Ribosomes: **Protein synthesis in slow motion**

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Using image reconstruction methods, electron microscopists can now visualize ribosomes at resolutions so high that the changes in the positions of ribosome-bound tRNAs which occur during protein synthesis can be seen.

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It has been known for 40 years that proteins are made by ribosomes. These large polymerases catalyze the construction of polypeptides by linking amino acids in the specific sequence directed by their mRNA templates. Prokaryotic ribosomes have molecular weights around 2.5×10^3 kDa; eukaryotic ribosomes are almost twice as big, and each ribosome synthesizes only one polypeptide at a time, regardless of origin (for review, see [1]). Although the template-directed polymerase action of ribosomes is analogous to that of RNA and DNA polymerases, the composition of ribosomes is remarkably different, being about 60% RNA and 40% protein.

Electron microscopy has made many contributions to the ribosome structure field. Ribosomes are big enough, (~250 Å) and their shapes distinctive enough, that useful information can be gleaned from negatively stained preparations. In the 1970s and 1980s, researchers such as Lake and Glitz at UCLA and the Stöfflers in Berlin deduced approximate shapes for the ribosome and its two subunits from images of negatively stained specimens. They also mapped the locations of landmarks on the ribosome surface using antibodies directed against ribosomal proteins and ligands as component-specific stains (for example, see [2,3]). These early ribosome models were not rigorous three-dimensional reconstructions, but, at about the same time, the quantitative analysis of ribosome images began and these studies have recently produced extremely important results.

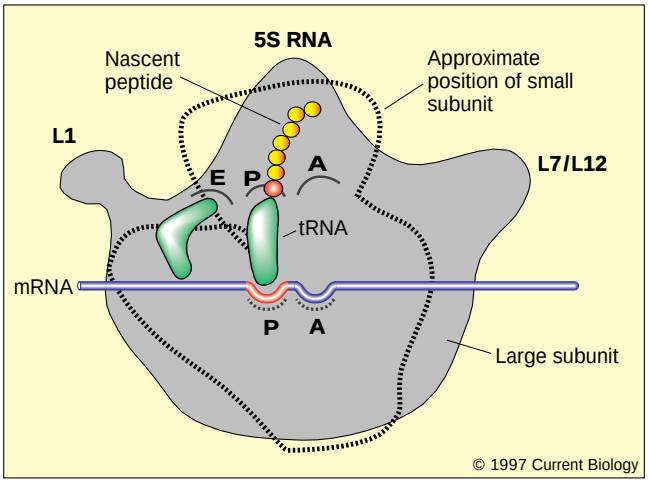
The easiest ribosome images to obtain are those of fields of randomly oriented particles. For the image analyst, however, it is easy to reconstruct the three-dimensional form of objects from projection images when their relative orientations are known in advance, but much harder when orientations must be deduced after the fact, as is the case here. It took years to develop the technology required, and, as it evolved, increasingly accurate ribosome reconstructions appeared, but the improvements were so gradual that they attracted little notice.

In 1995, there was a major breakthrough in the study of ribosomal structure. Two, independently derived, 20–25 Å resolution reconstructions of the 70S ribosome from *Escherichia coli* were published, one by Joachim Frank and coworkers [4] and the other by Marin van Heel's group [5]. Not only was there a great improvement in resolution, but the images analyzed were those of unstained particles embedded in vitreous ice, eliminating staining artefacts and revealing an amazing amount of new detail. These structures confirmed that the older, lower-resolution models of the ribosome were sound, and because the results of the two groups are similar, there is reason to believe their higher-resolution results are reliable also. Now, van Heel and colleagues [6] have extended their studies to analyze the movements of ribosome-bound tRNAs during protein synthesis.

The reader will recall that tRNAs, the molecules that carry amino acids to the ribosome, bind to ribosomes at three sites: the A site, the P site and the E site (see Fig. 1; for review, see [7]). Ribosomes make polypeptides one amino acid at a time, and the cycle of steps that extends growing polypeptides by one residue begins with the ribosome in the post-translocational state, with a deacylated tRNA bound to its E site, and a peptidyl-tRNA bound to its P site. An aminoacyl-tRNA carrying the next residue, which is selected out of the tRNA pool by interactions between its anticodon, the mRNA and the ribosome, is delivered to the A site by a GTP-binding (G) protein, elongation factor Tu (EF-1α in eukaryotes). The tRNA on the E site leaves the ribosome, and the peptide on the P-site tRNA is transferred to the amino acid on the A-site tRNA by an enzymatic activity intrinsic to the ribosome called peptidyl transferase. A ribosome in this condition is in the pre-translocational state, and it is a substrate for a second G protein, elongation factor G (EF-2 in eukaryotes), which promotes translocation, the process that returns it to the post-translocational state. During translocation, the peptidyl tRNA in the A site moves to the P site, the deacylated tRNA in the P site moves to the E site, and the mRNA advances by one triplet.

