

Reviews

Challenging the three-dimensional structure of ribosomes

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A prerequisite for understanding the detailed mechanism of the intricate process of protein biosynthesis is the determination of the molecular structure of ribosomes. This may be obtained by crystallographic analysis. Although such studies, in general, provide static pictures, they do indicate how to design subsequent functional and dynamic experiments. Thus, investigating models obtained by three-dimensional image reconstruction have stimulated further biochemical as well as crystallographic studies.

Ribosomes are composed of two subunits of unequal size which associate upon initiation of protein biosynthesis and dissociate at the termination of the process. A typical bacterial ribosome, e.g. from *E. coli*, is of molecular weight of 2.3×10^6 and migrates with a sedimentation coefficient of 70S. It contains 35 different proteins and two RNA chains in its large subunit (also named 50S, with a molecular weight of 1.45×10^6) and 21 proteins and one RNA chain in the small one (30S; mol. wt 8.5×10^5).

Crystals of active ribosomal particles

It was widely assumed that ribosomes would be intractable to crystallographic analysis. Their size, complexity, instability and flexibility were considered as obstacles to crystallization. Furthermore, it was predicted that even if crystals were obtained, they might not diffract at all or their diffraction patterns would be of poor resolution. Phase determination was also viewed doubtfully, and even if one found a way to phase the reflections, the resulting electron-density map would not be interpretable. Having successfully pursued the initial steps in crystallographic studies, we are now in a position to describe how some of

these problems have been overcome and how we believe the others can be solved.

A previous TIBS article¹ included a table describing the various crystal forms obtained until 1984. Those crystal forms which have now been fully or partially characterized are summarized here (Table I), and the current status is fully documented in Refs 2–9. It is worth noting that all the diffracting crystals are of ribosomal particles from thermophilic or halophilic bacteria, probably because ribosomes from these sources are rather stable and hardly deteriorate during preparation

and the long crystallization periods. The halophilic ribosomes present an extremely attractive system for crystallization, since they are stable and can be prepared as a homogeneous population at very high salt concentrations.

The current table contains a fair amount of information on the right-hand side, where the crystallographic (rather than the electron-microscopic) work is described. The column 'resolution' indicates the internal order of the crystals as reflected in their diffraction patterns with measurable reflections. Crystals of 50S ribosomal subunits from *Halobacterium marismortui* diffract to the highest resolution: 4.5 Å. Some diffraction patterns (of 50S subunits from *Bacillus stearothermophilus*) give indications of an even higher internal order. Occasionally, oriented arcs and distinct spots extending to 3.5 Å, with spacings similar to those measured from gels of ribosomes and extracted rRNA¹⁰, have been detected on a few diffraction patterns of single crystals or of samples containing many microcrystals^{2,7}. So far all usable crystals have been obtained from functionally active particles. Moreover, in contrast to the natural instability of single ribosomes, the crystalline material retains its integrity and biological activity for long periods. The preservation of activity in

Table I. Characterized three-dimensional crystals of ribosomal particles

Source	Crystal form ^a	Cell dimensions, (Å) determined by		Resolution	Comments	
		Electron microscopy	X-ray crystallography			
70S <i>E. coli</i>	A	340×340×590; P6 ₃				
70S <i>T.t.</i> ^b	M		524×524×306; P4 ₁ 2 ₁ 2	20 Å		
30S <i>T.t.</i>	M		407×407×170; P4 ₂ 2 ₁	7.3 Å	N, H ^c	
50S <i>H.m.</i> ^b	(1) P	310×350;	105°			
	(2) P	148×186;	95°	147×181;	13 Å	
	(3) ^d P	170×180;	75°	210×300×581; C222 ₁	4.5 Å	N, H
50S <i>B. st.</i> ^b	(1) A	130×254;	95°			
	(2) A	156×288;	97°			
	(3) A	260×288;	105°			
	(4) A	405×405×256;	120°			
	(5) A	213×235×315;	120°			
	(6) ^{d,e} A	330×670×850;	90°	360×680×920; P2 ₁ 2 ₁ 2	18 Å	N
	(7) ^{d,e} P			308×562×395; 114° C2	11 Å	N, H

^a A, M, P, AS = crystals were grown by vapor diffusion from low molecular weight alcohols (A), MPD (M), polyethylene glycol (P), or ammonium sulphate (AS).

