Hollows, voids, gaps and tunnels in the ribosome

Ada Yonath and Ziva Berkovitch-Yellin

Weizmann Institute, Rehovot, Israel and Max-Planck-Laboratory for Ribosomal Structure, Hamburg, Germany

Renewed discussion of concepts hitherto regarded as sacred icons has made the past year an exciting period in the study of the translation apparatus. In particular, reconstructed models of the ribosome have revealed key structural features, associated with empty or partially filled hollows, which, in turn, have stimulated functional assignments of varying degree of objectivity.

Current Opinion in Structural Biology 1993, 3:175–181

Introduction

Recent years in prokaryotic ribosome research may be characterized by major conceptual revisions, coupled with attempts at resolving ambiguities (e.g. [1,2,3•,4•]) and the introduction of substantial spatial rearrangements [5]. Thus, the ribosomal functions can no longer be attributed solely to the ribosomal proteins [6••], and the once favorable research object, the *Escherichia coli* ribosome, is gradually being substituted by more robust ones from thermophilic or halophilic bacteria [1,7••,8•]. In addition, compelling evidence has emerged indicating that polyphenylalanine, the product of the universal assay for ribosomal activity, may emerge from the ribosome by a path dictated by its special chemical nature, different from that of natural proteins, thus poorly representing the nascent proteins [9••]. Furthermore, the ribosome, traditionally conceptualized as a compact network, was found to contains a variety of hollows.

Reconstructing models for ribosomes

For over three decades, electron microscopy (EM) has been the method of choice for viewing ribosomal particles, despite its inherent subjectivity and the probable shape distortions which are introduced by the microscope vacuum and by the contacts with the flat microscopical grids. These efforts have proved to be both beneficial and destructive; they stimulated numerous sound functional experiments but at the same time introduced structural conceptions, frequently based on subjective interpretations, which lead to endless arguments about details of a level far beyond that justified by the nature of the experimental input.

There is no doubt that a molecular model for the ribosome will eventually be obtained by X-ray crystallography at reasonable (2.9 Å) resolution [7••,8•,10•]. Until then, and for assisting the initial phasing of the crystallographic data, low-resolution models have been reconstructed from tilt series of crystalline arrays [7••,8•,11,12]. This reconstruction procedure is fundamentally different from other EM methods in its inherent objectivity. Thus, diffraction patterns and not real images provide the initial data, and the reliability of the resulting models is determined by well established crystallographic, rather than visual, criteria. Furthermore, as the two-dimensional arrays are held by crystalline forces, the distortions introduced by the flat EM grids are reduced or even eliminated.

An appropriate example is given by the independently reconstructed models (at 27–28 Å resolution) from negatively stained crystalline arrays of 50S subunits from *Thermus thermophilus* (Y Fujiyoshi, personal communication) and *Bacillus stearothermophilus* (Fig. 1) [7••,11]. In both, the extended arm, a prominent feature in conventional models [13], is rather short. This dramatic deviation from the characteristic view was frequently not accepted, although the extended arm is known to be rather flexible. It is furthermore conceivable that the inherent flexibility of this arm, namely its ability to fold towards the main body of the subunit, enables the crystallization, as it is generally assumed that extremely asymmetric conformations are less favorable for crystallization. In addition, the substantial extension of this arm may result, in part, from its flattening on the EM grid. Indeed, this arm appears shorter also in the random-conical reconstructed model from non-crystalline *E. coli* 50S subunits embedded in amorphous ice, which are thus supposed to be free from contacts with the grid (Fig. 1; M Radamacher, S Srivastava, J Frank, abstract 19, European EM Conference, Granada, August 1992).

The advantages of the random-conical reconstruction procedure are noteworthy. This procedure is based on averaging the views of single particles at preferred orientations [14••], and hence is independent of the avail-

Abbreviations

EM—electron microscopy; **Ribosome-92**—The International Conference on the Translation Apparatus, November 1992, Berlin, Germany.

