THE FUNCTION AND SYNTHESIS OF RIBOSOMES

*Denis L.J. Lafontaine and David Tollervey**

Structural analyses of the large and small ribosomal subunits have allowed us to think about how they work in more detail than ever before. The mechanisms that underlie ribosomal synthesis, translocation and catalysis are now being unravelled, with practical implications for the design of antibiotics.

*FNRS, Université Libre de Bruxelles, Département de Biologie Moléculaire, IRMW - Campus CERIA, Avenue Emile Gryson 1, B-1070 Brussels, Belgium. *Wellcome Trust Centre for Cell Biology, The University of Edinburgh, King's Buildings, Mayfield Road, Edinburgh EH9 3JR, UK.
e-mails: denis.lafontaine@ulb.ac.be d.tollervey@ed.ac.uk

In all organisms, ribosomes form the core of the translation machinery. Translation is a key step in gene expression, converting the genetic information encoded in messenger RNAs (mRNAs) into contiguous chains of amino acids (polypeptides or proteins) with structural and/or catalytic properties. Ribosomes have two main functions — decoding the message and the formation of peptide bonds. These two activities reside in two large ribonucleoprotein particles (RNPs) of unequal size, the ribosomal subunits. Each subunit is made of one or more ribosomal RNAs (rRNAs) and many ribosomal proteins (r-proteins). The small subunit (30S in bacteria and archaea, 40S in eukaryotes) has the decoding function, whereas the large subunit (50S in bacteria and archaea, 60S in eukaryotes) catalyses the formation of peptide bonds, referred to as the peptidyl-transferase activity (BOX 1). The bacterial (and archaeal) small subunit contains the 16S rRNA and 21 r-proteins (*Escherichia coli*), whereas the eukaryotic small subunit contains the 18S rRNA and 32 r-proteins (*Saccharomyces cerevisiae*; although the numbers vary between species). The bacterial large subunit contains the 5S and 23S rRNAs and 34 r-proteins (*E. coli*), with the eukaryotic large subunit containing the 5S, 5.8S and 25S/28S rRNAs and 46 r-proteins (*S. cerevisiae*; again, the exact numbers vary between species).

During the past year, the ribosome has been revealed as a wonderfully complex RNA-based machine. Here, we give an overview of these results and integrate them with the mechanisms of ribosome synthesis.

Ribosome watching

The three-dimensional structures of isolated ribosomal subunits and the intact ribosome have recently been solved by X-ray crystallography with atomic resolution[1–4]. The analyses used ribosomes from the thermophilic bacterium *Thermus thermophilus* for the 30S subunit and the intact 70S ribosome, and the archaeon *Haloarcula marismortui* for the 50S subunit. Structural analyses followed and built on electron microscopy analyses of the overall structures of the subunits and functional complexes (see, for example, REFS 5–10) and several lower-resolution crystallographic analyses[11–16] (for further discussion see REF. 17).

Forty years of biochemical and genetic research on ribosomes from the bacterium *E. coli* had also led to the identification of many functionally important sites. Interpretation of the new structural data drew heavily on these analyses, revealing a high degree of agreement between the structural and biochemical data, and vindicating the efforts of a generation of biochemists in working out the function of the ribosome. In particular, the idea that the rRNAs might be the main functional element of the ribosome was not new[18,19], but this was confirmed by the finding that the site of peptide-bond formation is entirely surrounded by RNA. This established that the peptidyl-transferase activity of the ribosome is based on catalysis by RNA[20]. Such RNA enzymes are generally termed ribozymes — hence the much-used phrase, "the ribosome is a ribozyme".

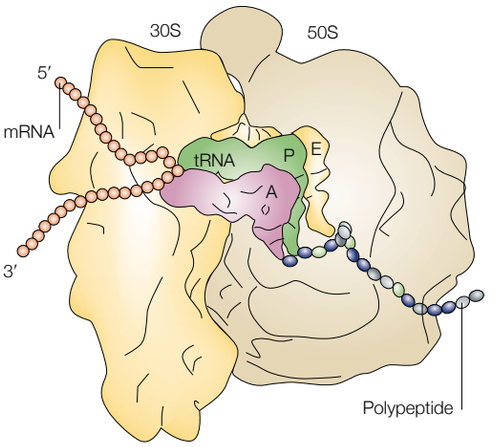
The overall shape of each subunit is largely determined by the structure of the rRNAs, which also contribute most of the mass. Ribosomal proteins cluster on the sides exposed to the solvent and on the periphery of the interface between the two subunits. The subunit interface itself — and, indeed, most sites with functional significance in translation — seem largely devoid of

proteins. At present, the primary function of the ribosomal proteins seems to be the stabilization of highly compact rRNA structures. To achieve this, proteins are intimately intertwined with the RNAs, making extensive interactions that often stabilize interhelical contacts between RNA domains, especially in the case of the more rigid 50S subunit. Many r-proteins have long, narrow basic extensions that snake through the rRNAs and reach deep into the RNA core of the subunit. These allow the very tight packing of the RNA that is seen around the active centre of the ribosome. As these extensions are poorly structured in the absence of the rRNA, they were generally not resolved in structural studies of individual components and their involvement in shaping the ribosome was underestimated. The principles of RNP structure that have been deduced from the ribosome are likely to be beneficial in understanding other RNA–protein complexes.

