predicted from the cDNA encoding the hypocretin precursor protein (the predicted cleavage sites are not consistent with the analysis of the native peptide). Orexin-B is identical to hypocretin-2 (*8*, *9*). Both ligand and receptor loci bear the *hypocretin* moniker; however, given that the structure of hypocretin-1 was incorrect (*9*) and that the receptors were identified by Yanagisawa's team (*8*), the nomenclature of this ligand-receptor system should probably be reconsidered—"narcoleptin" perhaps?

The hypocretin/orexin neuropeptide system has been proposed to regulate feeding and energy metabolism based on the anatomical expression pattern of the peptides and their effects on feeding behavior (*8*). The neurons are located in the lateral and posterior hypothalamus and project widely within the forebrain, limbic system, thalamus, hypothalamus, brainstem, and spinal cord (*10*). Thus, one obvious question to be addressed is the effect that inactivating the hypocretin/orexin precursor gene will have in mice. Yanagisawa and colleagues introduced a null mutation into this gene and carefully examined the mice for behavioral abnormalities during the daytime (*3*). But nothing appeared unusual. Nevertheless, knowing that mice are nocturnal and feed at night, the investigators observed the mice at night using infrared video recording. Incredibly, the mice appeared to have frequent episodes of behavioral arrest that resembled cataplexy (*11*). These "narcoleptic attacks" were usually triggered by ambulating and grooming. To determine whether the episodes could be epileptic seizures, they recorded EEG and electromyogram (EMG) activity and found that the mice did not appear to have a seizure disorder, but rather appeared to enter into REM sleep prematurely. Taken together these results suggest that the *orexin* knockout mice have the mouse equivalent of narcolepsy. Classical sleep physiologists remain cautious about the mouse phenotype (*12*) but, as a geneticist, I am persuaded by the beauty of the ligand and receptor phenocopy that is so reminiscent of the *steel/c-Kit* and *obese/diabetic* pairs of mouse mutants.

How are the hypocretins/orexins affecting sleep? It is too early to tell, but it is clear that a new and unexpected pathway has been linked to sleep. These studies also highlight the lateral hypothalamus as an important site of sleep regulation. The fact that a mutation in a G protein–coupled orexin receptor can cause narcolepsy immediately opens up the possibility of new drug discovery efforts with the future hope of therapeutics for the hundreds of thousands of narcoleptic patients who would like to take sleep for granted like the rest of us.

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PERSPECTIVES: STRUCTURE

Function Is Structure

Anders Liljas

To understand the function of a biological system such as the ribosome—the factory in the cell cytoplasm that makes proteins—it is first necessary to know the structure of its component parts. The ribosome, however, has not readily yielded its secrets to x-ray diffraction analysis. Now, four papers—two in this issue of *Science* (*1*, *2*) and two in a recent issue of *Nature* (*3*, *4*)—present the crystal structures of the 70*S* bacterial ribosome and its two component subunits, providing molecular insights that go way beyond our previous knowledge.

The large (50*S*) and small (30*S*) subunits of the ribosome are together composed of three types of ribosomal RNA (rRNA) and 54 different proteins. These subcellular factories translate the genetic message of mRNA into the amino acid sequence of the particular protein it encodes. The substrate in this enzymatic translation process is transfer RNA (tRNA), which has an anticodon at one end (that interacts with the mRNA bound to the 30*S* subunit) and the corresponding amino acid at the other (the 3′ end, which interacts with the 50*S* ribosome). As the ribosome moves along the mRNA, the mRNA codon forms base pairs with the anticodon of the corresponding tRNA. An accepted codon-anticodon match allows the growing string of amino acids attached to the ribosome-bound tRNA to be transferred to the amino acid of an incoming tRNA, so that the polypeptide is elongated by one amino acid residue at a time. This activity occurs in the peptidyl transfer site in the 50*S* ribosomal subunit (see the figure). Protein synthesis is catalyzed by a range of different translation factors that are active in the initiation, elongation, and termination phases of polypeptide production.