ISSN 0959-440X

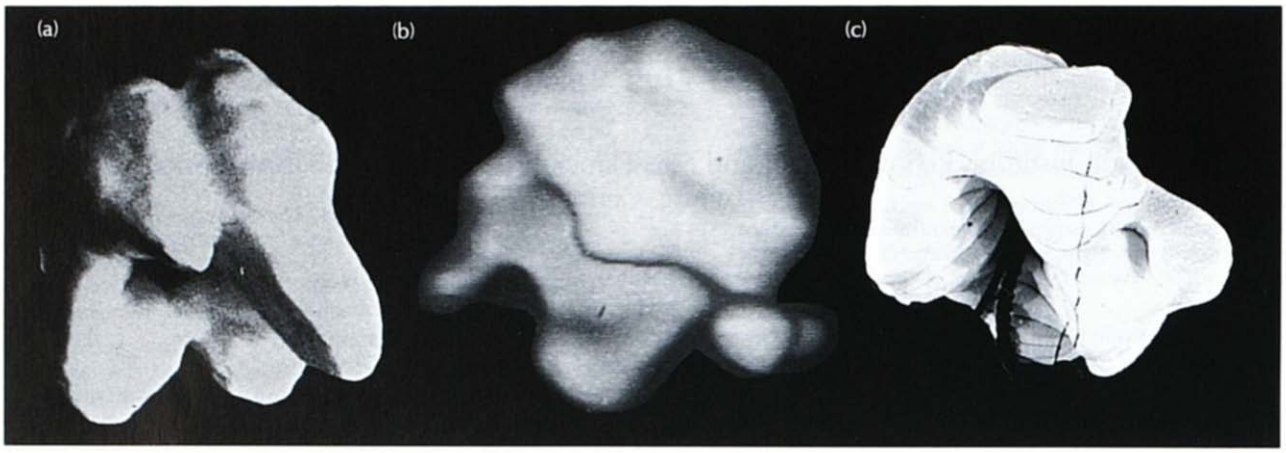

Fig. 1. Reconstructed models of the 50S subunit from **(a)** *B. stearothermophilus* [7••,11], **(b)** *E. coli* (M Radamacher, S Srivastava, J Frank, abstract 19, European Electron Microscopy Conference, Granada, August 1992) and **(c)** *T. thermophilus* (Y Fujiyoshi, personal communication). **(a)** and **(c)** were obtained from tilt series of crystalline arrays, **(b)** from ice-embedded single particles by the random-conical procedure. Note that none of the three models possesses a significantly extended arm.

ability of crystalline samples. As particles embedded in amorphous ice may assume many orientations, no tilting is required, and thus the reconstruction is not limited by the restrictive tilting range of the electron microscope, a major disadvantage in studies utilizing crystalline arrays. However, as the random-conical procedure requires subjective selection of images, the resulting models are only semi-objective.

Hollows, cavities, tunnels and voids

Although image reconstruction procedures are not free of limitations, they are clearly superior to conventional EM methods, as demonstrated by the conclusive detection of key features, otherwise unobserved, associated mainly with internal vacant or partially filled hollows. As early as 1983, a hole was detected within a model 50S subunit obtained by the optimized series expansion reconstruction method [15]. However, the concept of a ribosome containing holes, cavities, tunnels, gaps and voids became commonly accepted only recently.

The exit path of the nascent chain

More than two decades ago, as well as in recent years, biochemical studies showed that the latest synthesized 40–60 amino acids of the nascent proteins are not exposed to proteolytic enzymes, antibodies or similar substances added to the reaction mixture. Therefore, it was thought that this stretch is masked by the ribosome (for a review, see [7••]). A feature that may account for these observations was first seen as a narrow elongated region of low density in images reconstructed at very low (60 Å) resolution from 80S ribosomes of chick embryos [16]. Later, a tunnel of dimensions and location suitable to accommodate the nascent protein was clearly observed in images of 50S subunits of *B. stearothermophilus* [11] and *T. thermophilus* (Y Fujiyoshi, personal communication), reconstructed from negatively stained crystalline arrays at 27–28 Å. A similar feature could also be identified within the density map obtained from neutron-diffraction data at 30 Å resolution, collected from single crystals of 50S subunits from *Haloarcula marismortui* [17•]. It is noteworthy that two internal holes were detected in the random-conical reconstructed models of the 50S and 70S particles from *E. coli* [18,19••]. So far no role has been assigned to them. Visual examination indicated however that, at a suitable contour level, these holes may be connected to form a comparable tunnel [7••]. Interestingly, at this contour level, the length of the extended arm appears somewhat shorter than in the conventional models, closer to that observed for particles embedded in amorphous ice or by reconstruction from crystalline arrays (Fig. 1).

Subsequent computational [20•] and biochemical (described below and in [7••]) experiments were designed to analyze the width and the selectivity of the tunnel. Chemical and immunological probes, incorporated into the growing polypeptide chain, showed that the extending chain progresses from an rRNA-rich to a hydrophobic environment [9••], in agreement with earlier suggestions (for review, see [7••]), and that all investigated natural proteins, as well as polyserine and polyalanine, are indeed masked by the ribosome (AE Johnson, KS Crowley, GD Reinhart, abstract 10.5, Ribosome-92; B Hardesty, W Picking, OW Odom, abstract 7.6, Ribosome-92) [7••,9••] whereas polyphenylalanine and polylysine may choose different paths. (Ribosome-92 denotes The International Conference on the Translation Apparatus, held in Berlin, Germany in November 1992.)

