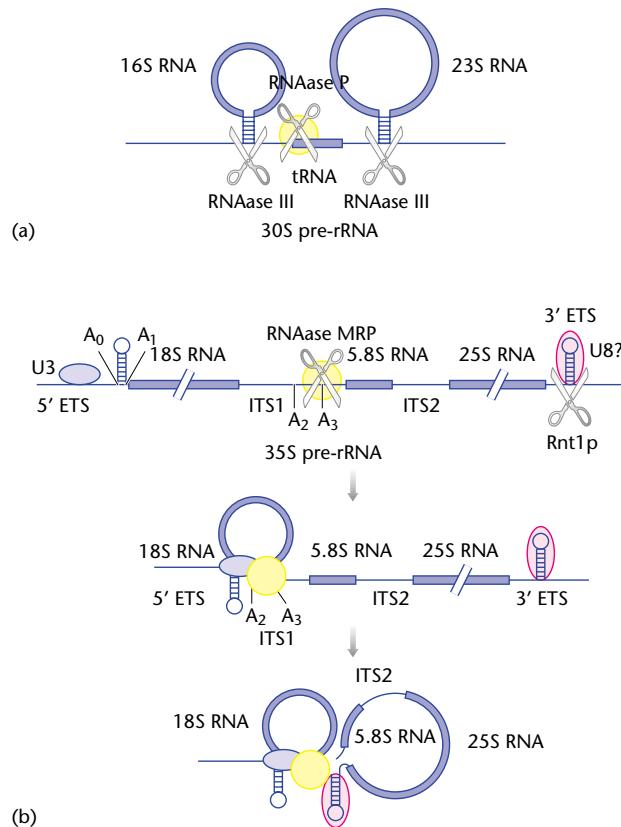


Figure 2 Pre-rRNAs in *Escherichia coli* (a) and *Saccharomyces cerevisiae* (b). Sites in the *E. coli* pre-rRNA that are cleaved by RNAase III and RNAase P are indicated. Sites in the *S. cerevisiae* pre-rRNA that are cleaved by Rnt1p and RNAase MRP are also indicated. RNAase MRP is homologous to RNAase P and Rnt1p is homologous to RNAase III. In *E. coli* processing at the ends of the mature 18S and 23S rRNAs is coupled, since base pairing is required to generate the RNAase III cleavage sites. In *S. cerevisiae* it has been proposed that interactions *in trans* with the small nucleolar RNAs (snoRNAs) U3 and U8 provides a similar coupling, although this has not yet been established. ETS, external transcribed spaces; ITS, internal transcribed spaces.

Pre-rRNA processing is less well understood in eukaryotes. In particular, many of the processing enzymes remain to be identified. Processing is posttranscriptional, with the exception of the initial cleavage by RNAase III (Rnt1p in yeast) in the 3'-ETS which, at least in yeast, is cotranscriptional. Subsequent processing shows a strong 5'→3' bias in the order of cleavage. In yeast, processing of the 18S rRNA involves four endonuclease cleavages, within the 5'-ETS and ITS1 and at the ends of the mature rRNA, but the endonucleases responsible have not been identified. ITS1 is also cleaved by an RNA-protein complex, RNAase MRP. This is substantially homologous to RNAase P, that cleaves the 5' ends of tRNAs. RNAase MRP cleavage allows entry for the 5' to 3' exonucleases Rat1p and Xrn1p that generate the 5' end of the 5.8S rRNA. The 3' processing of the 5.8S rRNA is also exonucleolytic and is carried out by a complex of eleven 3' to 5' exonucleases called the exosome.

As in Bacteria, the eukaryotic pre-rRNA-processing enzymes also have additional substrates. Yeast Rnt1p (RNAase III) processes precursors to small nucleolar RNAs (snoRNAs) and spliceosomal small nuclear RNA (snRNA). The 5' to 3' exonucleases Rat1p and Xrn1p degrade pre-rRNA spacer fragments and process the 5' ends of snoRNAs, and Xrn1p degrades mRNAs 5' to 3' in the cytoplasm. The exosome is involved in the degradation of nuclear pre-mRNAs and pre-rRNA spacer fragments, the processing of the 3' ends of snRNAs and snoRNAs and cytoplasmic mRNA turnover.