^b *B.st.* = *Bacillus stearothermophilus*, *T.t.* = *Thermus thermophilus*, *H.m.* = *Halobacterium marismortui*.

^c N, H = crystallographic data collected from native (N) and derivated (H) crystals.

^d Same form and parameters for crystals of a complex of 50S subunits + tRNA molecule and a segment (18–20 mers) of a nascent polypeptide chain.

^e Same form and parameters for crystals of large ribosomal subunits of a mutant (-L11 protein) of the same source.

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the crystalline state may be related to the natural aggregation of eukaryotic ribosomes in quasi-periodic arrays within the cells when the organism is exposed to physiological stress. These arrays are believed to show the strategy nature chooses for storage of potentially active ribosomes, to enable instant reactivation when the harsh conditions are removed.

For effective crystallographic studies, a constant supply of crystallizable material is essential. Suitable sources are those from which one can reproducibly obtain high-quality ribosomes in sufficient amounts for refinement of the crystallization conditions as well as for the production of large crystals needed for data collection. Currently, crystals can be grown from virtually all preparations of active particles mentioned in Table I. However, due to the intricate nature of particles, the exact conditions for the growth of large, well ordered crystals still must be varied for each ribosomal preparation. For example, preparations of the 50S subunits from *B. stearotherophilus* which yield good alcohol-grown crystals (crystal

form 6 in Table I) also lead to high-quality crystals when polyethylene glycol (form 7) is used. Thus, the basic requirements for crystallization depend more on the procedures for the preparation of the ribosomal particles than on the choice for the crystallization agent.

Despite extensive attempts to crystallize the ribosomal small subunits, crystals were obtained only recently⁴⁻⁸, probably because of the inherent instability and flexibility (for review see Ref. 11) of these particles. An insight into the comparative rigidity of the two ribosomal subunits was gained during studies aimed at the determination of the extent of protection provided by the ribosome to newly synthesized polyproline chains against proteolysis (see below). The reaction mixture contained, among other compounds, 70S ribosomes from *E. coli* and a commercial preparation of enzymes, including prolidase. At the end of the experiment, large variations in the resistance of the two subunits to proteolytic enzymes were observed. The 50S subunits remained intact, whereas the 30S subunits completely disintegrated (Evers and Gewitz, pers. commun.).

Cryo-biocrystallography

The crystals of ribosomal particles diffract X rays so weakly that intense synchrotron radiation is essential for crystallographic studies. This super-power beam is generated as a by-product in accelerators where high-energy-particle experiments are carried out.

On exposure to X-ray radiation of any intensity, the crystals of ribosomal particles exhibit extreme sensitivity. The reflections of resolution beyond about 18 Å decay within the first few minutes of exposure at ambient temperature. For a long time, using 'traditional' methods, we were not aware of the existence of the higher resolution reflections. Consequently we mistakenly believed and published², that our crystals diffracted to much lower than their actual resolution. Furthermore, in a tedious experiment aimed at collecting data, 260 crystals had to be irradiated. These were aligned only visually, to save radiation time. Despite the large number of exposed crystals, only a partial diffraction data set could be constructed, containing substantial overlap, but because of the high mosaic spread, not even a single fully recorded reflection.

The development of a novel strategy

for data collection was therefore essential. Crystals were shock-cooled to cryotemperature (~ 85 K) and kept cold throughout data collection¹². Under these conditions the crystals acquired 'eternal life' and showed virtually no radiation damage even after days in the synchrotron X-ray beam (Fig. 1). As a result, complete data sets could be collected from individual crystals. Although the cryogenic treatment (termed cryo-biocrystallography) does not offer any improvement in resolution or in mosaic spread, for crystals of ribosomal particles it provides the only practical route to data collection found so far.

An iterative procedure for phasing

For the presentation of the three-dimensional structure of a crystalline compound by Fourier synthesis, all the reflections present in the diffraction pattern of the crystal need to be summed. Each reflection is a wave characterized by its direction, intensity and phase. What keeps it from being a trivial computational problem is the fact that the directions and amplitudes of the reflections can be measured, whereas phases cannot be directly determined. Thus, even when a macromolecule of average size is studied, the determination of the phases involve indirect approximations.