The MS2 coat protein is a most suitable candidate for studying the fate of nascent chains, not only because of the availability of its mRNA but also because in its native conformation its amino terminus points towards the solution [21]. By attaching fluorescent and/or antigenic moieties to the amino terminus, it was found that a length of ~120 amino acids was needed for exposure to the probe's antibody, whereas a 60-amino-acid sequence was

sufficient for detection by the corresponding Fab fragment (B Hardesty, personal communication). Furthermore, measurements of the energy transferred between labels attached at various positions along the nascent protein indicated proximity between the amino-terminal and other positions along the chain (e.g. Cys47), thus suggesting initial folding within the ribosome (B Hardesty, W Picking, OW Odom, abstract 7.6, Ribosome-92), presumably at a folding domain close to the exit site, which may be accommodated within the funnel- (or delta-) shaped region observed at the end of the exit tunnel (Fig. 2) [11].

The separation between the ribosomal subunits

An intersubunit free space, estimated to occupy 15–20% of the total volume of the ribosome, was first detected in models reconstructed from crystalline arrays of 70S ribosomes from *B. stearothermophilus* [7••,8•,12]. A similar feature was recently revealed also in the model reconstructed from single 70S ribosomes from *E. coli* by the random-conical procedure (Fig. 3) [19••]. Phylogenetic studies have provided further evidence for a wide opening, showing no support for direct base pairing between the two subunits [22•]. Interestingly, even the tight intersubunit rRNA clustering, suggested by crosslinking and fingerprinting studies, could be accommodated, after slight rearrangements, into the limited area available for the subunit interface in the reconstructed models [23].

Interpretations of the reconstructed models

Two fundamentally different approaches have been followed in interpreting the models of the 70S ribosome. The first, which was applied to the reconstructed models from crystalline arrays, is based almost solely on similarities in specific features observed in several reconstruction experiments. It was found that all models of 50S subunits reconstructed from crystalline arrays are almost undistinguishable (Fig. 3) [7••,11], whereas those of the 70S ribosome are of comparable dimensions and share common features but differ in bulkiness (Fig. 3). The interpretation of the most detailed and therefore most restrictive thinnest ('thin') model (Fig. 3) provides the basis for further functional assignments, which were found to fit even the bulkiest ('thick') model (Fig. 3). The 50S subunit was fitted to the 70S ribosome according to its shape and to the direction of its longest tunnel (t1 in Fig. 3). The part left for the 30S subunit contained a groove, which was assigned as the path of mRNA. A tRNA molecule, in its crystallographically determined conformation, was model-built into the intersubunit void with its anticodon near the mRNA groove and its CCA terminus close to the tunnel (Fig. 3) [7••,8•]. Steric considerations showed that this void is spacious enough to accommodate up to three tRNA molecules together with other non-ribosomal components that participate in protein biosynthesis; thus, the void might provide the location for the various enzymatic activities of protein biosynthesis. The suggestion that the tRNA is positioned within the intersubunit space is further supported by a recent finding, showing that upon binding to 70S ribosomes, the "entire" tRNA molecule becomes inaccessible even to hydroxy radicals [24•].

Despite the speculative nature of these assignments, they have significantly advanced the current crystallographic studies, as complexes have been designed in which the intersubunit void should be fully or partially filled (containing charged tRNA molecules and mRNA analogues). These yielded crystals of a quality higher than that of native 70S ribosomes [7••,8•]. The prospects for determining the structure of these complexes are rather high, as an undecagold cluster can be quantitatively bound to the tRNA molecule without hampering its binding to ribosomal particles [25].