Movement of the subunits drives translocation

The bacterial 30S subunit consists of three relatively flexible globular domains that are organized in a Y shape around a thin neck. Consistent with previous lower-resolution data, these gross morphological regions are now seen to consist largely of individual domains of RNA structure: the 5′ domain of the 16S rRNA forms the 'body'; the central region of the rRNA forms the 'platform'; and the 3′ region forms the 'head'[3] (BOX 1). Active sites on the small subunit, where codons in the mRNA are recognized and the transfer RNAs (tRNAs) bind, are generally formed from elements of different structural domains.

Box 1 | How ribosomes work in translation



Translation is an iterative process, adding one amino acid to the growing polypeptide chain at each round, at a rate of ~15–20 residues per second in bacteria. Amino acids are carried to the ribosome attached to the 3′ end of a specific class of small RNAs, the transfer RNAs (tRNAs). There are three binding sites for tRNAs on the ribosome. In the aminoacyl site (A site), a tRNA charged with an amino acid (aminoacyl-tRNA) is presented to the messenger RNA (mRNA) being translated. The peptidyl site (P site) carries the growing peptide chain attached as a peptidyl-tRNA complex. The exit site (E site) contains empty tRNAs on their way out of the ribosome.

On the small ribosomal subunit (30S), the anticodon of an aminoacyl-tRNA is matched by base-paired interactions to a nucleotide triplet or codon on the mRNA. The difference in binding energy between cognate and near-cognate interactions is not enough to account for the high accuracy of translation, and a key function of the small subunit is to discriminate against aminoacyl-tRNAs that do not match the codon on the message[48]. This crucial step in the decoding process was poorly understood until the demonstration that the conformation of several residues on the 16S rRNA is sensitive to the structure of the minor groove of the first two base pairs in the codon–anticodon interaction[30]. These should always show perfect Watson–Crick complementarity and mismatches alter the geometry of the minor groove. The induced change in the 16S rRNA therefore allows discrimination against non- or near-cognate interactions.

This decoding function of the 30S subunit is assisted by a GTPase, the elongation factor EF-Tu (EF1-α in eukaryotes). In fact, each amino acid is targeted to the A site as part of an aminoacyl-tRNA–EF-Tu–GTP ternary complex, and hydrolysis of the bound GTP accompanies and signals correct tRNA–mRNA matching.

At the interface between the ribosomal subunits, a canyon in the large subunit (50S) accommodates and orientates the 3′ ends of the aminoacyl-tRNA and peptidyl-tRNA. The growing peptide chain is cleaved off the peptidyl-tRNA in the P site and joined to the amino-acid moiety on the A-site tRNA. This is the peptidyl-transferase reaction, which is catalysed by the 23S rRNA and results in the transfer of the growing polypeptide to the tRNA in the A site, leaving an empty tRNA in the P site. During this reaction, the mRNA is clamped in place by narrow entrance and exit channels that run between domains of the 30S subunit (REFS 4,22). After peptidyl transfer, the translation factor EF-G binds to the ribosome and the 30S rotates relative to the 50S, accompanied by opening of the mRNA entrance and exit channels[22]. The ribosome can now move forward on the mRNA to read the next codon in a process called translocation. This movement is associated with GTP hydrolysis by EF-G[22,24]. During translocation, the peptidyl-tRNA, still bound to the mRNA, moves into the P site and the uncharged tRNA that previously carried the peptide chain moves to the E site, ready to exit the ribosome[4,21]. The EF-G–GDP complex occupies the A site, which might help drive the translocation reaction[24–26]. This works because the three-dimensional structure of EF-G–GDP is generally similar to that of the aminoacyl-tRNA–EF-Tu–GTP complex, a phenomenon termed macromolecular mimicry[49]. Finally, EF-G–GDP is displaced, leaving the A site empty and ready to accept the next aminoacyl-tRNA, and the 30S rotates back, clamping the mRNA. The steps of elongation are well conserved between prokaryotes and eukaryotes, although in fungi (but apparently not other eukaryotes) tRNA release from the E site additionally requires ATP hydrolysis by EF-3.

