Structural aspects of ribonucleoprotein interactions in ribosomes

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Impressive progress has been made towards the determination of the molecular structure of the ribosome and the illumination of its functional features. The highlights include reaching almost atomic resolution in crystallography of intact ribosomal particles and isolated ribosomal components and the assignments of several functional centers.

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Introduction

The increasing sophistication in instrumentation, the implementation of powerful genetic techniques and the use of ribosomes of halophilic and thermophilic bacteria have resulted in major accomplishments which could not be foreseen earlier. Most of the recent studies are reviewed in three volumes of *Biochemie*, two of which are dedicated to the late HG Wittmann and one to A Spirin's retirement, as well as in the proceedings of the International Conference on 'The Translational Apparatus' held in Berlin in November 1992, which below is called 'Ribosome-92'. We have selected a subset of studies which, in our opinion, reflects the characteristic trends in the past year's structural research in protein–RNA interactions. Studies on ribosomes as a cellular assemblage will be addressed in the next issue of *Current Opinion in Structural Biology*.

Turning point in ribosomal crystallography

Intact particles

Perhaps the most striking and unexpected achievement is the growth of crystals of 50S ribosomal subunits from *Haloarcula marismortui*, which diffract well to almost atomic resolution, 2.9Å (Fig. 1) [1], despite the apparent heterogeneity in the genes encoding ribosomal RNA (rRNA) of these ribosomes (S Mylvaganam, PP Dennis, abstract 232, Ribosome-92) [2]. Reasonable expectations for phasing the crystallographic data are based on: recent chemical improvements of a potential heavy-atom derivative, the undecagold cluster, aimed at minimizing its steric freedom in the crystal (S Weinstein *et al.*, abstract 142, Ribosome-92); the advanced stage of the sequencing [3]; the genetic insertion of exposed cysteines on the surface of these subunits (E Arndt, C Paulke, F Franceschi, abstract 86, Ribosome-92); and the crystallization of particles lacking a few proteins [4•].

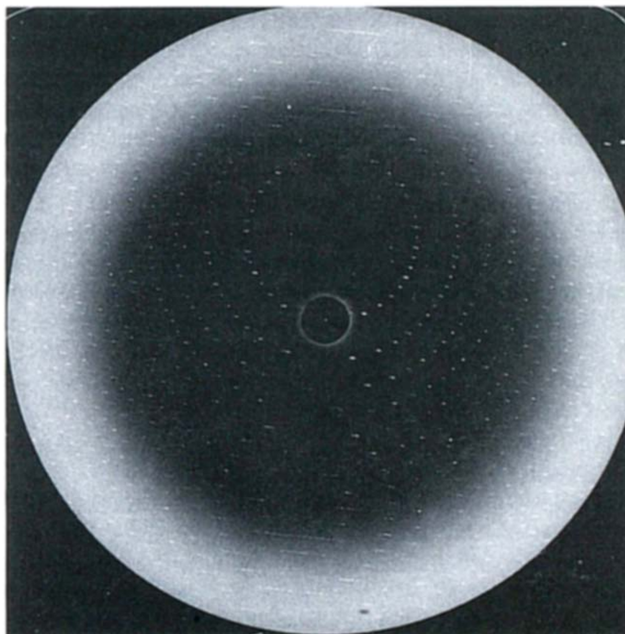

Fig. 1. A rotation photograph of a crystal of 50S subunits from *H. marismortui* grown as described in [1]. The pattern was obtained at 90 K at Station F1/CHESS, operating at 5.3 GeV and 50 mA. Crystal to film distance = 220 mm; collimator = 0.1 mm; wavelength = 0.9091 Å.