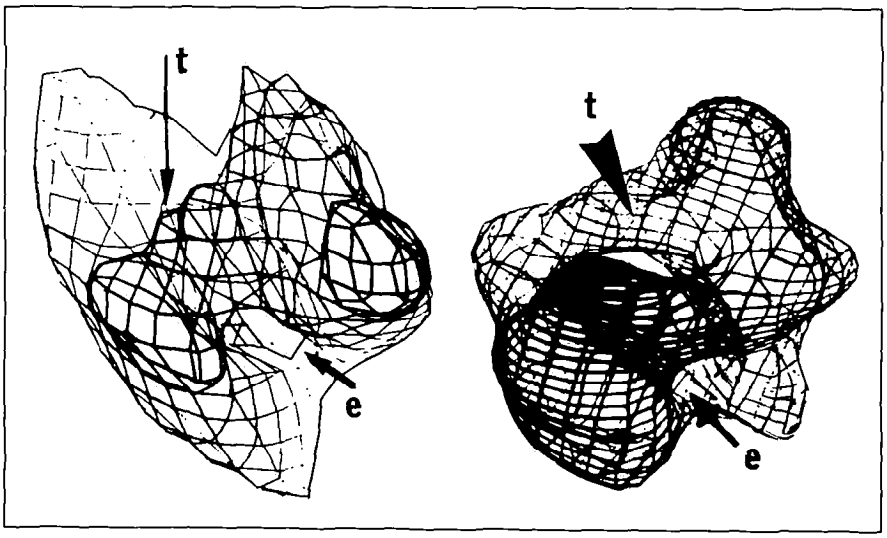

Fig. 2. A computer graphic display of the reconstructed 50S subunit from *B. stearothermophilus* [7••,11]. Two perpendicular views are shown. Labels identify the entrance to the internal tunnel (t) and the funnel-shape end of the tunnel (e), which may provide the site for the initial folding of nascent chains. Adapted from [11].