Like most G proteins, EF-Tu and EF-G show limited inherent GTPase activity. This is stimulated several fold by an accessory factor that is part of the 50S subunit and is now referred to as the GAR (GTPase-associated region).

ACID–BASE CATALYSIS
A Brønsted–Lowry acid is a substance that donates a proton (hydrogen ion, H^+); a Brønsted–Lowry base is a substance that accepts a proton.

ACID-DISSOCIATION CONSTANT (K_a). The strength of a given acid (its ability to donate a proton in water) is expressed by its acidity constant (K_a). A stronger acid has a higher K_a.

On the basis of electron microscopy reconstructions[21–24], the physical movements of these domains, coupled with rotation of the small subunit relative to the more rigid large subunit, are believed to have a key function in translation. A ratchet mechanism[22] is proposed to drive movement of the mRNA and associated peptidyl-tRNA complexes through the translating ribosome, in a process termed translocation (BOX 1). Movement of the 30S subunit, in turn, is driven by binding of translation factors, notably EF-G, and structural changes in these factors are powered by the energy of GTP hydrolysis[25,26].

The importance of flexibility in the small subunit underlies the action of several clinically important antibiotics[3,27–30]. These stabilize individual structural domains in local conformations that inhibit their movement, thereby altering the balance between the conformational states of the 16S rRNA as it goes through the translation cycle (BOX 2).

Box 2 | Re-visiting antibiotic action

Many antibiotics work by inhibiting bacterial, but not human, protein synthesis. But some bacteria have developed resistance to clinically important antibiotics and other potential antibiotics are not sufficiently specific towards bacterial ribosomes to be suitable for human or veterinary medicine. Most sites with functional significance in translation are targeted by the antibiotics, including the decoding and peptidyl-transferase centres and the GTPase-associated region (BOX 1). Recent structural analyses have clarified the molecular basis for the inhibitory effects of many antibiotics.

Co-crystallization with the 30S subunit has shown that several antibiotics bind directly to the 16S rRNA at positions close to the mRNA- and tRNA-binding sites, where there is substantial movement during decoding and translocation. Antibiotic binding is predicted to reduce the subunit flexibility that is crucial for the structural rearrangements that normally occur during translation. Gentamycin and streptomycin, which affect translational accuracy, would stabilize the 16S rRNA structure around the decoding site in the ribosome ambiguity (ram) conformation that favours binding of non-cognate aminoacyl-tRNAs, leading to misincorporation[4,28]. Spectinomycin, a rigid molecule with a fused ring system, would inhibit EF-G-mediated translocation by binding close to the pivot point of the head and sterically blocking its movement[28]. Antibiotic-resistant mutations in r-protein S5 might reduce the stability of the head–body interaction, so that mobility is maintained even in the presence of the antibiotic.

Paromomycin binds to a loop that is involved in the induced-fit recognition of cognate tRNAs (BOX 1)[28,30,50]. By favouring the structure normally provoked by the correct codon–anticodon interaction, paromomycin binding lowers the activation energy, reducing the stringency of recognition[28,30,50]. Hygromycin B binds close to paromomycin and sequesters the tRNA in the A site, perhaps by preventing the conformational changes that are required during translocation[27].

Tetracycline directly inhibits binding of aminoacyl-tRNAs to the A site by binding to an overlapping site on the ribosome, leading to the release of aminoacyl-tRNA after GTP hydrolysis by EF-Tu. It might also reduce fidelity by favouring the ram conformation by binding at other sites[27,51]. Tetracycline resistance often involves chemical modification of the drug and, with the atomic definition of its binding pocket, the chemical modifications known to abolish antibiotic properties of tetracycline interfere strongly with its interactions with the A site. In addition, the bacterial specificity of the drug could be explained by the poor conservation of its binding pocket in eukaryotic rRNA.

Even where co-crystallization data are not available, chemical crosslinking data and resistance mutations can now be more clearly interpreted. This has been seen for evernimicin, an oligosaccharide antibiotic that interacts with the large ribosomal subunit at a site distinct from the peptidyl-transferase centre[52], and also for linezolid, which binds at the peptidyl-transferase site[34].

In addition to movement of the ribosome itself, the translation factors and GTPase, EF-Tu and EF-G undergo substantial conformational changes. The structures of EF-Tu bound with two otherwise unrelated antibiotics[53,54] indicate that both act by inhibiting the structural changes required for cycling between the GTP- and GDP-bound conformations.