In both yeast and *E. coli* (the two organisms in which RNA processing is best understood) RNA-processing enzymes are not specific to a single pathway, but have a range of RNA substrates. The spacer regions of RNA precursors change very rapidly during evolution and RNA-processing systems appear to have evolved to recruit enzymes from a pool of factors as needed, rather than by developing specific enzymes for each substrate.

Relationships between pre-rRNA processing in Bacteria and eukaryotes

There are extensive similarities in pre-rRNA processing across evolution. The bacterial, archaeal and eukaryotic pre-rRNAs are essentially colinear and several pre-rRNA-processing enzymes are conserved from bacteria to eukaryotes (**Figure 2**).

RNAase III

RNAase III is a protein endoribonuclease that cleaves both sides of imperfect double-stranded RNAs. In the bacterial pre-rRNA, RNAase III cleaves the stems that are formed by the sequences that flank the 16S and the 23S rRNAs (see **Figure 2a**), producing the substrates for subsequent trimming reactions. In the eukaryotic (yeast) pre-rRNA, the

homologue of RNAase III (Rnt1p) carries out the first step in processing by cleaving a stem in the 3'-ETS (see **Figure 2b**).

RNAase MRP and RNAase P

RNAase MRP and RNAase P are endoribonucleases that consist of RNA–protein complexes. RNAase P processes the 5' ends of pre-tRNAs in all organisms and cleaves the 5' end of the tRNA located in the ITS region in the bacterial and archaeal pre-rRNA (see **Figures 1a** and **2b**). RNAase MRP has only been identified in eukaryotes and in the eukaryotic (yeast) pre-rRNA RNAase MRP cuts within ITS1. The bacterial RNAase P consists of an RNA molecule together with a single protein molecule. In contrast, eukaryotic (yeast) RNAase P has nine protein components together with a single RNA molecule. The RNA components of RNAase P and MRP show similarities in their predicted structures and share eight protein components; each also has one unique protein component. It is likely that RNAase MRP arose from RNAase P in an early eukaryote and became specialized for pre-rRNA processing.

The RNA component of RNAase P from many Bacteria and Archaea shows *in vitro* activity in the absence of its protein cofactor, although the salt conditions required for this activity are far from physiological. An RNA molecule that shows enzymatic activity in the absence of proteins is termed a ribozyme. It is believed that the RNA component of bacterial RNAase P functions as a ribozyme in RNA cleavage *in vivo*. The protein component aids the binding and release of the substrate, and allows cleavage of wider range of substrates than with the RNA alone. In contrast, attempts to demonstrate ribozyme activity for the RNA components of eukaryotic RNAase P or MRP have been unsuccessful, suggesting that some or all of the enzymatic activity has been taken over by the protein components.

Role of the small nucleolar RNAs in eukaryotic pre-rRNA processing

Eukaryotes contain a large number of snoRNA species (approximately 150 in human cells). With the exception of the RNA component of RNAase MRP, all of these species can be divided into two groups. These are designated as 'box C + D' and 'box H + ACA' on the basis of conserved sequence elements that are believed to be sites of protein binding (**Figure 3**). Each class of snoRNA is associated with a set of common proteins and members share conserved features of predicted secondary structure. Most of these snoRNAs act as site-specific guides for the modification of nucleotides within the rRNAs. The box C + D snoRNAs select nucleotides at which the 2'-hydroxyl positions of the sugar residues undergo methylation (2'-O-methylation), while the box H + ACA snoRNAs select positions at which

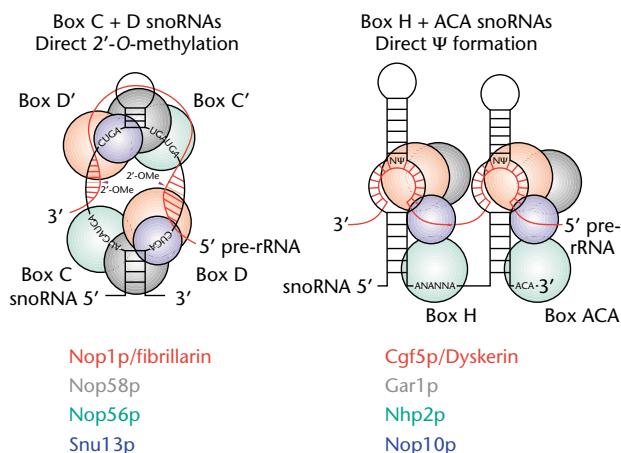


Figure 3 The small nucleolar RNAs (snoRNAs) and their associated proteins. The two major families of snoRNA are each associated with a specific set of proteins. For the box C + D snoRNAs, these are Nop1p/fibrillarin, Nop58p, Nop56p and Snu13p. The H + ACA snoRNAs are associated with Cgf5p/dyskerin, Gar1p, Nhp2p and Nop10p. Nop1p and Cgf5p are the putative catalytic subunits. The colours of other components of the two classes of snoRNP do not indicate functional homology.

