# Ribosomes: **Cashing in on crystals**
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**Structures of the ribosome and its two subunits have been determined by X-ray crystallography at resolutions sufficient to reveal interactions between RNA and protein in the subunits and orientations of substrates at the subunit interface in the intact ribosome.**

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The ribosome has long been subject to intense scrutiny by molecular and cell biologists because of its central role in protein biosynthesis. Despite several decades of study, however, the mechanistic details of how proteins are made have remained elusive. The structural complexity of the ribosome — which comprises two non-equivalent subunits containing RNA and protein — and other components of the translation machinery reflect a process that is both complicated and highly regulated in the cell. The high level of structural conservation in ribosomes from the three kingdoms of life — prokaryotes, archaea and eukaryotes — suggests that the ribosome has a common function and common evolutionary origins. Three recent papers [1–3] have reported X-ray crystallographic structures of the ribosome and its subunits from bacteria and archaea, providing insights into the interactions that take place within and between the two subunits.

All ribosomes are 1:1 complexes of two unequal subunits, the larger about twice the size of the smaller. In bacteria, the small subunits (30S) contain a single copy of an RNA of ~1500 nucleotides — 16S ribosomal RNA (rRNA). Large subunits (50S) contain a single RNA of ~2900 nucleotides (23S rRNA), and one copy of a 120 nucleotide RNA (5S rRNA). In *Escherichia coli*, the small ribosomal subunit contains single copies of at least 20 different proteins, whereas the large subunit contains more than 30 different proteins. Eukaryotic ribosomes are qualitatively similar, but they contain more proteins and larger rRNA molecules. The rRNA molecules play a key role in ribosome architecture, and perhaps directly in the catalysis of translation: the ratio of molecular weight of RNA to that of protein in all ribosomes is about 60:40, and disruption of rRNA diminishes or destroys translation activity.

The ribosome translates the sequences of messenger RNAs (mRNAs) by polymerizing amino acids, supplied in the form of aminoacylated transfer RNA molecules (tRNAs), in the order dictated by the genetic code. The small subunit binds to mRNA and the large subunit, and determines when a match has been made between the trinucleotide codon for an amino acid on the mRNA template and the complementary anticodon of an incoming tRNA substrate carrying the cognate amino acid. The large subunit catalyzes the formation of a peptide bond between the growing peptide chain and the incoming amino acid and interacts with protein factors that couple catalysis with translocation of the mRNA template and the incoming tRNA substrates.

The importance of the ribosome in gene expression, its formidable size of 2.5–4.5 megadaltons, and the intimate involvement of RNA in all of the ribosome's functions have attracted structural biologists wielding a variety of techniques (reviewed in [4]). Electron microscopy provided some of the first images of the ribosome at low resolution, revealing distinctive shapes for both subunits. Positions of ribosomal proteins within the particle were determined by small-angle neutron scattering, which involves measuring the scattering difference between a fully protonated ribosome and a ribosome containing two deuterated proteins in an otherwise protonated background. Furthermore, as nearly all ribosomal proteins present epitopes on the surface of the ribosome, antibodies against them were used as specific markers to visualize the positions of these proteins by negative staining and electron microscopy. Sequencing and phylogenetic comparison of rRNA molecules led to secondary structures for the 16S and 23S rRNAs of bacterial ribosomes.

Remarkably, it proved possible to crystallize ribosomes and their component subunits. Yonath and coworkers obtained the first crystals of the 50S ribosomal subunit in 1979: this group demonstrated high resolution X-ray diffraction from these crystals and developed methods for measuring such diffraction data (reviewed in [4,5]). Given the difficulty of ribosome purification, the large size of the crystallographic unit cell and the sensitivity of the ribosome crystals to radiation-induced decay, this was a phenomenal achievement. Crystals of the 30S ribosomal subunit, and of the intact 70S ribosome, were subsequently reported, heightening the anticipation of forthcoming structures [4].

To solve these structures, phases for the observed X-ray diffraction patterns had to be determined. The typical procedure of identifying specifically bound heavy atoms within the crystals, for which positions can be determined and used to bootstrap to a complete set of phases, proved