RNA at the core: the peptidyl-transferase site

In contrast to the small subunit, the large subunit appears as a compact monolithic block about 25 nm in diameter, with a fairly even mass distribution. The face of the large subunit that is in contact with the small subunit lacks pronounced structural features, with the exception of a large canyon flanked by an RNA ridge. This ridge is formed from a single RNA domain (domain V of 23S rRNA) and shows less flexibility during the translation cycle than does the small subunit RNA. The canyon is large enough to accommodate the 3′-aminoacyl acceptor stems of three tRNA molecules (located in the A, P and E sites; BOX 1), and it contains the active site for the formation of peptide bonds[1,4,20]. The structure of the 50S subunit associated with analogues mimicking substrates for the A and P sites revealed that the functional groups involved in the peptidyl-transfer reaction (FIG. 1) are all tightly packed into an RNA pocket within domain V of the 23S rRNA[1,4,20]. This structure is formed from nucleotides that are >95% conserved across all three kingdoms of life, supporting its key functional role. The domain is stabilized by the long extensions of four r-proteins, but these are too far away to participate directly in catalysis. From this, it was concluded that the peptidyl-transfer reaction is catalysed by RNA[1,20].

On the basis of the structure of the 50S subunit in complex with analogues that mimic the substrates for the A and P sites, an adenine residue (A2451 in *E. coli*), located ~0.3 nm from the peptide bond to be formed, was proposed to have a key role in catalysis[20,31]. A2451 was thought to function as a general base, with N3 removing a proton from the α-amino group of the aminoacyl-tRNA, promoting its attack on the peptidyl-tRNA, and then donating the proton back to stabilize the leaving group after peptidyl transfer. This is ACID–BASE CATALYSIS, and the principles involved are well known from protein enzymes.

For this trick to work at physiological pH, A2451 requires a highly elevated pK_a (which can be derived from the ACID-DISSOCIATION CONSTANT), as N3 is normally protonated only at very acid pH. The mechanism proposed was a charge-relay mechanism involving interactions between A2451 and G2447, and between G2447 and the phosphate of A2450, which is entirely buried in the rRNA structure. These interactions could result in the stabilization of rare, TAUTOMERIC forms of G2447 and A2451 and a consequent increased negative charge density on N3 of A2451. Such a system would resemble the charge-relay mechanism determined for serine proteases (for instance, chymotrypsin). This model is supported by chemical probing[31], showing that the pK_a of A2451 is indeed highly perturbed in the intact ribosome. More circumstantial support came from the *in vitro* selection of a ribozyme with peptidyl-transferase

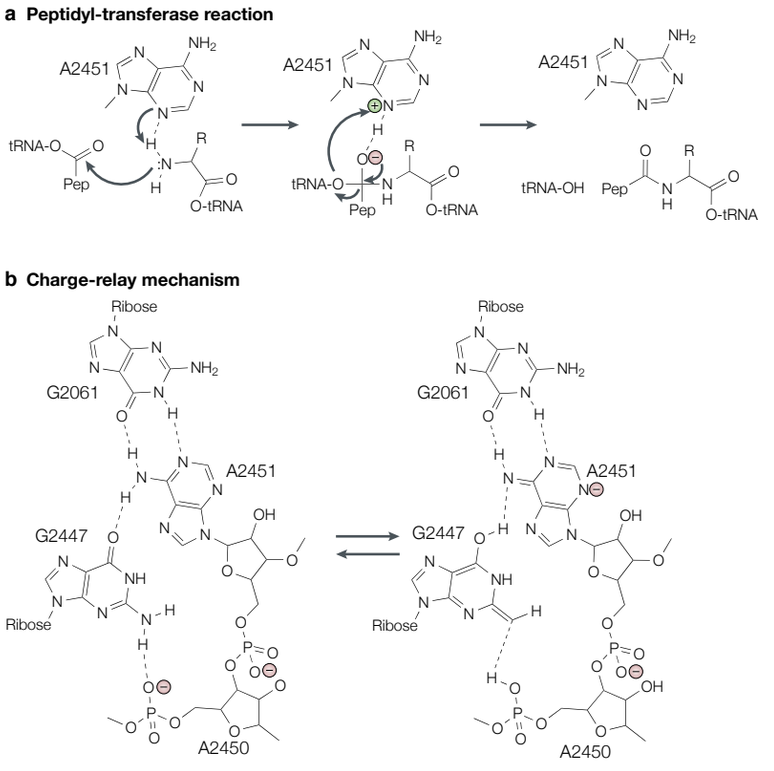


Figure 1 | **Peptide bond formation. a** | The peptidyl-transfer reaction starts by a nucleophilic attack on the CARBONYL carbon on the peptidyl-transfer RNA (tRNA) by the α-amino group of the aminoacyl-tRNA. This results in the acetylation of the 3′-hydroxyl group of the peptidyl-tRNA and concomitant formation of a tetrahedral intermediate at the carbonyl carbon. The tetrahedral intermediate resolves to yield a peptide extended by one amino acid esterified to the A-site-bound tRNA and a deacylated tRNA in the P site. **b** | The proposed charge-relay system. The proposed charge-relay system that may allow N3 of A2451 to be negatively charged at neutral pH, permitting it to act as a proton acceptor and donor during the peptidyl-transfer reaction.