Because of the extreme complexity of the ribosomes, an algorithm for iterative determination of phases has been developed (Fig. 2). At the initial stages of phasing, low-resolution information may be obtained by placing approximate models in the crystallographic unit cell. A search for best positioning based on the agreement between the calculated with the observed intensities will follow, similar to the commonly used 'molecular replacement method', in which an envelope of a protein molecule serves as the starting approximate model. With this aim in mind, models of ribosomal particles have been reconstructed using two-dimensional sheets viewed by electron microscopy.

The enormous size of the ribosome, which is an obstacle to crystallographic studies, is advantageous for electron microscopy. Tilt series of ordered two-dimensional arrays of 50S subunits¹³ and of 70S ribosomes¹⁴ were used for Fourier synthesis which led to low-resolution models at 30 and 47 Å, respectively (Fig. 3). These reconstructed models are of volumes which accord well with those measured by

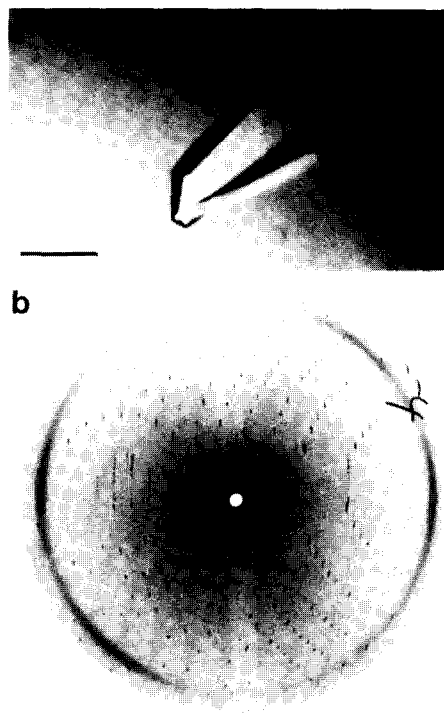


Fig. 1. (a) Crystals of 50S subunits of *Halobacterium marismortui* grown as described in Ref. 3. Bar = 0.2 mm. (b) A 1° rotation pattern (recorded on film) of a crystal similar to the one shown in (a) but soaked in a solution containing the components used for its growth and 18% ethylene glycol. The pattern was obtained after 25 h of irradiation at -180°C with synchrotron X-ray beam (X11 port at EMBL/DESY). Wave length: 1.488 Å. Exposure time: 7 min. Crystal to film distance: 205 mm. Resolution: 4.5 Å.