Stark *et al.* [6] now report 20 Å resolution reconstructions of the pre-translocational and post-translocational ribosome, both with the two tRNAs bound that are specified by an appropriate mRNA. These new structures are a more refined version of the study reported in 1996 by

Figure 1



Approximate locations of the tRNA binding sites on the (bacterial) ribosome. The large subunit is shown in grey, with its subunit interface towards the reader. Its L7/L12 arm is to the right, its L1 arm is to the left, and its central protuberance, which is mostly 5S rRNA, is on top. The outline of small subunit is shown with broken lines in the approximate position it occupies in the two-subunit couple. The purple horizontal line indicates mRNA, and tRNAs are depicted as green boomerang-shaped objects, one of them with a nascent peptide bound (orange). A codon and its corresponding amino acid are shown in red. The large subunit has three tRNA sites, which are, from left to right, the E site, the P site and the A site. The small subunit is believed to have only a P site and an A site. (I thank John Czworkowski for providing the original version of this figure.)

Frank's group [8], which imaged ribosomes with deacylated tRNA bound to all three sites.

It has long been known that tRNAs bind to the ribosome on the face of the large subunit that binds the small subunit, and the reconstructions from both groups show that the subunit interface has a cavity large enough to accommodate three tRNAs. The large subunit surface of the cavity has the peptidyl transferase region, and its small subunit surface includes the mRNA-binding region. As expected, this is where the 'extra' density appears that both groups identify as tRNA by comparing images of empty ribosomes with images of full ribosomes.

The tRNA-associated density detected by Frank and coworkers [8] is an irregular region not quite big enough to accommodate three tRNAs. In both of van Heel's reconstructions [5,6], the tRNA-associated density consists of two 'blobs', one for each tRNA bound. The blobs appear to be the size of half a tRNA molecule, and they clearly move during translocation. In the pre-translocational image, there is a blob in the middle of the cavity, and another on its L7/L21 side (see Fig. 1). In the post-translocational image, the middle blob stays put, the L7/L12-side blob disappears, and is replaced by a blob on the L1 side of the cavity. The interpretation is obvious. The ribosome's P site is full in both states, and so the middle blob must be peptidyl-tRNA bound to the P site. Other evidence indicates that the A site must be on the L7/L12 side of the P site, and that the E site is close to L1; the blobs that change are where tRNAs bound to those two sites should be. We are looking at snapshots of protein synthesis in progress.

Although some aspects of the distribution of tRNAs are difficult to discern from the published figures — even at this high resolution — it does appear that van Heel's three blobs [6] are encompassed by the tRNA density observed by Frank and coworkers [8]. The data from the two groups therefore appear to be compatible; where they disagree is in their interpretation. Frank places the centers of gravity of the three tRNAs at about the same locations as van Heel. Furthermore, both orient their tRNAs so that the acceptor ends of the tRNAs bound to the A and P sites are near the peptidyl transferase site on the 50S subunit, and their anticodon ends are close to the mRNA region on the 30S ribosome — as they must be — and both think that tRNAs may change conformation somewhat when they bind to the ribosome. The two groups part company, however, over the angle between the planes defined by tRNAs bound to the A and P sites, and more grossly in the placement of the E site.

In some respects, Frank's tRNA placements [8] deviate from those anticipated from earlier data more than those proposed by van Heel [6], but I am not persuaded by van Heel's argument that Frank's work should be disregarded because ribosomes with three deacylated tRNAs bound are physiologically irrelevant. Neither group has visualized its tRNAs at a sufficiently high resolution to specify their orientations unambiguously, and as van Heel sees less density for each tRNA than Frank, his proposals are less restrained by data than Frank's.

One additional point: the non-tRNA parts of van Heel's images are nearly the same, and hard to distinguish from those of empty ribosomes (Frank has noted the same for his full and empty ribosomes). This is a surprise. Hydrodynamic and scattering data suggest that there are small conformational differences between pre-translocational and post-translocational ribosomes [9], and one might have thought a change that appears modest hydrodynamically would be easy to see at 20 Å resolution. However, van Heel's observation is consistent with the chemical modification results of Noller and coworkers [10], who have reported no alterations in the chemical reactivity of rRNA that cannot be explained by changes in the occupancy of tRNA sites. At 10 Å, a resolution van Heel suggests electron microscopists may achieve, answers to all of these questions should be forthcoming, and to many more besides.

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