**Figure 1**
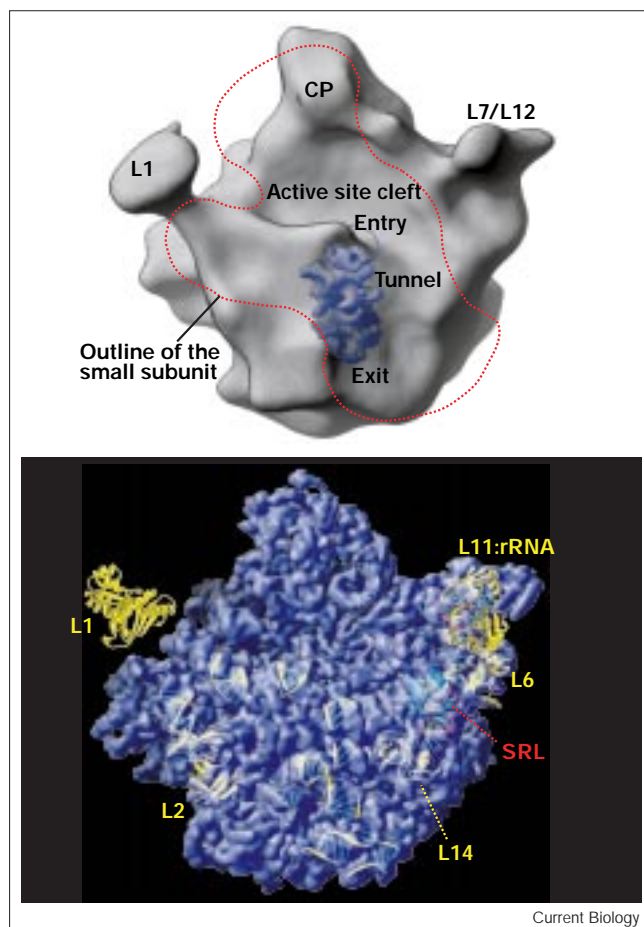


Overview of the large ribosomal subunit as seen from the side of the 30S subunit interface. **(a)** Schematic diagram showing the disposition of gross features as previously visualized by electron microscopy, including the central protuberance (CP) and the proteins L1 and L7/L12. The tunnel through which incoming tRNA substrates pass is shown, with the presumed entry and exit sites marked. The approximate outline of the small subunit binding to the large subunit is indicated. **(b)** Solid surface representation of the 5 Å resolution map of the large ribosomal subunit. Proteins and 23S rRNA fragments with known three-dimensional structures that have been located and fitted to the map are shown as well as a vast distribution of generic duplex RNA. The sarcin-ricin loop (SRL) is an essential segment of ribosomal RNA that is a target of several antibiotics.

challenging because of the size of the ribosome and hence the large number of heavy atom binding sites. Three groups, however, have now independently solved the phase problem for the 50S, 30S and 70S ribosome crystals, respectively, and have recently reported the resulting electron density maps [1–3]. These are the largest asymmetric crystal structures solved to date, and interestingly, each group used a different strategy for phase determination. Ban *et al.* [1] initially used a model of the 50S subunit derived from cryo-electron microscopy to obtain low resolution phases, which were useful for locating heavy atoms

to provide higher resolution phases [6]. Clemons *et al.* [3] were able to soak heavy atoms into pre-formed crystals without changing the arrangement of ribosome molecules in the crystals (multiple isomorphous replacement). Cate *et al.* [2] used the technique of multiwavelength anomalous diffraction (MAD), in which the difference in diffraction properties of a crystal-bound heavy atom at different wavelengths of X-rays is used to determine phases.

Following on from their previous 9 Å resolution electron density map of the 50S subunit from *Haloarcula marismortui* [6], a member of the archaea, Ban *et al.* [1] have now obtained a 5 Å resolution map that reveals rRNA helices crisscrossing the subunit. Even though earlier images of the 50S subunit determined by cryo-electron microscopy revealed its overall shape (at approximately 15–20 Å resolution), the 5 Å map shows its knarled surface, complete with numerous nooks and crannies (Figure 1). The electron-dense phosphate backbone of rRNA is distinctive at 5 Å resolution, enabling Ban *et al.* [1] to model more than 300 base pairs of duplex RNA into their map. Several other familiar RNA features were identified as well, including GNRA tetraloops (where N is any of the four nucleotides and R is a purine), side-by-side helical packing and many regions of single-stranded RNA. Although more difficult to identify at this stage, twelve ribosomal proteins have been located in the map. Atomic coordinates for four closely related bacterial ribosomal proteins have been fitted to the electron density. Most of these proteins crosslink two or more rRNA rods, consistent with previous biochemical results indicating an important role for ribosomal proteins in stabilizing ribosome conformation.

Two portions of the map and its current model are particularly interesting. A long discontinuous rod of duplex rRNA rims a deep cleft at the entrance to the peptidyl transferase active site, otherwise known as the 'P' site, where the peptide chain is transferred from the peptidyl tRNA bound at this site to the incoming aminoacylated tRNA bound to the so-called 'A' site. At the bottom of the active site cleft is a tunnel running straight through the subunit, thought to be the path of the newly synthesized protein chain as it passes from the peptidyl transferase center to the point where it exits the ribosome. The other region of the subunit that has been modeled is the 'factor-binding center', which binds to two translation elongation factor proteins, EF-G and EF-Tu, as well as to the initiation factor protein IF-2 and the release factor protein RF-3. Three ribosomal proteins and two rRNA fragments that form a major part of the factor-binding center have been positioned, and this positioning agrees with earlier chemical protection and crosslinking data. The two elongation factors and an aminoacylated tRNA were then positioned in this site in a manner consistent with cryo-electron microscopy and crosslinking data. The resulting models for factor binding predict several additional interactions

between the bound factors and the 50S subunit that can now be tested. As data from 50S subunit crystals can be measured to 3 Å resolution, a high resolution electron density map and a complete atomic model may be available in the near future.