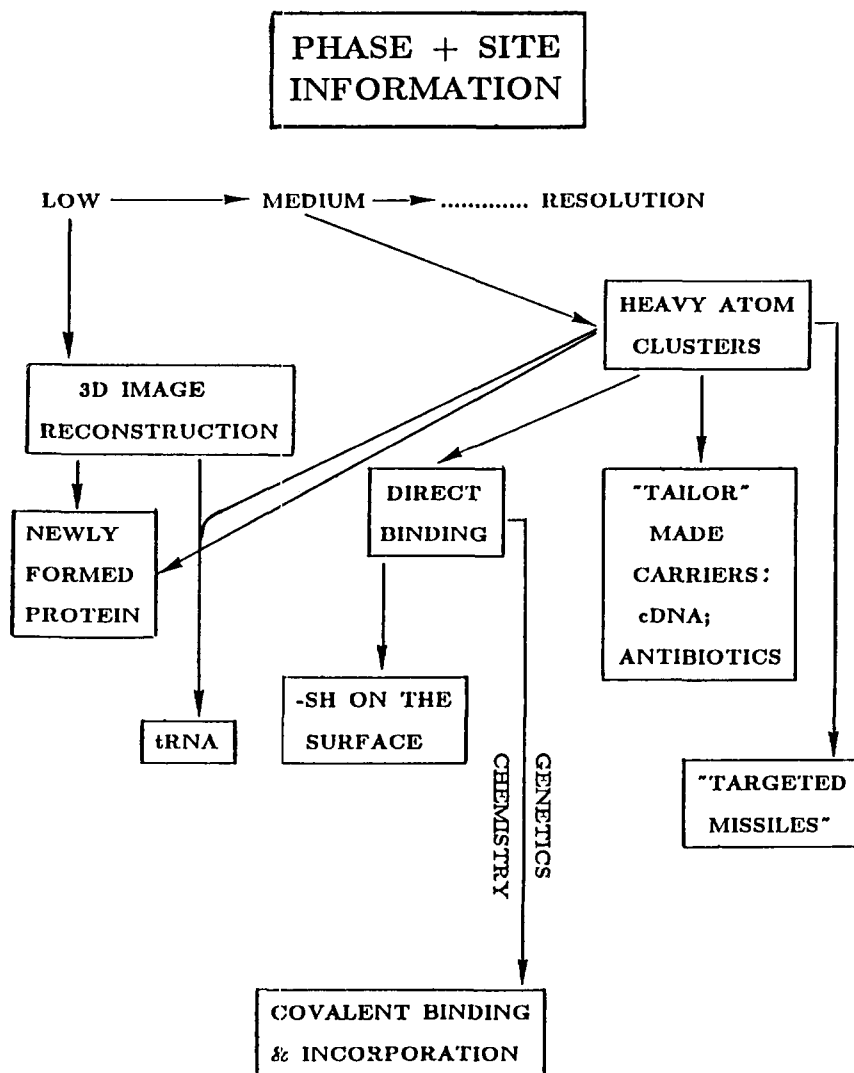


Fig. 2. A flow-chart of the algorithm for iterative phasing, showing how information obtained by different methods (genetic, biochemical, organometallic, crystallographic, electron-microscopical and by image reconstruction), can be used for the determination of phases together with the location of specific sites.

other physical methods or calculated from the known compositions of ribosomes¹⁵. The available biochemical information, leads us to suggest functional roles to some of the features detected by these reconstructions.

A void and a tunnel

A significant portion (15–20%) of the volume within the envelope of the ribosome is an empty space. This void is large enough to contain the components involved in protein biosynthesis and thus may provide the site for the actual process. Another prominent feature is a tunnel, about 100 Å in length and up to 25 Å in diameter, which spans the 50S subunit (Fig. 4). It has been known for more than twenty years that the growing polypeptide chain is protected by the ribosome from enzymatic digestion, until the nascent protein is large enough to fold rigidly and protect itself (for examples see Refs 16,

17; also summarized in Ref. 13). The tunnel may well provide the protected exit path of the growing polypeptide chain: it originates at the presumed site of protein biosynthesis, terminates at a location which appears compatible with that assigned for the 'exit' of the nascent chain by immuno electron microscopy¹⁸, and its diameter and length are large enough to accommodate a peptide of 25–40 amino acids of any sequence, at any conformation.

During the last few years the procedures for preparation of samples as well as of image reconstruction have been improved. Consequently, models of ribosomes which show reasonable detail have recently become available. A tunnel in the large ribosomal subunit was first reported in 1986, when 80S ribosomes, packed in two-dimensional arrays were reconstructed¹⁹. Most recently, a similar feature was located when isolated 70S ribosomes from

E. coli were subjected to related reconstruction studies²⁰. The tunnel was clearly observed in all the reconstructions of 50S particles¹³ but is somewhat less well resolved in those of assembled ribosomes^{14,19,20}, probably because their tunnels are partially filled with nascent protein chains.

The possible location of the exit tunnel stimulated a series of experiments aiming at investigating the nature of this path as well as providing a new tool for determination of phases. It was found that newly synthesized chains of polyphenylalanine bind readily to 50S subunits from *E. coli*²¹, *B. stearothermophilus* and *H. marismortui*²², indicating a degree of hydrophobicity at the walls of the tunnel. Also, despite the excess positive charge of the ribosomal proteins, a newly formed chain of polylysine could be firmly bound to the large subunits of *B. stearothermophilus*²², hinting that the walls of the exit tunnel are rich also in RNA. A consequence of these experiments was the crystallization of complexes of 50S subunits with a tRNA molecule and a short polypeptide fragment⁹. These may be used for subsequent attempts to derive phases (see below and Fig. 2).

The ability of the ribosome to protect the newly formed protein is not sequence dependent. In addition to naturally occurring proteins^{16,17} (as well as polylysine and phenylalanine



Fig. 3. The reconstructed models of 70S ribosome (top) and 50S subunit (bottom). Bar = 20 Å.