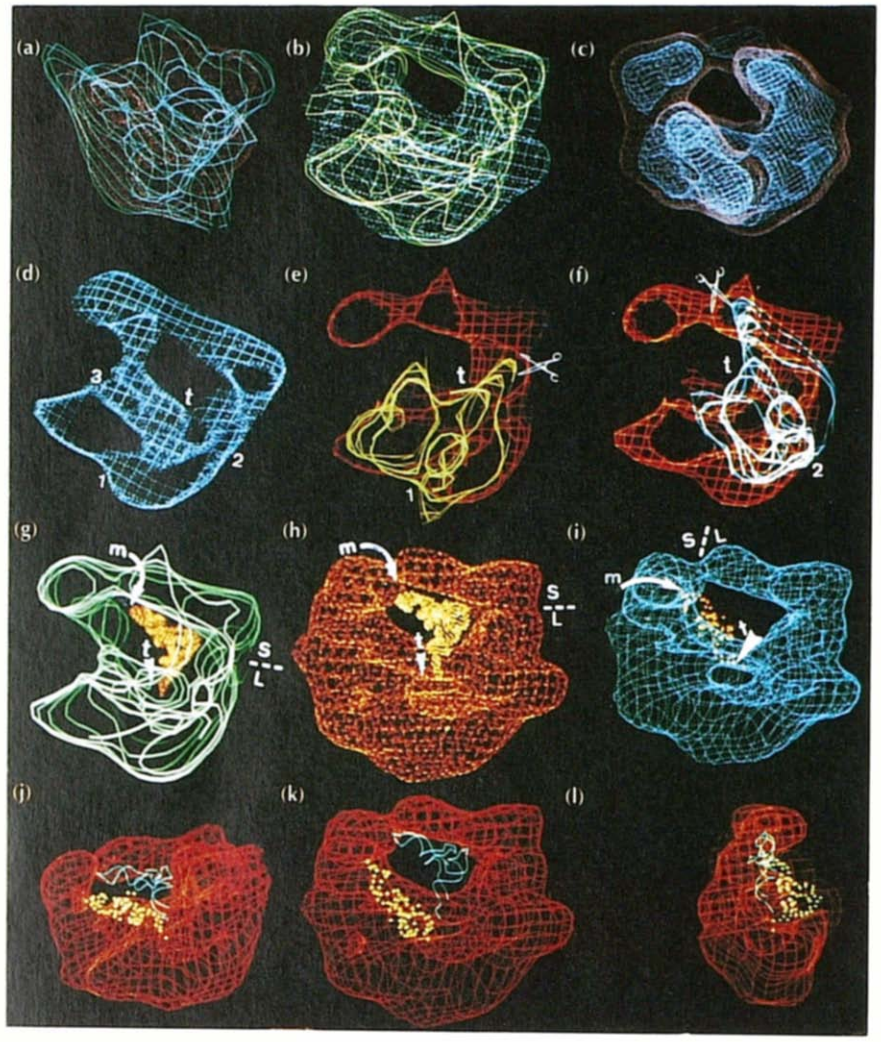

Fig. 3. The two alternative assignments in the reconstructed 70S ribosomes. First row **(a–c)**: **(a)** Superposition of the two most deviating models of 50S subunits, reconstructed from crystalline arrays [7••,11]. **(b)** Superposition of the two most deviating models of the 70S ribosome [7••,12], reconstructed from crystalline arrays (the 'thick' is shown as a blue net, the 'thin' in yellow lines). **(c)** The model of the 70S ribosome reconstructed by the random-conical procedure [19••]. Second row **(d–f)**: t shows the entrance to the tunnels detected in the 50S subunit; 1, 2 and 3 show the other ends of the tunnels (1 being the longest one). The scissors point at the assumed contact region between the 30S and the 50S subunits. All views show a slice of about 50 Å in depth of the 'thin' 70S ribosome. **(d)** The three internal tunnels revealed in the 'thin' 70S ribosome. **(e,f)** Superposition of the reconstructed models of the 50S subunit and the 'thin' 70S ribosome (shown as an orange net), according to the original alignment, based on the direction of tunnel 1 **(e)**, and according to the alternative interpretation, based on the direction of tunnel 2 **(f)**. Third row **(g–i)**: The broken white lines show the approximate directions of the interface between the S (small = 30S) and L (large = 50S) subunits. t is the entrance to the tunnel. The presumed positions of the bound part of the mRNA chain are marked by arrows and the letter m. The model-built tRNA molecule according to the original interpretation is shown in the 'thin' **(g)** and 'thick' **(h)** models. **(i)** The position of the tRNA molecules according to the alternative interpretation. Fourth row **(j–l)**: Three views of the 'thick' 70S ribosome, with its intersubunit free space containing two tRNA molecules; the one shown as a yellow chain of plus signs (+) is placed according to the alternative interpretation.

Assignments in the random-conical reconstructed model

The random-conical reconstructed model was interpreted according to a second approach. Hints for this interpretation were taken from the exterior shape of the 70S reconstructed ribosome and from its internal mass distribution, as high-density regions were interpreted as rich in rRNA [19••]. First, some prominent contours within the 70S ribosome were matched with the 'consensus' [13] shape of the 30S subunit. The rest were compared with features such as the 'L1 arm' and the 'stalk base', characteristic of the shape of the 50S subunit, viewed in the electron microscope [13] or reconstructed by the random-conical procedure at a resolution comparable to that of the 70S ribosome, i.e. 40 Å [18].

It is rather surprising that this interpretation of the 70S ribosome was anchored on the shape of the 30S subunit, as the only available models for it were obtained by conventional EM, the subjective nature of which was the motivation behind the use of the more sophisticated and objective reconstruction procedures. Furthermore, the 30S subunit is rather flexible and known to undergo substantial conformational changes upon association with the 50S subunit; thus, its shape within the 70S ribosome may significantly differ from that of the isolated subunit. It is also unclear why the rather reliable model for the 50S subunit reconstructed from crystalline arrays at a resolution of 28 Å [11], i.e. higher than that of the 70S ribosome (40 Å) [19••], was not used for the interpretation.

The potential of the random-conical protocol has been demonstrated by its ability to detect ligands complexed with ribosomes. Thus, the eukaryotic initiation factor 3 was found to bind to the 40S subunit away from its assumed interface with the large subunit [26], and tRNA, to which the complex biotin–avidin was bound, was detected on 30S subunits, pointing away from the particle (A Verschoor *et al.*, abstract 7.4, Ribosome-92).

The validity of the assignments in the random-conical reconstructed model was assessed by comparisons of specific rRNA-rich regions within the 70S ribosome with otherwise derived corresponding features [19••], some of which are of only marginal relevance to the overall structure of the 70S ribosome. Thus, despite the inaccuracy and the variability of the shapes observed for naked rRNA, and although they may not reflect the *in situ* conformation [27], an effort was made to detect similarities between them and features of the reconstructed model. In parallel, no obvious differences were detected in comparisons of the rRNA-rich features with the topologies of rRNA determined 10 years ago at 70 Å resolution in crystalline eukaryotic ribosomes [28]. Interestingly, features detected by phosphorous imaging in the rather flexible 30S subunits resembled the shape of the part identified as the 16S molecule in the random-conical model, whereas only very little similarity was found for the so assigned 23S component, the major component of the more rigid 50S subunit.

The provision of an apparently detailed model, coupled with the natural tendency for overinterpretation, has stimulated further experiments of a varying degree of sophistication, objectivity and maturation. Some of these have led to vigorous discussions, resembling those of the early days of ribosomal structural research (e.g. A Spirin, V Lim, R Brimacombe, abstract 5.5, Ribosome-92). Other efforts have been aimed at an accurate identification of rRNA cross-links at the subunit interface [23]. Furthermore, a super-detailed description has been proposed for the stereochemistry of the tRNA–mRNA interaction, utilizing the intersubunit void for the peptidyl transferase activity, which includes some unresolved major dilemmas, such as the locations and the size of several ribosomal proteins [29•].