CARBONYL GROUP
C=O; an important functional group in organic chemistry.

TAUTOMERISM
Mechanism by which enols and ketones rapidly interconvert. The keto–enol equilibrium usually favours the ketone product, and enols are rarely isolated. In FIG. 1b, the ketone form is on the left and the unusual enol tautomer is on the right.

POLYCISTRONIC RNA
An RNA transcript that contains the sequence of more than one functional RNA.

RNA HELICASES
A large, highly conserved family of RNA-dependent ATPases, generally thought to catalyse rearrangements in RNA structure. Some members can separate a base-paired RNA helix.

activity that contained all the catalytic residues involved in the charge-relay system[32,33].

This attractive model was recently tested by mutagenesis of key residues, A2451 and G2447. Mutations in G2447 that were predicted to inhibit the charge-relay mechanism in fact confer resistance to the antibiotic linezolid *in vivo*[34], presumably indicating that the ribosomes are functional, and mutation of neither A2451 nor G2447 blocked peptidyl-transferase activity *in vitro*[35]. These results seem to show that the charge-relay system is not required for peptidyl-transferase activity, although it might nonetheless normally be involved *in vivo*. Accurate relative positioning of the reacting groups by the 23S rRNA might be enough to allow peptidyl transfer to occur spontaneously — that is, there would be no need for RNA-mediated chemical catalysis[35]. A problem in interpreting all of these data is that peptidyl-transferase activity is not rate limiting in translation, making its rate in intact ribosomes difficult to estimate; it might be that even a substantial reduction in its activity would have little effect on growth or measured translation rates (see REF. 36 for further discussion). It is clear that the last word has not been written on this subject.

The nascent polypeptide leaves the ribosome through an exit tunnel around 10-nm long and 1–2-nm wide. This extends from the centre of the canyon where the peptidyl transferase centre lies, through to the back of the large subunit. The tunnel can contain up to 50 amino acids and shows conspicuous constrictions and bends, which might constrain folding of the polypeptide until it reaches solvent and binds to chaperone proteins. Folding within the tunnel would necessarily be limited to α-helix formation, the largest structure that could (barely) be accommodated. The narrowest segment is formed by the non-globular extensions of r-proteins L4 and L22. These form a gated 1.2-nm opening, which might sense the nascent chain as it is formed and relay a signal to the surface of the particle through their globular domains[1].

Making ribosomes

In keeping with the conserved structure of the mature particles, ribosome synthesis shows a high degree of evolutionary conservation. In almost all organisms, the mature rRNAs are generated by post-transcriptional processing from a POLYCISTRONIC precursor rRNA (pre-rRNA). Both the organization of the pre-rRNAs (FIG. 2) and aspects of the pre-rRNA processing pathway (FIG. 3) are well conserved. The key steps in ribosome synthesis are: transcription of the pre-rRNA; covalent modification of the mature rRNA regions of the pre-rRNA; processing of the pre-rRNA to the mature rRNAs; and assembly of the rRNAs with the ribosomal proteins. In eukaryotes, additional steps include the import of r-proteins from the cytoplasm to the nucleus and the export of the ribosomal subunits from the nucleolus through the nucleoplasm and nuclear pore complexes to the cytoplasm (BOX 3).

The compact nature of the RNA structures seen in the ribosomal subunits (see above) presents clear problems for the biosynthetic machinery. To allow access to processing, modification and assembly factors there must be a strict temporal order during ribosome synthesis. Final folding of the rRNA must be prevented until late in the pathway, maintaining key regions of the pre-RNAs in a relatively loose structure. After these steps, the rRNA must be refolded into the mature structure. The ATPase activity of the *E. coli* RNA HELICASE DbpA is specifically stimulated by binding to a fragment of the peptidyl-transferase centre[37], indicating a possible role in the formation of the catalytic core of the 50S subunit. Synthesis of the *E. coli* 30S involves a major structural isomerization that controls the formation of the central pseudoknot, a long-range interaction that is a core feature of 16S rRNA folding. This interaction connects the three main domains of the mature small subunit — the head, platform and body. In the pre-rRNA, the 5′ region of the 16S rRNA is base paired to a flanking sequence in the 5′ EXTERNAL TRANSCRIBED SPACER (ETS) region, preventing pseudoknot formation and presumably maintaining an open structure in the 16S rRNA[38].