The halophilic ribosomes present an attractive system for investigating protein–RNA interactions because they function at salt concentrations which usually cause the dissociation of nucleoprotein assemblages. It is noteworthy that despite their 'exotic' properties, the halophilic ribo-

Abbreviations

r-protein—ribosomal protein; **rRNA**—ribosomal RNA. The following letters represent the bacterial source when they are placed to the left of the protein identification number: E—*Escherichia coli*; B—*Bacillus stearothermophilus*; T—*Thermus thermophilus*; H—*Haloarcula marismortui*.

somes show reasonable structural similarity with those of eubacteria and eukaryotes [5••]. A complex isolated from 50S subunits of *H. marismortui* is particularly illuminating. It contains a fragment of 23S RNA and protein HL1 (where H identifies the bacterial source, and L1 is the protein identification number, i.e. protein 1 from the large ribosome subunit; see Abbreviations), which are interchangeable with their *E. coli* counterparts (U Evers *et al.*, abstract 123, Ribosome-92). The halophilic ribosomes are also optimal candidates for evolution and comparative studies [5••] and for elucidating fine factors governing the translational activity [6].

Isolated components

The difficulties inherent in crystallizing of isolated components of *E. coli* ribosomes, and the observations that some of them loose their *in situ* conformation upon isolation, led to the assumption that the conformation of all ribosomal components is dictated by its supporting environment. The recent 'quantum jump' in the crystallization of isolated thermophilic rRNA and ribosomal proteins (r-proteins) suggests that components possessing intrinsic characteristic fold may crystallize in isolation, provided that they have not been damaged during their preparation, a condition fulfilled better when thermophilic ribosomes are used. It remains to be seen whether the structures of the isolated ribosomal components bear resemblance to their *in situ* conformation.

For over two decades, the crystallization of isolated, fragmented or complexed rRNA molecules gave rise to considerable frustration, as repeating efforts yielded crystals useful only for preliminary analysis [7••,8]. The determination of the 2.6 Å resolution structure of a synthetic ribonucleic oligomer of 12 base pairs, which imitates helix A of 5S rRNA is therefore remarkable (VA Erdmann *et al.*, abstract 4.4, Ribosome-92) and gives rise to further expectation for structural studies on rRNA.

Until 1992, the structures of only one ribosomal protein (BL30) and a fragment of another (EL7/12) were known, whereas alot more are being investigated this year. The structure of r-protein BS5 has been published [9], and other r-proteins from two sources have been analyzed: from *Bacillus stearothermophilus*, BL6 (at 3.2 Å), BL9 and BL17 (V Ramakrishnan, S White, abstract 4.1, Ribosome-92), and from *Thermus thermophilus* TL1 and TS6 [10•]. The former has almost been determined at 3.0 Å, whereas the latter's 2.3 Å electron-density map was only tentatively interpreted because of the lack of complete sequence (E Brazhnikov *et al.*, abstract 91, Ribosome-92). It is noteworthy that the latter barrier is soon to be removed as the *T. thermophilus* r-proteins are currently being sequenced (T Choli *et al.*, abstract 93, Ribosome-92). In order to prepare amounts sufficient for crystallographic studies, in some cases, advantage was taken of the convenience of overexpressing thermophilic r-proteins in *E. coli* efficient cellular systems [11,12], in contrast to the obvious difficulties in overproducing *E. coli* r-proteins in *E. coli* cells.

Protein BS5 is involved in binding aminoacyl-tRNA. Its conformation is different from the common fold of BL30 and EL7/12. Analysis of several mutants led to plausible suggestions for the specific interactions and allosteric effects associated with this protein. The determination of its structure may lead to further structural investigations, as it shares sequence identity with protein S2 in rat (Arg-Ser-Ser-Phe), which has also been detected in nucleoproteins associated with chromatin and preribosomal particles [13].

Structural aspects of the catalytic activities of ribosomal RNA

Until recently, it was assumed that the catalytic activities of the ribosome are performed solely by proteins, and that the rRNA molecules have a more passive role in transferring genetic information, providing the scaffold for the ribosome and involve in some undefined manner in several ribosomal functions.