Assessing the reliability of interpretations at low resolution

An exercise demonstrating the uncertainties involved in interpretations of globular and relatively undetailed models, even when only objectively determined features are involved, was recently reported [30]. As the shape of the random-conical reconstructed 70S ribosome [19••] is almost identical to those reconstructed from crystalline arrays (Fig. 3) [7••,8•,12], the latter were screened for various tentative assignments, all based solely on the external shape and the internal features revealed in the reconstructed model of the 50S subunit [11]. It was found that positioning the reconstructed 50S subunit in the thin 70S model at any location other than the original one (Fig. 3) [7••,8•], leaves hardly any density for the 30S subunit. In contrast, the thick model possesses sufficient density for the 30S subunit in almost any orientation, but not one orientation shows a convincing fit between the shape of the 50S and its assigned part within the 70S ribosome. An almost satisfactory fit was obtained by aligning the direction of the main 50S tunnel with one of the shorter tunnels observed in the 70S reconstructed model (t2 in Fig. 3). The resulting 30S subunit contains a groove suitable to accommodate the mRNA, in a position permitting a tRNA molecule to bridge the distance between it and the entrance to the tunnel (Fig. 3).

This exercise yielded several important lessons: the current models do not possess the level of details required for unequivocal positioning of the two subunits; the size and shape of the intersubunit void permits the accommodation of the components associated with protein synthesis in several sterically reasonable arrangements; and the assignment of this void as the site of protein biosynthesis does not critically depend on the accurate locations of the small and the large subunits.

Conclusions and prospects

The intensive activity in reconstructing models for ribosomes has led to impressive progress, despite occasional overinterpretations and even though it is clear that neither of the current models is of sufficient detail to provide unequivocal functional assignments.

Fig. 4. A monolayer of 50S subunits from *B. stearothermophilus*, obtained on a lipid–water monolayer (W Chiu, H Schmidt, TL Guan, T Arad, A Yonath, J Piefke, F Franceschi, unpublished data).

It is gratifying that all recent interpretations utilize the intersubunit void as the center of activity of the ribosome. This void, first reported in 1987 [12], was until recently either ignored or opposed. Regardless of the reliability of the different interpretations, the mere detection of the void has already led to a significant progress in the crystallographic studies, as it has stimulated the crystallization of complexes of ribosomes at defined functional states [7••,8•].

The impressive contributions of the reconstructed models to the development of the ribosome research show that the days of traditional EM are over, although studies of models obtained by these methods are still being undertaken and published [2,31–33]. It is also evident that models of a higher resolution, coupled with careful biochemical studies, are essential for more accurate assignments. Next to crystallography, cryo-temperature image reconstructions from unstained crystalline arrays is the most promising procedure to yield such detail. Preliminary efforts in this direction have already led to the growth of arrays of 50S subunits, which diffract to ~15 Å resolution (Fig. 4).

Acknowledgement

The writing of this review was supported by research grants from the United States National Institutes of Health (GM34360), the German Ministry for Research and Technology (MPBO 180), The German Space Agency (50QV 86061) and the Kimmelman Center for Bimolecular Structure and Assembly. A Yonath holds the Martin S. Kimmel Professorial chair at the Weizmann Institute.

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. YONATH A, FRANCESCHI F: **Structural Aspects of Ribonucleoprotein Interactions in Ribosomes.** *Curr Opin Struct Biol* 1993, 3:45–49.

2. SHATSKY IN, BAKIN AV, BOGDANOV AA, VASILIEV VD: **How Does the mRNA Pass Through the Ribosome?** *Biochemie* 1991, 73:937–945.

3. • RINKE-APPLE J, JÜNKE N, STADE K, BRIMACOMBE R: **The Path of mRNA through the *E. coli* Ribosome: Site Directed Cross-Linking of mRNA Analogues Carrying a Photo-Reactive Label at Various Points 3′ to the Decoding Site.** *EMBO J* 1991, 10:2195–2202.

See [4•].

4. • DONTSOVA O, KOPYLOV A, BRIMACOMBE R: **The Location of mRNA in the Ribosomal 30S Initiation Complex: Site Directed Cross-Linking of mRNA Analogues Carrying Several Photo-Reactive Labels Simultaneously on Either Side of the AUG Starting Codon.** *EMBO J* 1991, 10:2613–2620.

In these two papers [3•,4•], the authors document their attempts at pinpointing the mRNA-binding mode by site-directed cross-linking. Despite the promising potential of this approach, it has so far led to somewhat conflicting results. These publications may serve as an example of the current state of confusion, even when compared with similar studies performed in the same laboratory within the past three years.