In eukaryotes, premature formation of the central pseudoknot might be prevented by binding of the 5′ end of the 18S rRNA to the U3 SMALL NUCLEOLAR RNA

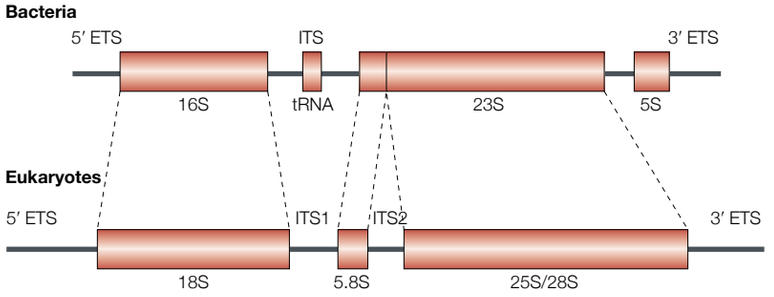


Figure 2 | **Conserved organization of the pre-rRNA.** The pre-ribosomal RNAs (rRNAs) are collinear in most organisms from all three kingdoms. The small subunit rRNA (16S in bacteria and archaea; 18S in eukaryotes) and large subunit rRNA (23S in bacteria and archaea; 25S/28S rRNA plus 5.8S rRNA in eukaryotes) are co-transcribed as a polycistronic precursor. In the pre-rRNA, the mature rRNA sequences are flanked by external transcribed spacers (5′ ETS and 3′ ETS) and separated by one or more internal transcribed spacers (ITS). Although there are many exceptions, the 5S rRNA is generally present in the common rRNA precursor in bacteria and archaea, but is independently transcribed by RNA polymerase III in eukaryotes. In eukaryotes, ITS2 is inserted into the 5′ region of the ancestral 23S rRNA, separating the 5.8S rRNA from the 25S/28S rRNA. In many bacteria and archaea, the ITS region contains a transfer RNA (tRNA), the 5′ end of which is cleaved by RNase P. Eukaryotes lack the tRNA but retain a cleavage site for a homologous endonuclease, RNase MRP, at this position (see FIG. 3).

(snoRNA), rather than to the pre-rRNA spacer as seen in *E. coli*. An RNA helicase associated with the U3 snoRNA might participate in this activity[39]. Strikingly, yeast ribosome synthesis involves no less than 17 putative RNA helicases[39–41], indicating a requirement for extensive structural reorganization.

Functional bacterial ribosomes can be assembled *in vitro* from the rRNAs and proteins, although specific conditions are required[42,43]. The analysis of *in vitro* assembly revealed a well-defined order of addition of the r-proteins and substantial cooperativity in r-protein binding, with assembly nucleated by a small number of primary rRNA-binding proteins[44,45]. For the 50S subunit, clear evidence was obtained for a structural rearrangement, which required incubation at an elevated temperature[43]. The assembly pathway has, as yet, been largely unaddressed by structural analyses. However, in one example, binding of the S15 protein was shown to reorganize and stabilize a complex structure in the rRNA that was required for the subsequent binding of two other r-proteins[46]. It is very probable that many other such cycles of structural rearrangement and stabilization, followed by specific protein binding, will occur during ribosome assembly.

In addition to processing, the rRNAs in all organisms undergo extensive covalent nucleotide modification at sites that cluster near the active core of the ribosome. Although not yet reported from structural studies, these modifications will probably aid the observed tight packing of the rRNAs through TERTIARY INTERACTIONS.