The demonstration of the catalytic abilities of RNA in several biological systems, the accumulation of data showing substantial conservation in some rRNA regions, and recent site-directed mutagenesis experiments which uncovered specific roles for rRNA, have stimulated the design of experiments challenging the above dogma. Those that might contribute to structural analyses or are based on structural consideration are highlighted here.

GTPase activity

The GTPase center is associated with two highly conserved components: a stretch of 23S RNA and the protein L11. Although the latter promotes GTPase activity, cells lacking it are viable and their ribosomes do not undergo major conformational changes, and yield crystals similar to those derived from the intact cells [7••]. In addition, extensive mutagenesis in the RNA stretch indicated fold-dependent recognition [14•], raising the intriguing question why are both components of the GTPase center so highly conserved when most of the bases in the RNA stretch are not essential for recognition and how is the functional activity preserved even when protein L11 is totally removed.

A deeper understanding of the GTPase center may be obtained by investigating cellular activities associated with it, such as the production of guanosine 3′5′-bispyrophosphate, which is regulated by the availability of aminoacyl-tRNA. As RelA, the protein responsible for this reaction, was recently overexpressed [15], its structural investigation has become feasible. Interestingly, the *E. coli* protein binds well to *T. thermophilus* ribosomes, paving the way for their co-crystallization in a fashion similar to other 70S complexes [7••].

Peptidyl transferase activity

The suitability of the thermophilic bacteria for providing solid results was clearly demonstrated in exciting functional studies, which, when performed with *E. coli*, gave

rise to ambiguous findings. Thus, only 20–40 % of the original activity of a model assay for peptidyl transferase was retained in *E. coli* 50S subunits from which most of the protein mass was removed, whereas the *T. acuarticus* ribosomes exhibited 80 % of their original activity after the same treatment [16••]. As this model assay was inhibited by some antibiotics which inhibit the peptidyl transferase activity, it was assumed that it resembles the natural peptide-bond formation. This experimental system is still not fully determined, as 5 % of the total protein mass remained bound to the rRNA. These may account for stoichiometric amounts of a few r-proteins and/or their fragments. Nevertheless, these findings indicate the direct participation of rRNA in the peptidyl transferase function.

Modelling attempts

As the enzymatic role of rRNA became evident [17••], its specific structural features became the topic of many studies. For example, the large number of existing experimental procedures have been augmented by the use of Pb(II) to induce hydrolysis of rRNA–r-protein complexes at interhelical and loop regions [18,19]. In the absence of experimentally determined molecular models, sophisticated computing procedures, which combine results of chemical, physical and functional experiments, have been employed. Protocols based on distance geometry [20] or energy minimization [21•] were used for modelling 16S RNA. Although the comparison between the two models suffers from the lack of a common base, major differences in the placements of the structural domains are clearly observed. Both models are compatible in shape with that emerging from electron microscopy and in volume with that reported in preliminary crystallographic studies. It is noteworthy that both were compared with models based on experimental results, some of which have undergone major rearrangements during the course of these computations [22•].

Incorporation of biochemical information with computational results led to the suggestion that 5S RNA of *E. coli* and *Xenopus laevis* are built of three distinct domains, not connected by tertiary interactions. On the basis of their shape similarity, it was argued that these models represent the universal structure of 5S RNA [19,23]. The rRNA chains were also modelled according to a radically different algorithm, namely extracting secondary and tertiary structural elements from aligned sequences of a large number of phylogenetically diverse sources, followed by comparisons with experimental results [24•,25•].

The assembly process

With the aim of detecting the intermediate steps during *in vitro* folding of rRNA, the appearance of folding domains was monitored by visual inspection of their morphology (using traditional electron microscopy) and the determination of their mass and radius of gyration [26]. The uncertainties in monitoring dynamic processes by electron microscopy cannot be overlooked, as it was shown, using tunneling electron microscopy, that natural populations of isolated rRNA contain more than one morphology [27]. Despite this and the lack of detail, these physical measurements indicated significant reorganization of 16S RNA during its *in vitro* assembly, in accord with the considerable differences in the Pb(II) cleavage patterns of 5S RNA from *E. coli* induced by binding protein EL18 [18].