5. DÖRING T, CREUER B, BRIMACOMBE R: **The Topology of the 3′-Terminal Region of *E. coli* 16S Ribosomal RNA; an Intra-RNA Cross-Linking Study.** *Nucleic Acid Res* 1992, 20:1593–1597.

6. •• NOLLER FH, HOFFARTH V, ZIMNIAK L: **Unusual Resistance of Peptidyl Transferase to Protein Extraction Procedures.** *Science* 1992, 256:1461–1469.

An almost conclusive proof for the peptidyl transferase function of rRNA.

7. •• BERKOVITCH-YELLIN Z, BENNETT WS, YONATH A: **Aspects in Structural Studies on Ribosomes.** *CRC Crit Rev Biochem Mol Biol* 1992, 27:403–444.

A detailed review, summarizing more than a decade of crystallography and three-dimensional reconstructions of ribosomes.

8. • YONATH A: **Approaching Atomic Resolution in Crystallography of Ribosomes.** *Annu Rev Biophys Biomol Struct* 1992, 21:77–93.

A relatively short summary of the state of ribosome crystallography at the end of 1991.

9. •• PICKING W, PICKING WD, HARDESTY B: **The Use of Synthetic tRNAs as Probes for Examining Nascent Peptide on *E. coli* Ribosome.** *Biochemie* 1991, 73:1101–1107.

The authors document an elegant approach for following the progression of the nascent chain, by attaching fluorescent and/or immunological probes to various positions on it.

10. • VON BÖHLEN K, MAKOWSKI I, HANSEN HAS, BARTELS H, BERKOVITCH-YELLIN Z, ZAYTZEV-BASHAN A, MEYER S, PAULKE C, FRANCESCHI F, YONATH A: **Characterization and Preliminary Attempts for Derivatization of Crystals of Large Ribosomal Subunits from *Haloarcula marismortui*, Diffracting to 3 Å Resolution.** *J Mol Biol* 1991, 222:11–15.

The first documentation of crystals of intact ribosomal subunits, diffracting to almost atomic resolution.

11. YONATH A, LEONARD KR, WITTMANN HG: **A Tunnel in the Large Ribosomal Subunit Revealed by Three-Dimensional Image Reconstruction.** *Science* 1987, 236:813–817.

12. ARAD T, PIEFKE J, WEINSTEIN S, GEWITZ HS, YONATH A, WITTMANN HG: **Three-Dimensional Image Reconstruction from Ordered Arrays of 70S Ribosomes.** *Biochimie* 1987, 69:1001–1006.

13. WITTMANN HG: **Architecture of Prokaryotic Ribosomes.** *Annu Rev Biochem* 1983, 52:35–65.

14. •• PENCZEK P, RADERMACHER M, FRANK J: **Three-Dimensional Reconstruction of Single Particle Embedded in Ice.** *Ultramicroscopy* 1992, 40:33–53.

The authors describe and explain clearly the random-conical reconstruction procedure, using the ribosome as an example.

15. VOGEL RH: *EMBL Annual Report.* Heidelberg: European Molecular Biology Laboratory; 1983.

16. MILLIGAN RA, UNWIN PNT: **Location of the Exit Channel for Nascent Proteins in 80S Ribosomes.** *Nature* 1986, 319:693–696.

17. • EISENSTEIN M, SHARON R, BERKOVITCH-YELLIN Z, GEWITZ HS, WEINSTEIN S, PEBAY-PEYROULA E, ROTH M, YONATH A: **The Interplay between X-Ray Crystallography, Neutron Diffraction, Image Reconstruction, Organo-Metallic Chemistry and Biochemistry in Structural Studies of Ribosomes.** *Biochemie* 1991, 73:879–886.

The authors document their integrated efforts in employing a variety of techniques for elucidating a reliable model of the ribosome.

18. RADERMACHER M, WAGENKNECHT T, VERSCHOOR A, FRANK J: **Three-Dimensional Structure of the Large Ribosomal Subunit from *E. coli*.** *EMBO J* 1987, 6:1107–1114.

19. •• FRANK J, PENCZEK P, GRASSUCCI R, SRIVASTAVA S: **Three-Dimensional Reconstruction of the 70S *E. coli* Ribosome in Ice: the Distribution of Ribosomal RNA.** *J Cell Biol* 1991, 115:597–605.

A description of a model for the 70S ribosome obtained by the semi-objective random-conical reconstruction procedure from unstained sin-

gle particles embedded in amorphous ice, showing the envelope of the particle, its internal free space and the distribution of rRNA-rich regions. Although the interpretation of this model is based on the comparisons with subjectively determined features, some sound propositions are made, and the potential of this approach is clearly demonstrated.