A similar level of atomic detail is seen in the 5.5 Å resolution map of the 30S ribosomal subunit from the bacterium *Thermus thermophilus*. Clemons *et al.* [3] produced a map of the small subunit with the characteristic shape seen by electron microscopic studies. A large number of rRNA double helices were located by visual inspection of the map and assigned to regions of 16S rRNA on the basis of available biochemical data. The seven proteins of known high-resolution structure have been placed in the density, assisted by a variety of data from previous studies indicating the approximate locations of these proteins within the 30S subunit. Five additional proteins whose detailed structures are not yet known have been identified on the basis of their location and their probable structural features, such as α helices. Most striking in the map is a long, protein-free stretch of double-helical density at the subunit surface, which Clemons *et al.* [3] assigned to the penultimate stem of 16S rRNA believed to be located at the interface between the two ribosomal subunits. The almost complete absence of protein at the interface surface is consistent with predictions that the subunit interface is composed of RNA.

The interface is likely to be the most important part of the ribosome in terms of function, as this is where tRNA molecules bind and interact both with the P site and the A site. Hence, the predominance of RNA in this region is consistent with the idea that a primordial ribosome may have contained no protein at all [7]. The central domain of the 30S subunit includes a platform of rRNA essential for tRNA binding and subunit association, as well as a 3′ region of rRNA involved in translational accuracy. Now completely modeled by Clemons *et al.* [3], this central domain is observed to have an elongated, curved architecture that wraps partially around the body of the small subunit. As seen elsewhere in the subunit and also in the 50S subunit, the domain contains numerous short rRNA helices packed together. A three-way junction of helical rRNA forms the heart of the central domain and is stabilized by several protein–RNA interactions. Further details of the structure await the 3.6 Å resolution electron density map that the authors are currently working on.

Beyond the determination of ribosomal crystal structures — a demanding endeavor in itself — lies the challenge of understanding the functional states and potential dynamic motions of both of the ribosomal subunits during peptide elongation. Cate *et al.* [2] begin to address these issues with their 7.8 Å electron density map of the intact 70S ribosome from *T. thermophilus*. It was possible to co-crystallize the 70S ribosome with relevant substrates, or to soak substrates into preformed crystals and determine their positions by calculating the difference in electron density between the bound and free ribosome crystals. The overall architecture of the 70S ribosome seen here is similar to that observed previously by cryo-electron microscopy. Furthermore, structural features seen in the 30S and 50S electron density maps are also observed, such as the long rRNA helix in the 30S subunit at the interface. By correlating the electron density maps with features of the RNA visible at 7.8 Å resolution, it was possible to determine the interface between the subunits in the 70S ribosome. Peripheral contacts involve RNA–protein interactions, whereas the platform and penultimate stem region, described above for the 30S subunit, involve a complex network of RNA–RNA and RNA–protein interactions. One of these, a double-helix of RNA that protrudes from the 50S subunit and makes direct contact with the protein S15 in the 30S subunit, was elegantly deciphered using directed chemical footprinting [8]. In this experiment, the S15 protein was specifically tagged and its contacts in the 50S subunit were mapped. The resulting model fits beautifully with the observed electron density, demonstrating the interplay between biochemical analysis and crystal structure determination, particularly at the medium resolution of the current electron density maps of the ribosome. Despite the close packing of subunit surfaces, however, the extent of the interface is surprisingly modest, perhaps reflecting the functional importance of subunit motion and reversible association.

Complexes of mRNA template and tRNA molecules with the ribosome reveal the locations of these substrates as they bind in the active site at the subunit interface. The A site is rather open, with minimal contacts seen between the mRNA–tRNA complex and the ribosome electron density. In contrast, the tRNA anticodon appears tightly clamped to the message at the P site, perhaps to maintain the correct reading frame of the genetic code. Fingers of electron density contact the minor helical groove and the backbone in a way that would be universal to all tRNAs, explaining how different substrates can be bound in the same site. The single-stranded end of P-site tRNA that carries the growing peptide chain is poised at the entrance to the presumed peptide exit channel, marking the location of the peptidyl transferase center. The tantalizing question of the identity of this center may soon be answered following further interpretation of the map.

With these structures comes the hope of insights into some of the most intriguing and difficult questions about ribosome function and evolution. How does the ribosome actually read the genetic code and distinguish between cognate and non-cognate tRNA molecules? Is the ribosome at heart a ribozyme, using RNA to catalyze the chemistry of protein synthesis with proteins providing the stabilizing 'glue'? It has long been imagined that a primordial ribosome was

composed entirely of RNA (reviewed in [7]), and detailed structures of modern ribosomes may provide important clues to their evolutionary history. Finally, structures of the ribosome will provide an unprecedented view of RNA structure and RNA–protein interactions. Once-fanciful goals of achieving atomic resolution structures of the ribosome, and understanding the ribosome's moving parts and substrates, are now within reach — exciting prospects indeed.

### References

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