uridine is converted into pseudouridine (Ψ) by rotation of the base. SnoRNA homologues have recently been identified in Archaea where they also seem to be involved in rRNA modification.

In addition, a small number of box C + D and box H + ACA snoRNAs are required for pre-rRNA processing (U3, U14, snR10 and snR30 in yeast; U3, U8, U14 and U22 in vertebrates). Genetic analyses showed that the yeast snoRNAs are all required for the pre-rRNA cleavage steps, in the 5'-ETS and ITS1, on the pathway of 18S rRNA synthesis. In *Xenopus* (frog) oocytes, U3, U14 and U22 are similarly required for 18S rRNA synthesis, while U8 is required for processing in the 3'-ETS and in ITS1 on the pathway of 5.8S/28S rRNA synthesis. In the case of the yeast box C + D snoRNAs U3 and U14, compensatory mutations have demonstrated that they must base pair with the pre-rRNA to function in ribosome synthesis.

The requirement for these snoRNAs in processing does not appear to be related to roles in rRNA modification and the snoRNAs also do not appear to act catalytically in processing. Rather, they are thought to mediate changes in the structure of the pre-rRNA, possibly establishing the correct conformation for recognition by the endonuclease(s). In *E. coli*, the coordinated processing of the 5' and 3' ends of the 16S and 23S rRNAs is ensured by the requirement that the flanking sequences base pair to generate the cleavage site for RNAase III (see **Figure 2a**). No equivalent base pairing can be drawn for eukaryotes; instead it is speculated that interactions *in trans* with snoRNAs brings the processing sites together and ensures their coordinated cleavage (see **Figure 2b**). The U3 snoRNA is thought to provide this function for the coordination of

processing in the 5'-ETS and ITS1, while *Xenopus* U8 may coordinate processing in the 3'-ETS and ITS1.

Structural rearrangements of the rRNAs during ribosome synthesis

In the *E. coli* pre-rRNA, the 5' end of the 16S rRNA is engaged in a base paired interaction with the 3' region of the 5'-ETS. This interaction must be broken in order for the 5' end of 16S rRNA to assume its mature conformation – this involves a long-range interaction between the loop of the 5' stem–loop structure and nucleotides around position 917, an interaction referred to as the central pseudoknot. This structure is conserved throughout evolution and is likely to play a crucial role in the overall folding of the rRNA. In the pre-rRNA of eukaryotes, the U3 snoRNA base pairs to the 5' stem–loop structure of the 18S rRNA, preventing formation of the central pseudoknot. Formation of the pseudoknot is presumably an irreversible step and the alternative structure may block premature creation of this long-range interaction until the correct stage in the assembly process is reached. In yeast, a putative RNA helicase (an enzyme that can open RNA structure) is found stably associated with U3 and may catalyse this structural isomerization.

In eukaryotes, large numbers of modification guide snoRNAs base pair with the pre-rRNA. The conformation of the pre-rRNA in the snoRNA-associated form must be very different from its mature structure, and extensive structural rearrangements are inevitable. Remarkably, 17 different putative RNA helicases that are required for ribosome synthesis in yeast. These are likely to play essential roles in such structural remodelling.