The solving of the first tRNA structures by x-ray crystallography in 1974 began to unravel the mystery of how proteins are synthesized (*5*). The distance between the anticodon and the acceptor end of tRNA (where the amino acids are attached) was found to be very large (75 Å). Any new model explaining how the ribosome works had to accommodate this essential fact. Next came the structure (at 2.7 Å resolution) of tRNA bound to the polypeptide elongation factor Tu (EF-Tu) (*6*). In this complex, the amino acid at the acceptor end of tRNA was found to be firmly bound to and buried in the elongation factor and so could not be added to the polypeptide chain until its release from EF-Tu. Then came the discovery that the structure of the complex between tRNA and EF-Tu is mimicked by that of elongation factor G (EF-G)—the ribosomal translocase that moves the tRNA together with the mRNA to expose a new codon (*6*, *7*). The simple explanation for this similarity is that these elongation factors bind to the same site on the ribosome.

Despite these advances, solving the structures of the 50*S* and 30*S* subunits of the bacterial ribosome has been a challenge for more than 30 years. Electron microscopy, fluorescence resonance energy transfer, and neutron scattering, together with bifunctional cross-linking, affinity labeling, chemical footprinting, and other chemical techniques have enabled the ribosome to be analyzed at low resolution. Recently, cryo-electron microscopy (cryo-EM)—which can be thought of as crystallography performed on single particles—has improved the resolution of ribosomal structures dramatically (*8*), and other techniques have enabled the visualization of high-resolution details of some ribosomal components.

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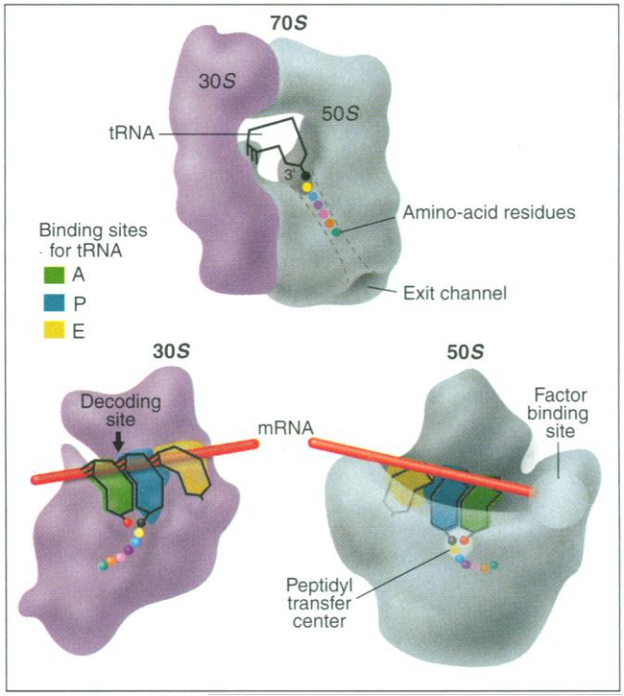
The production of crystals of ribosomes and their subunits that diffract x-rays much more efficiently prompted the expectation that the ribosome's structure would soon be revealed in great detail (*9*). This expectation was finally realized at a meeting in June this year with the presentation of the crystal structures of the bacterial ribosome and each of its subunits at resolutions below 8 Å, at which vital details relevant to the ribosome's function were able to appear (*10*). The *Nature* papers report the structures of the large (*3*) and small (*4*) subunits at resolutions of 5.0 and 5.5 Å, respectively. In addition, the location of mRNA in the small 30*S* ribosomal subunit has been identified (at 4.5 Å resolution) by labeling with heavy metal atoms (*11*). The reports on pages 2095 and 2133 of this issue present crystal structures of the bacterial ribosome complexed with mRNA and various tRNAs at a resolution of 7.8 Å (*1, 2*). The richness of detail in all of these structures is set to solidify our understanding of the minute details of protein synthesis.