Do these patterns reflect the *in vivo* assembly of ribosomes? Recent studies which questioned the self-assembly of *E. coli* ribosomes indicate, although not conclusively, the involvement of a chaperonine (the product of the *dna-k* gene), in the *in vivo* ribosome assembly. Using two thermosensitive mutants of this gene product, ribosomal particles with abnormal sedimentation constants were accumulated. Although still not characterized, their mere exsistance suggests the involvement of non-ribosomal cell products in the assembly process (JH Alix, J Guerin, abstract 4.5, Ribosome-92).

Probing rRNA function using antisense DNA

Probing exposed single-stranded rRNA using cDNA oligomers has recently gained considerable popularity in mapping the ribosome surface. In studying the initiation step, it was found that AUG- and dATG-containing oligomers promoted the non-enzymatic binding of f_{Met}-tRNA with similar properties, and formed initiation complexes which are fully reactive with puromycin [28]. In parallel, to illuminate the formation of the initiation complex, the competition between a variety of cDNA oligomers and poly(U), poly(AUG), $tRNA^{Phe}$, $tRNA^{fMet}$ and the initiation factors was monitored [29].

In principle, cDNA may be used for the derivatization of ribosomal crystals, as a heavy atom may be attached to them before their hybridization and subsequent co-crystallization. DNA oligomers that target naturally exposed single-strand rRNA regions and those that become exposed by the removal of r-proteins, were synthesized but neither led to stoichiometric binding, a property which is seldomly monitored in functional studies. The non-quantitative binding may result from the inherent conformational flexibility of these rRNA stretches, which has also been detected in the α-sarcin/ricin domain by probing the universally conserved dodecamer in the α-sarcine loop of *E. coli* [30••]. The α-sarcin/ricin domain is involved in aminoacyl-tRNA and elongation factor binding as well as GTPase activity. In eukaryotic cells, it is cleaved by α-sarcin and ricin at adjacent nucleotides. As the structural elements that these two inhibitors recognize are not only different, but incompatible, it was suggested that the factor and the inhibitors induce the conformational changes needed for their binding in this rRNA stretch [30••].

Antisense DNA has proved instrumental in monitoring dispositions of normal and altered *E. coli* 16S rRNA [31].

It has also been used for the accurate identification of cross-linked rRNA segments in an experiment which led to major revision of the model of the 16S rRNA chain [22•]. cDNA probing experiments originated a controversy, concerning inter-protein distances. These, combined with photo-affinity labeling, were used for probing two regions of 16S RNA from *E. coli*. Comparisons of the distances determined this way with those obtained by triangulation of deuterated proteins pointed to a discrepancy of ~65 Å (B Cooperman, personal communication).

It is conceivable that the contradicting results described above stem from the inherent instability and flexibility of 30S subunits from *E. coli*, as found in biochemical experiments and crystallization efforts [7••]. Because, in general, the 50S subunit is more rigid and stable, it is expected that more conclusive information will emerge from the massive attempts at positioning its r-proteins, using contrast variation of protonated r-proteins reconstituted into a deuterated ribosomal matrix [32].

Conclusions and perspectives

The large volume of experimental procedures and results discussed here manifest the growing interest in the ribosome and the drive to reveal its extremely high inherent complexity. Even the severe discrepancies revealed recently using different methods are, in a way, encouraging, as they are bound to lead to more critical experimental approaches, dictate the use of conformationally uniform populations, and the design of reliable controls. At the same time, this review clearly demonstrates that structural information of a much higher detail is essential even for the initial steps in understanding the forces holding the ribosome together and controlling its function.

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