It has been speculated that the primary function of the modification guide snoRNAs is to assist the correct folding of the pre-rRNA. RNAs or proteins that function to promote the correct folding of another factor are termed chaperons. In this model, the rRNA modifications are a side product that serve as signals that the snoRNA has bound (and has presumably done its work). Formation of the modification would trigger a helicase to dissociate the snoRNA/pre-rRNA base pairing. There are, however, few data to support this model at present.

rRNA Function

Primary and secondary structure of rRNAs in different species

Inspection of the structure of rRNAs in distant organisms from the three domains of life reveals that despite substantial differences primary sequence, both the small subunit rRNA (SSU-rRNA) and large subunit rRNA (LSU-rRNA) display remarkable conservation of their

secondary, and probably tertiary, structures. Core structures can be drawn for the SSU- and LSU-rRNAs which can accommodate the secondary structures of all described rRNAs. The most conserved elements are presumed to be of functional significance. These have been proposed to represent the active core of the ribosome and the portion of modern ribosomes which was first established in the course of evolution. Notably, almost all the rRNA posttranscriptional modifications (base and ribose methylation as well as conversion of uridine to Ψ) fall within these conserved core regions of the rRNAs.

Initial approaches to the structure of the rRNAs involved the analysis of isolated fragments of the rRNA by chemical probing and RNA–RNA crosslinking experiments. These analyses generated a great deal of data on the functional interactions within the ribosome but were less informative on the mechanisms of translation. A powerful technique for the determination of secondary structure was based on phylogenetic comparisons. The principal approach is to look for compensatory mutations in regions predicted to base pair. Compensatory mutations arise when pairs of changes in sequence occur such that the base-pair potential is retained with different nucleotides. The identification of multiple compensatory mutations in predicted stem structures provides strong evidence for the existence of actual base pairing. Backed by the crosslinking data, this allowed the overall secondary structure of the conserved regions of the rRNAs to be established with good confidence. The structures of both ribosomal subunits from Archaea have recently been determined by X-ray crystallography, offering the first view of ribosome structure at atomic resolution. This structure has already helped to rationalize several decades of biochemical and genetic data. A major breakthrough for the whole field of RNA biology is that the structure provides decisive support for the view that the peptidyl-transferase reaction (the reaction by which amino-acid residues are attached to each other to form proteins) is catalysed by the rRNA itself.

In the primary sequences of the rRNAs, the conserved sequences are separated by variable regions, in which the primary and secondary structures diverge more rapidly in evolution. The overall length of these regions is also poorly conserved and is generally longer in eukaryotes; they are therefore often referred to as expansion segments. The core structures of the SSU- and LSU-rRNAs contain 10 and 18 such variable regions, respectively. In general, the variable regions are more dispensable for ribosome function.

Functional domains in the rRNAs

The combination of biochemical approaches, mostly crosslinking experiments and chemical footprinting of rRNAs bound to tRNAs or to various antibiotics, with the genetic analysis of *E. coli* strains bearing mutations in their

rRNAs led to the definition of several functional domains in the rRNAs. As summarized in **Table 1**, domains were ascribed to the most basic functions of the ribosome (i.e. decoding or codon–anticodon recognition and peptidyltransferase activity) as well as to antibiotic binding and interactions with ribosomal proteins and translational factors. The general picture which emerged from these studies is that the decoding centre (the specific recognition of the codon by the tRNA) of the ribosome lies within its small subunit and the peptidyltransferase activity (addition of the new amino acid to the growing polypeptide) is carried out by the large subunit. The accuracy of translation is determined by components of both subunits, probably reflecting interactions of the tRNAs with both ribosomal subunits.

The analysis of mutations in the rRNAs has long been hindered by the large copy number of rDNA genes. In *E. coli* this problem was partially overcome by the over-expression of the mutated rDNA copy from a high copy plasmid construct providing a mixed rRNA population, with about 50% each of the wild-type and mutant ribosomes. In yeast, a number of more sophisticated systems for the conditional expression of mutant and wild-type rRNAs have been devised, allowing the analysis of the processing and function of the rRNA. These techniques have been extensively used for the analysis of the effects of

cis-acting mutations in the spacer regions on pre-rRNA processing. The effects of mutations on the function of the rRNA is less well characterized. However, it appears that functional domains, particularly the accuracy centre, as well as features of the rRNA that are recognized by aminoglycoside antibiotics, have been highly conserved throughout evolution. Our understanding of the structure and function of ribosomes in eukaryotes remains much less advanced than in *E. coli*.

Catalytic activities of the rRNAs during translation

The identification of the ribosomal components involved in peptide-bond formation has been a longstanding challenge in ribosome research. This area was mostly explored in *E. coli*, making use of systems for *in vitro* reconstitution of the subunits.