Fig. 4. The outline of the model of the 50S subunit, showing the entrance (T) to and the exit (E) from the tunnel. Bar = 20 Å.

mentioned above), sequences rich in proline, obtained by coding with poly (C) or random polymers of (C,A) or (C,U), were also found to be protected by the ribosome (Evers and Gewitz, unpublished). The various polypeptide sequences differ in their affinities for ribosomes. Thus, polyproline whose rate of synthesis is much lower than that of other polypeptides, adheres to the tunnel most firmly, perhaps owing to its rigid conformation. It was recently shown that it is possible to incorporate a rather bulky group (biotin) into the nascent chain²³. The diameter of the tunnel is large enough to accommodate such a group.

Immuno electron microscopy has recently shown that the N-terminus of a nascent chain can be detected on two patches on the ribosomes²⁴. One is close to the subunit interface, and the

all shape of the particle and on the direction of the exit tunnel (Fig. 5). This was followed by a computerized refinement. There are two regions in which the model of the large subunit and its assigned location within the reconstructed 70S ribosome show a rather poor fit. It has yet to be determined whether this is a consequence of the low resolution of the reconstructed models, or whether it reflects cooperative conformational changes occurring upon association of the two subunits.

We have assumed that the remainder of the 70S ribosome represents the small subunit. This model (Fig. 6) is similar to the model of the small subunit obtained by investigating single particles by electron microscopy¹⁵. However, isolated 30S subunits appear somewhat wider than those reconstructed within the 70S ribosomes in

second is at the other end of the particle, hinting that the N-terminus is exposed when either 2–6, or more than 40 amino acids have been synthesized. It is conceivable that during the initial stage of protein biosynthesis, the first few amino acids of the newly formed protein may not be fixed in space. Only if the growing chain finds its way into the tunnel, does the process of protein biosynthesis continue. This may explain why only 40–50% of any given population of ribosomes are active in protein biosynthesis, whereas almost all bind tRNA (Rheinberger and Nierhaus, submitted).

A possible site for mRNA – tRNA interaction

A portion of the 70S ribosome was identified as the large subunit by a visual correlation, based on the over-

the orientation dictated by the interparticle contacts. The broadening of the isolated subunits may well be caused by contact with the flat electron microscope grid. The difference between the isolated 30S subunit and that reconstructed within the crystalline 70S ribosomes may also reflect conformational changes occurring upon association of the two subunits.

The small and the large ribosomal subunits are well separated in all reconstruction studies of whole ribosomes^{14,19,20}. However, the void between the subunits, clearly resolved in our studies (Figs 3, 5 and 7), is less well defined in the reconstruction of the two-dimensional sheets of 80S ribosomes¹⁹ and of the single 70S ribosomes²⁰. This might be due to the lower internal order of the arrays of the 80S ribosomes¹⁹, as well as to flattening and/or collapse of the single 70S particles, investigated at their most frequent orientation on the electron-microscopy grid²⁰.

In the two dimensional arrays of the 70S ribosomes, regions rich in RNA can be detected using uranyl acetate as a partial positive stain. Of special interest is a region located on the 30S which contains a groove. In accord with biochemical and 'model-building' studies²⁵, we assigned this groove as the presumed mRNA binding area. The spatial relationship between this groove and the tunnel would accommodate a molecule of tRNA, reading at one end the codon from mRNA, and participating at its CCA end, in formation of a polypeptide chain (Fig. 7). This tRNA molecule is at a position permitting further interactions with the surface of the ribosome at its internal empty space. These interactions may account for the non-cognate ribosome-tRNA recognition. The void between the two subunits is large enough to accommodate at least two molecules of tRNA as well as other components (i.e. elongation factors) which take part in protein biosynthesis.