The crystallographic achievements displayed in the *Science* reports are significant. These structures are the largest asymmetric objects ever solved by crystallography (molecular masses: 30*S*, 900 kD; 50*S*, 1600 kD; 70*S*, 2500 kD). To be able to calculate (by Fourier summation) the electron density of a crystal, one needs to know not only the amplitudes (related to the intensities of the diffracted x-ray beams), but also the so-called phase angles for each diffracted beam. Until now, the initial phase angle determination has been a major obstacle to solving large structures by x-ray diffraction. But low-resolution structures obtained with cryo-EM have been used to identify the position and orientation of the ribosomal particles in the crystals (*1*). This has helped to identify the location of heavy-atom clusters, which, when studied at several x-ray wavelengths with synchrotrons, become a very powerful means for determining phase angles (*1–4, 11*).

The new structures have been invaluable for working out where the 54 proteins and more than 4500 nucleotides of rRNA are located in the ribosome. For example, a protrusion on the 50*S* subunit and its association with rRNA was identified by labeling it from protein S15, which sits in the 30*S* subunit close to the protrusion (*2*). The contacts between the two ribosomal subunits are surprisingly limited (*1, 2*). This explains the relative ease with which ribosomes can be dissociated and is consistent with rearrangements of the relative orientation of the subunits. One of the few connections between the large and small subunits is a long rRNA helix (identified as the penultimate helix of 16*S* rRNA) that runs along the length of the 30*S* subunit (*1, 4*).



Rotund marvels. Structure of the 70*S* ribosome and its functional center. **(Top)** The tRNA molecules span the space between the two subunits; the channel in the 50*S* subunit through which the growing peptide chain protrudes is shown in dashed lines. **(Bottom)** The 30*S* **(left)** and 50*S* **(right)** subunits have been opened up to give a better view of the three binding sites for tRNA, the A, P, and E sites. The 30*S* subunit shows the approximate location of the site where the codons of the mRNA are read by the anticodons of the tRNAs. The 50*S* subunit has the tRNA sites shown from the opposite direction. The acceptor ends of the A- and P-site tRNAs are close to each other in the peptidyl transfer site, which is close to the exit channel located behind a ridge in the 50*S* subunit. The binding site for EF-G and EF-Tu is located on the right-hand protuberance of the 50*S* subunit.

The functional centers of the ribosome have now been clearly identified. Thus, the ribosomal binding sites for tRNA (A, P, and E) form bridges between the subunits (see the figure). The relative orientation of the A- and P-site tRNAs seems to be more parallel than originally inferred by cryo-EM (*1, 8*). Regions of the penultimate helix of 16*S* rRNA—previously identified as positions where the codons of mRNA are decoded by tRNA—have now been further characterized (*1, 4*). The anticodon in the P site is held firmly in place by its interactions with the ribosome. In contrast, the A-site anticodon projects into an open cavity, giving it the flexibility it needs for reorientation of its acceptor end after release of the tRNA from EF-Tu, which enables the molecule to take up its functional position in the peptidyl transfer site (*1*). This site in the 50*S* subunit—where the amino acid of the A-site tRNA approaches the P-site tRNA for peptide transfer—has been accurately pinpointed. However, the identification of the exact components of the peptidyl transfer site, the regions of 23*S* rRNA involved, and the proteins that are close enough to participate in peptide bond formation remain to be established (*1, 3*). As soon as the components of the peptidyl transfer site are definitively identified, the decade-long debate about whether the ribosome is a ribozyme (an RNA molecule with enzyme activity) will come to an end. The channel through which the synthesized polypeptide chain protrudes can be seen clearly in the crystal structure of the 50*S* subunit (see the figure) (*3*). In addition, the binding sites for several of the translation factors, including EF-Tu and EF-G, have been identified and a tentative model for their interaction presented (*3*).

Future crystallographic and cryo-EM studies should allow the structure of the ribosome to be solved at even higher resolutions. However, it is of the utmost importance that all of the structural data be made available in the Protein Data Bank as soon as possible (*12*). Attempting to obtain detailed structural impressions from the illustrations provided in the literature is of little value.

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