Pioneering work used a simple peptidyltransferase (PT) assay in which 50S subunits were mixed with two minisubstrates that mimicked tRNAs bound at the A and P site (i.e. their structures resembled the tRNA carrying the incoming, activated amino acid and the tRNA carrying the elongating polypeptide chain, respectively), allowing the formation of a single peptide bond. The authenticity of the reaction relied on the efficient inhibition

Table 1 Functional domains within the *E.coli* rRNAs

Functional domain	Region of rRNA	Major functions	Functionally related antibiotics
C1400 region	16S rRNA (1400–1500)	Decoding Translocation	Paromomycin
530 loop	16S rRNA (500–545)	Decoding EF-Tu binding	Streptomycin
Helix 34	16S rRNA	Decoding EF-G function and translocation	Spectinomycin
912 region	16S rRNA (885–912)	Translational accuracy	Streptomycin
Helix 45 (colicin fragment)	16S rRNA (1494–3'end)	Shine–Dalgarno interaction Initiation factor binding Subunit interface	Kasugamycin
Domain II (GTPase region)	23S rRNA	EF-G-dependent GTP hydrolysis ppGpp synthesis in stringent response	Thiostrepton Micrococin
Domain V (PT centre)	23S rRNA	Peptide bond formation Interaction with tRNA 3' ends Translational accuracy	Chloramphenicol Erythromycin
Domain IV (1916 loop)	23S rRNA	Interaction with anticodons and tRNA 3' ends Translational accuracy Subunit interface	
α sarcin loop	23S rRNA (2653–2667)	EF-Tu and EF-G binding site	Sarcin, ricin

by PT-specific antibiotics such as chloramphenicol and carbomycin. These early studies established that the PT activity is not dependent on mRNA, 30S subunit, translational factors, guanosine triphosphate (GTP), adenosine triphosphate (ATP) or even intact tRNAs. Large ribosomal subunits lacking individual or multiple ribosomal proteins were tested in this assay and showed to be equally active. Only five proteins appeared to be essential for PT activity, two of which were shown to be required for 50S subunit assembly. In a different set of experiments, ribosomes were subjected to harsh protein extraction conditions. The particles were stripped nearly to completion but maintained their PT activity. These experimental approaches did not determine whether the two or three proteins that remained bound to the rRNA are involved in the PT activity *per se* or whether they are required to maintain a minimally competent rRNA structure.

In the high resolution ($\sim 3 \text{ \AA}$) structure of the archaeal LSU the peptidyl transferase (PT) region is seen to be surrounded by a domain of tightly packed rRNA. The proteins are generally located on the exterior of this structure, although some project into the rRNA domain, making extensive protein–RNA contacts and stabilizing the tight structure of the rRNA around the catalytic active site. These crystallographic studies also confirmed that PT activity lies solely with the rRNA; no ribosomal proteins were found within 18 \AA of the PT centre (to have an impact on catalysis a protein would have to be within 3 \AA). An RNA-based mechanism for peptide bond formation has been proposed involving the N³ position of an adenine residue (A2251 in *E. coli*) and a charge relay system. This first activates the incoming peptide by accepting a proton, and then neutralizes the charge on the leaving group after peptide bond formation. In essence, this is analogous to the reverse of the acylation step observed in serine proteases (e.g. chymotrypsin) during peptide hydrolysis and suggests that RNA have learned the chemical principles of catalysis before protein enzymes.

The current view is therefore that the catalytic activity of the ribosome lies in its RNA component while the ribosomal proteins act as chaperones in ribosome assembly and as cofactors to increase the efficiency of the RNA-mediated PT reaction and the accuracy of translation.

In vitro reconstitution of fully functional *E. coli* small ribosomal subunits has long been achieved, either from *in vitro* transcribed or purified 16S rRNA and pools of ribosomal proteins. The situation was more complex for large ribosomal subunits, however. Active large ribosomal subunits can only be reconstituted if an authentic fragment of at least 80 nucleotides containing several posttranscriptional modifications is added *in trans*. This suggests that the full activity of the large ribosomal subunit critically requires one or more modified nucleotides.