Combining genetic, biochemical and metalorganic procedures

The most common method for estimating phases in crystallography of biomacromolecules is that of multiple isomorphous replacement (MIR). Phases are determined from at least two derivatives containing heavy atoms. The crystals of the derivatized compounds have to be isomorphous to the native ones and the additional electron-dense group must be located

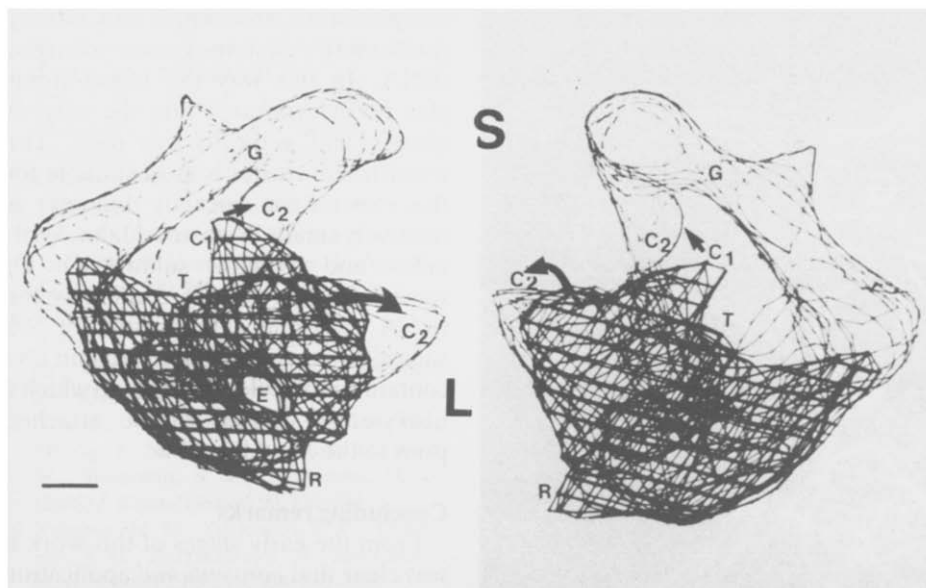


Fig. 5. Superpositions of two views of computer graphic displays of the outline of the reconstructed models of the 70S ribosome (in lines) and of the 50S ribosomal subunit (drawn as a net). L and S indicate the 50S and the 30S subunits, respectively. G represents a groove, rich in RNA, in the small (30S) subunit. T shows the cleft and the entrance to the tunnel, and E the presumed exit site of the nascent chain, both on the 50S subunit. The extra density R is a consequence of the different resolutions at which the two models were reconstructed (30 and 47 Å resolution). C₁ and C₂ indicate regions of extra density in the models of the 50S subunit and of the 70S ribosome, respectively. These may be a result of conformational changes upon association. The arrows indicate a possible direction for a cooperative movement of these regions. Bar = 20 Å.

at the same site in every unit cell of the crystal. Useful derivatives may be obtained by soaking native crystals in a solution of the heavy-atom compounds, by co-crystallization of the protein with a heavy-atom compound, or by covalent binding of a heavy atom to specific sites prior to crystallization. The latter approach requires sophistication and long preparation periods but is bound to be more suitable for ribosomes since it may reveal the locations of selected sites (Fig. 2). Also, it eliminates non-specific attachment of the clusters to multiple sites on the ribosomes, which might complicate phase determination or make it impossible.

No matter which of these approaches is taken, it is clear that because of the enormous size of ribosomes, extremely heavy and dense chemical moieties have to be used. Clusters, which contain a core of several metal atoms linked directly to one another, comprise systems of especially high electron density. Monofunctional reagents of two clusters: undecagold (mol. wt 6200) and tetrairidium (mol. wt 2100) atoms were prepared (Weinstein, Jahn, Wittmann and Yonath, submitted). The functional moiety, a maleimido group is relatively short lived (~ 6 h), and at pH 5.4 it reacts selectively with sulfhydryl groups. We could

determine conditions under which no more than two -SH groups are readily available on the surface of any ribosomal species crystallized by us. Thus, soaking of native crystals in solutions of the reactive clusters, named by us 'tar-semi-quantitative attachment.

Specific binding was achieved either by direct interaction of a heavy-atom cluster with a chemically active group on the surface of the ribosome prior to crystallization, or by binding of a cluster to an isolated ribosomal component which was subsequently reconstituted into the ribosome.