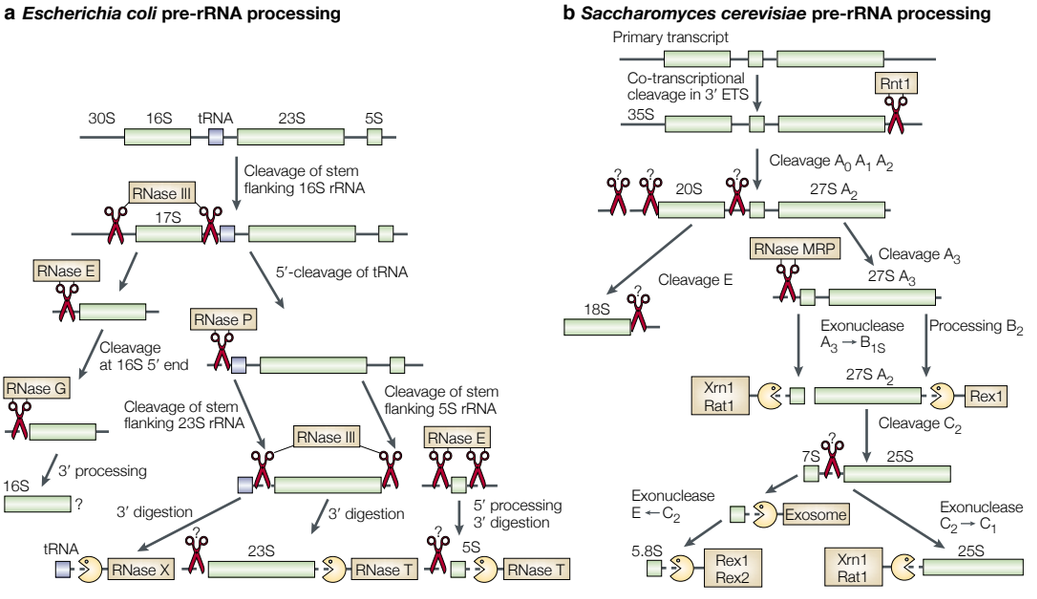


Figure 3 | **Conserved features of pre-rRNA processing in bacteria and eukaryotes.** The pathways presented are for the two best-characterized organisms, the bacterium *Escherichia coli* and the eukaryotic budding yeast *Saccharomyces cerevisiae*, but extensive conservation is expected throughout bacteria and eukaryotes. In both cases, the mature ribosomal RNAs are generated by sequential endonuclease cleavage, with some of the mature rRNA termini generated by exonuclease digestion. Some components are clearly homologous between bacteria and eukaryotes: first, the double-stranded endonucleases RNase III and Rnt1; second, the endonucleases RNase P and RNase MRP, which are themselves ribonucleoprotein complexes; and last, the 3′–5′ exonuclease RNase T is related to Rex1 and Rex2. The transfer RNA can be 3′ processed by any one of several 3′–5′ exonucleases, although RNase T and RNase PH are probably the most effective; these are collectively designated as RNase X. Scissors with question marks indicate that the endonuclease responsible is unknown. 5′ processing of *E. coli* 23S and 5S rRNA is assumed to be endonucleolytic as no 5′–3′ exonuclease has been identified in *E. coli*, in contrast to the roles of the 5′–3′ exonucleases Rat1 and Xrn1 in yeast. The mechanism of 3′ processing of the *E. coli* 16S rRNA is not known and several steps in yeast pre-rRNA processing involve cleavages by unidentified enzymes. In yeast, an alternative pathway generates a minor 5′ extended form of the 5.8S rRNA; for simplicity this has been omitted from the figure. For further details, see REFS 60–64.

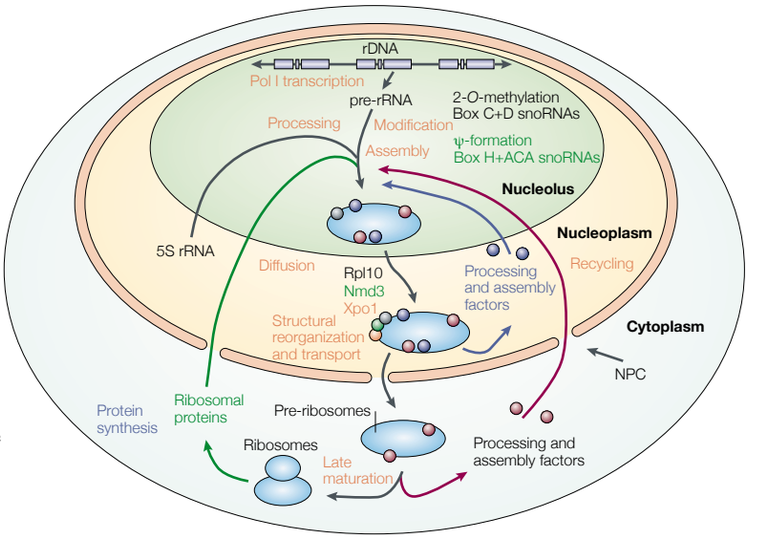
EXTERNAL AND INTERNAL TRANSCRIBED SPACERS (ETS and ITS). Regions of the ribosomal RNA precursors that do not form parts of the mature rRNAs or ribosomes, and are removed by processing.

SMALL NUCLEOLAR RNAS (snoRNAs). A set of small, stable RNAs, from 60 to 600 nucleotides in size. Most species form base-paired interactions with the pre-ribosomal RNAs that select sites of modification of the rRNAs. A smaller number (including U3) are required for processing of the pre-rRNA.