20. • NAGANO K, TAKAGI H, HAREL M: **The Side by Side Model of Two tRNA Molecules Allowing the α-Helical Conformation of the Nascent Polypeptide during the Ribosomal Transpeptidation.** *Biochemie* 1991, 73:947–960.

Attempts to include an impressive amount of data for the stereochemical explanation for the negatively linked allosteric interactions between the A and PE sites.

21. VALEGARD K, LILJAS L, FRIBORG K, UNGE T: **The Three-Dimensional Structure of the Bacterial Virus MS2.** *Nature* 1990, 345:36–41.

22. • LARSEN N: **Higher Order Interactions in 23S rRNA.** *Proc Natl Acad Sci USA* 1992, 89:5044–5048.

This paper shows a phylogenetic analysis of possible tertiary interactions of rRNA based on base pairing and conservation. This paper is also recommended for tabulating all chemical and enzymatic accessibilities on the ribosome.

23. MITCHELL P, OSSWALD M, BRIMACOMBE R: **Identification of Intermolecular RNA Cross-Links at the Subunit Interface of the *E. coli* Ribosome.** *Biochemistry* 1992, 31:3004–3011.

24. • HUETTENHOFER A, NOLLER HF: **Hydroxyl Radical Cleavage of tRNA in the Ribosomal P Site.** *Proc Natl Acad Sci U S A* 1992, 89:7851–7855.

An elegant approach leading to the conclusion that the P site of tRNA is located between the two ribosomal subunits, rather than on the surface of the ribosome.

25. WEINSTEIN S, JAHN W, LASCHEVER M, ARAD T, TICHELAAR W, HAIDER M, GLOTZ C, BOECKH T, BERKOVITCH-YELLIN Z, FRANCESCHI F, YONATH A: **Derivatization of Ribosomes and of tRNA with an Undecagold Cluster: Crystallographic and Functional Studies.** *J Cryst Growth* 1992, 122:286–292.

26. SRIVASTAVA S, VERSCHOOR A, FRANK J: **Eukaryotic Initiation Factor 3 Does Not Prevent Association through Physical Blockage of the Ribosomal Subunit–Subunit Interface.** *J Mol Biol* 1992, 226:301–304.

27. MANDIYAN V, TUMMINIA SJ, WALL JS, HAINFELD JF, BOUBLIK M: **Assembly of the *E. coli* 30S Ribosomal Subunit Reveals Protein-Dependent Folding of the 16S rRNA Domains.** *Proc Natl Acad Sci USA* 1991, 88:8174–8178.

28. KUHLBRANDT W, UNWIN PNT: **Distribution of RNA and Proteins in Crystalline Eukaryotic Ribosomes.** *J Mol Biol* 1982, 156:611–617.

29. • LIM V, VENCLOVAS C, SPIRIN A, BRIMACOMBE R, MITCHELL P, MÜLLER F: **How Are tRNAs and mRNA Arranged in the Ribosome? An Attempt to Correlate the Stereochemistry of the tRNA–mRNA Interaction with Constraints Imposed by the Ribosomal Topography.** *Nucleic Acid Res* 1992, 20:2627–2637.

Despite its rather premature nature, this publication is recommended as an intellectual exercise, in which the authors try to merge an impressive amount of data of varying quality.

30. FRANCESCHI F, WEINSTEIN S, EVERS U, ARNDT E, JAHN W, HANSEN H, VON BÖHLEN K, BERKOVITCH-YELLIN Z, EISENSTEIN M, AGMON I *ET AL*: **Towards Atomic Resolution of Prokaryotic Ribosomes: Crystallographic, Genetic and Biochemical Studies.** In *The Translation Apparatus.* Edited by Nierhaus K, Franceschi F, Subramanian E, Erdmann V, Wittmann-Liebold B. New York: Plenum Press; 1993.

31. HARAUZ G, FLANNIGAN D: **Electron Microscopical Projections of the Small Ribosomal Subunits from *Thermomyces lanuginosus.*** *Biochem Biophys Acta* 1992, 1130:289–296.

32. HARAUZ G: **Electron Microscopical Projections of the Large Ribosomal Subunit from *Thermomyces lanuginosus.*** *Biochem Biophys Acta* 1992, 1132:58–66.

33. KYLE KM, HARAUZ G: **Structure of Ribosomal Subunits from *Saccharomyces cerevisiae.*** *Micron Microsc Acta* 1992, 23:273–286.

A Yonath and Z Berkovitch-Yellin, Department of Structural Biology, Weizmann Institute, Rehovot, 76100, Israel.