Genetic procedures for obtaining ribosomes of *B. stearothermophilus* in which one protein is missing were developed by us, based on previous studies on *E. coli* (for review see Ref. 26). Ribosomes from a mutant which lacks one ribosomal protein (BL11) in its large subunit have been isolated. The mutated subunits yielded crystals isomorphous with those of two forms of 50S subunits from the wild type^{7,9}. This shows that, for both crystal forms, the missing protein, BL11, is not involved in the network of crystal contacts and that its absence does not induce gross conformational changes. Similar attempts to crystallize 50S particles lacking protein BL12 failed, perhaps because its absence influences the over-

all structure of the ribosome²⁷.

Protein BL11 has one sulfhydryl group which is accessible on the surface of the 50S particle and is suitable for binding the clusters. Both the gold and the iridium clusters were covalently and quantitatively bound to the isolated protein, which was subsequently incorporated into the core of the mutated particles to form cluster-labeled 50S subunits. These were crystallized isomorphously with the native ones⁹.

Protein BL11 is an example of a ribosomal component having a different conformation in isolation than *in situ*.

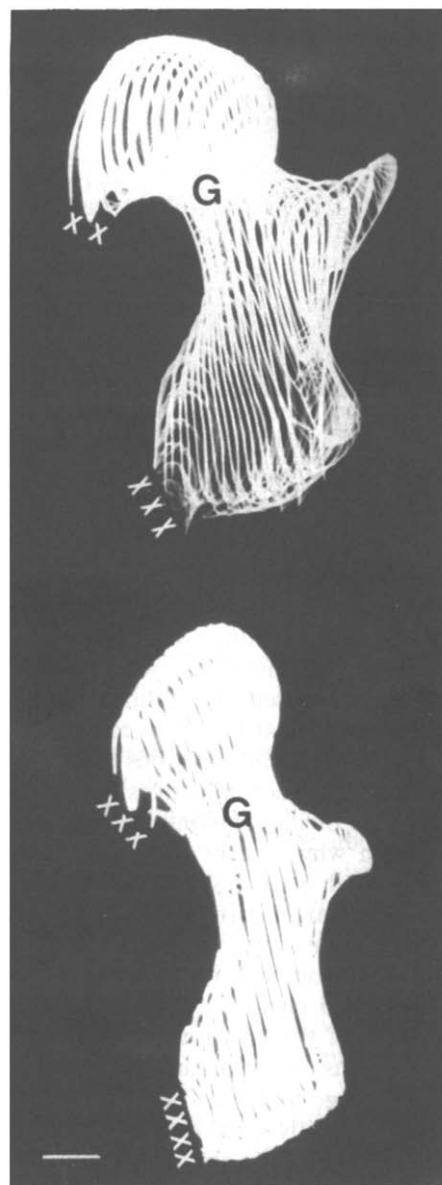


Fig. 6. The outline of the 30S ribosomal subunit obtained by subtracting the 50S subunit from the 70S ribosome. The interaction regions with the 50S subunit are indicated by (XXX). Bar = 20 Å. Two views are shown: the bottom is related to a view taken perpendicular to the plane of the sheet, the top view was taken from the side, at an angle of about 35°.



Fig. 7. A 'model-built' molecule of tRNA (in yellow) integrated into the reconstructed model of the 70S ribosome so that its anticodon is attached to the mRNA binding site at the groove G on the 30S subunit and its CCA-end is near the entrance to the exit tunnel T. The 50S subunit is superimposed as a net. Bar = 20 Å.

In solution its -SH group is reactive only under denaturing conditions (Weinstein, Jahn, Wittmann and Yonath, submitted), whereas this group is exposed on the surface of the ribosome (see above). It is conceivable that the ribosome provides a network of contacts which influences the conformations of its components. This may explain why, except for a few cases, ribosomal particles crystallize more readily than their isolated proteins.

Quantitative removal of a few given proteins from the ribosome can also be performed chemically, by a stepwise change of the concentrations of additives (salts and/or organic materials) to the storage buffers. These procedures are frequently employed in ribosomology of eubacteria and were adopted by us for large-scale preparation of a few proteins, among them BL11²⁸. In order to apply a similar procedure to halophilic ribosomes we took advantage of our previous studies, showing that the stability, compactness and biological activity of halophilic ribosomes depend strongly on a delicate equilibrium

between the mono- and divalent ions in their media. It was found that there is a critical point at which these particles undergo significant conformational changes resulting in loss of activity and gradual unfolding²⁹. After initial difficulties it became possible to detach selectively a few ribosomal proteins from the 50S subunits of *H. marismortui*. These could subsequently be incorporated into the core particle.