TERTIARY INTERACTIONS
In addition to stem structures formed by base pairing, RNAs can interact using alternative interactions between nucleotides. These are important in the overall folding of RNA molecules, and are collectively known as tertiary interactions.

Box 3 | Key steps in eukaryotic ribosome synthesis

After transcription of the pre-ribosomal RNAs, most steps in eukaryotic ribosome synthesis occur within the nucleolus. Here, the pre-rRNAs are processed to yield the mature rRNA species (FIG. 3), which also undergo extensive covalent modification. In bacteria, rRNA modifications are made by conventional enzymes, but in eukaryotes most modification involves methylation of the sugar 2′ hydroxyl group (2′-*O*-methylation) or pseudouridine (ψ) formation, which occur at sites that are selected by base pairing with a host of SMALL NUCLEOLAR RIBONUCLEOPROTEIN (snoRNP) particles[55]. Human cells contain over 100 species of snoRNP, and each pre-rRNA molecule must transiently associate with a member of each species. During pre-rRNA transcription and processing, many of the 80 or so ribosomal proteins assemble onto the mature rRNA regions of the pre-RNA. Many mutations known to inhibit ribosome synthesis in yeast are believed to act mainly at the level of ribosome assembly, but this process is poorly characterized.



During maturation, the pre-ribosomal particles are released from association with nucleolar structures, and are believed to diffuse to the nuclear pore complex (NPC). In yeast, nuclear export of pre-60S particles is mediated at least in part by the small GTPase Ran and the export factor Xpo1/Crm1, which binds to the ribosomal protein Rpl10 through an adaptor protein, Nmd3 (REFS 56–58). Export of the pre-40S subunit also requires Ran[59], but no specific export factors have been identified. Passage through the NPC is likely to be preceded by structural rearrangements and the release of pre-ribosome-associated proteins, including processing and assembly factors. It seems likely that further ribosome synthesis factors will be released during late structural rearrangements in the cytoplasm that convert the pre-ribosomal particles to the mature ribosomal subunits. Failure to reorganize the pre-ribosomes and the consequent deficit in recycling of processing factors might underlie the observation that almost all mutations that lead to the under-accumulation of cytoplasmic 60S subunits also inhibit early pre-rRNA processing steps[60,61]. Names of specific factors are taken from budding yeast, *Saccharomyces cerevisiae*, but we predict that the steps are conserved throughout eukaryotes.

Several pre-rRNA processing activities remain to be identified, particularly in the yeast *S. cerevisiae* (indicated by question marks in FIG. 3). Notably, all of the nucleases indicated in FIG. 3, from both *E. coli* and yeast, process other RNAs in addition to the pre-rRNAs. It is likely that, when the enzymes responsible for the remaining processing activities are identified, they too will be found to process other substrates.

Perspectives

The continuing structural analyses are greatly increasing our understanding of how ribosomes work. In the immediate future we can expect to see direct tests to discriminate between alternative models for catalytic activity. A clearer understanding of the interactions between the ribosomal subunits should also emerge, with direct tests of models for the movement and functional interactions between the subunits. But it will be a long time before we fully understand the way in which these intricate machines are put together and how they function.

Recent structural analyses have given clear insights into the mechanisms of several antibiotics, and more analyses can be expected, leading to the development of new, design-based drugs. The characterized antibiotics bind at many sites and inhibit various steps in the translation cycle. A particular virtue of future, structure-based antibiotic design will be the ability to target additional sites and activities not targeted by existing antibiotics. This might have the advantage that no naturally occurring resistance activities will exist to seed the development of drug-resistant pathogens.

Structural understanding of eukaryotic ribosomes is lagging far behind that of their bacterial counterparts; the best structure available at present is a 1.75-nm structure of the yeast 80S ribosome[47]. However, the high conservation of the rRNAs means that the mechanisms and structures involved will be fundamentally similar. Initial steps will be computer modelling or 'threading' of the eukaryotic rRNAs into the bacterial structures, followed, presumably, by crystallographic structures.

The synthesis of ribosomes is not directly addressed by the recent structural data. However, an understanding of the mature subunits is likely to be crucial to understanding the assembly pathway. The analysis of smaller regions is already revealing that many sequential steps lie on the pathways by which the entire, amazing structure comes together.

SMALL NUCLEOLAR RIBONUCLEOPROTEINS (snoRNPs). Complexes between the snoRNAs and specific proteins.

Links

DATABASE LINKS EF-Tu | Rex1 | Rex2 | Rat1 | Xrn1 | Crm1 | Rpl10 | Nmd3

FURTHER INFORMATION Tollervey lab

ENCYCLOPEDIA OF LIFE SCIENCES Ribosome structure and shape | Bacterial ribosomes | Eukaryotic ribosomes

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