Functional interactions between the rRNAs, mRNA and tRNAs

In Bacteria, the 3' end of the 16S rRNA base pairs with the mRNA (see also **Table 1**). This is termed the Shine–Dalgarno interaction and is crucial for translation initiation. In all organisms, the mRNA and tRNA interact in the codon–anticodon recognition which is reiterated throughout the whole translational process. This interaction, which involves only three base-paired nucleotides, is stabilized by a number of interactions between the tRNA and the rRNAs. Interactions important for the catalysis of peptide bond formation are provided by the recognition of the universally conserved 3' CCA end of aminoacyl-tRNA substrates by 23S rRNA. In particular, a Watson–Crick base pair forms between a universally conserved residue, G2252, close to the PT centre in the 23S rRNA and C74 at the acceptor end of tRNAs. In *E. coli*, an rRNA–mRNA interaction is proposed to play a role in the recognition of the termination triplet.

Interactions of the rRNAs with antibiotics

Antibiotics have been of great use in ribosome research, particularly where their site(s) of interaction with the ribosome could be correlated with specific translational defects, providing putative functions for particular sections of rRNAs (see also **Table 1**).

Most characterized antibiotics appear to bind primarily to the rRNAs. In some cases, the methylation status of specific rRNA residues correlates with antibiotic resistance or sensitivity. For example, the N⁶-dimethylation of A2058 in the 23S rRNA PT centre by the ErmE methyltransferase confers resistance to lincosamide and streptogramin B and variety of macrolide antibiotics, including erythromycin. Erythromycin binds to A2058 and neighbouring nucleotides, and mutation of these bases also confers antibiotic resistance. Similarly, resistance to thiostrepton is conferred by ribose methylation or substitution of A1067 in the GTPase region of the 23S rRNA, within the thiostrepton-binding site. However, both thiostrepton and erythromycin also make functionally important contacts with ribosomal proteins (with L11 and L15, respectively). In contrast, the N⁶-dimethylation of the A1518A1519 doublet at the 3' end of 16S rRNA by the KsgA methylase is required for sensitivity to kasugamycin. Atomic structures of ribosomal subunits bound to antibiotics have recently been reported. These studies are starting to shed an entirely new light on the interactions between antibiotics and rRNAs. For instance, several small subunit-specific antibiotics appear to exert their inhibitory effects through the stabilization of individual ribosomal domains which would otherwise show a degree of flexibility relative to each other essential for decoding and possibly for the translocation process.

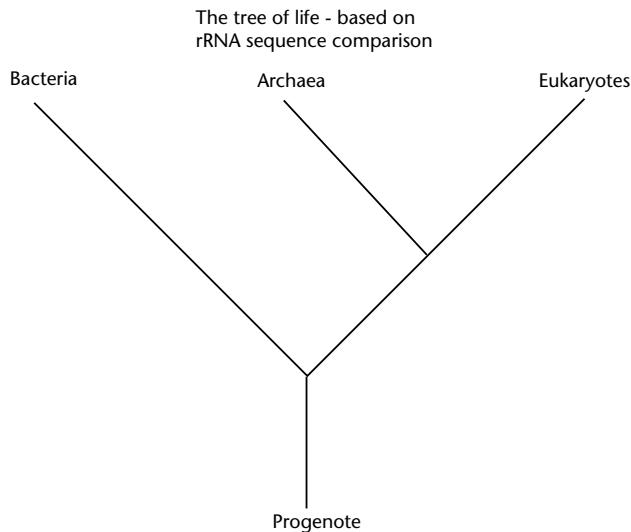


Figure 4 The tree of life. Universal phylogeny derived from comparisons of rRNA sequences.

rRNA and Phylogeny

Highly conserved in structure and presumed function across all of evolution, the rRNAs, and particularly the small subunit rRNA, have become the most commonly used markers for establishing phylogenetic relationships between organisms. Mutations in the conserved core regions of the rRNA are heavily biased toward nucleotide substitution, rather than deletion and insertion. This, together with the existence of a universal secondary structure, considerably facilitates the sequence alignment process.

Molecular phylogeny (phylogeny derived from sequence comparison) based on the rRNA sequence led to the

conclusion that there are three domains of life: Bacteria (previously termed Eubacteria), Archaea (previously termed Archaeobacteria) and Eukaryotes (also termed Eukarya). Most models predict that during evolution from the Progenote (the first organisms able to replicate their genomes) the Archaea and eukaryotes arose from a common ancestor, following their separation from the bacterial line (see **Figure 4**). Trees with different topology have been derived based on a variety of protein sequences, probably as a consequence of the relatively high levels of lateral gene transfer that is now thought to have occurred during evolution.

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