The clusters may also be attached to tailor-made carriers with high affinities for specific sites on ribosomes, such as antibiotics or DNA oligomers complementary to exposed single-stranded rRNA regions³⁰. Since most of the interactions of such carriers are characterized biochemically, the crystallographic location of the heavy-atom compounds will be used not only for phase determination but also to reveal the location of functional sites on the ribosome.

A natural extension of our ability to crystallize complexes of 50S subunits with fragments of the nascent polypeptide⁹ will be to bind a heavy-atom

compound to an amino acid moiety (preferably cysteine) on charged tRNA. In this way the heavy-atoms may be incorporated into the nascent protein and mark its exit path. The tetrairidium cluster is most suitable for this experiment since its diameter is relatively small (Weinstein, Jahn, Wittmann and Yonath, submitted). Of special interest in this context is the tRNA molecule. The crystals of 50S subunits bearing the nascent chain also contain a molecule of tRNA, to which a heavy-atom cluster can be attached prior to the crystallization.

Concluding remarks

From the early stages of this work it was clear that *conventional* application of X-ray crystallography to the determination of the structure of ribosomes will not be possible. Even after the first obstacles have been removed, the way to structure determination is still hampered by a large number of conceptual and technical difficulties. However, the use of genetic procedures together with the vast amount of available information concerning the function as well as the chemical and physical properties of ribosomes, should provide sophisticated tools to overcome some of the limitations of the traditional approach. The adoption of this attitude, combined with the 'quantum jump' achieved recently in instrumentation for X-ray crystallography, should lead to the determination of the three-dimensional structure of the ribosome.

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How do proteins recognize specific RNA sites? New clues from autogenously regulated ribosomal proteins

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Some ribosomal proteins which bind specifically to ribosomal RNA also act as translational repressors and recognize their encoding messenger RNAs. The messenger- and ribosomal-RNA binding sites for four of these proteins are now well defined, and striking similarities in primary and secondary structure are apparent in most cases. These 'consensus' structures are useful clues to the features proteins use to recognize specific RNAs.

There is a long list of RNA-protein interactions that are known to be important for gene expression and cell function. Many of these interactions appear in the important machinery needed to express proteins (e.g. spliceosomes and ribosomes), while others regulate steps in the pathway from gene to protein. These interactions raise the question of how proteins recognize specific RNA sites. Are some kinds of RNA structures (helices, loops, bulges, mismatches, pseudoknots) particularly favored as recognition features? Do proteins sense specific sequences, particular backbone conformations, or some combina-

tion of sequence and structure? To answer these questions the RNA features recognized by a variety of binding proteins must be compared. However, defining a protein-recognition site in an RNA can be tedious work: the nucleotides that form the recognized structure may be dispersed at several locations through a long RNA sequence. A useful way to identify such binding sites is to compare two or more RNAs which both bind to the same protein site. Any similarities in sequence or secondary structure then define a 'consensus' site and provide clues to sequences or structures detected by the protein.

Finding an RNA consensus site

For some ribosomal proteins consensus RNA binding sites can be derived in

two different ways. Homologous rRNAs from evolutionarily distant organisms will sometimes bind the same protein; only the conserved features can be important for recognition¹. Even more informative are the ribosomal proteins which bind both rRNA (in ribosome assembly) and to mRNA (as translational repressors). In these cases two RNA-binding sites with very different functions can be compared, and similarities between them should be limited to essential features recognized by the protein*.

The autoregulation hypothesis

The translational repressor activity of some *E. coli* ribosomal proteins was noticed by several groups about ten years ago^{2,3}. Most ribosomal protein operons contain one protein which behaves as a repressor: if protein synthesis outstrips rRNA synthesis, the accumulating pool of free repressor will bind to the mRNA and repress translation of all the ribosomal proteins in that operon.

Translationally regulated ribosomal protein operons are listed in Table I, along with their identified repressor proteins. In most of these cases the repressor protein has been shown specifically to recognize a single target site in

*The term 'homologous' is restricted to RNAs sharing an evolutionary relationship, while 'similar' describes two RNA structures with analogous function but independent origin.

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