1. Introduction

Protein accounts for roughly half the dry mass of all organisms and, in large measure, the morphologies and physiologies of organisms are macroscopic manifestations of the properties of the proteins they synthesize for themselves. This issue of the Philosophical Transactions of the Royal Society consists of a series of articles that will provide the reader with an overview of what is known today about the way the information encoded in the nucleotide sequences of genomes is translated into the amino acid sequences of proteins. Its focus is the ribosome, the enzyme that catalyses that transformation in all organisms, because the reputation of the individual whose scholarly activities this issue celebrates, Venki Ramakrishnan, derives from the many memorable contributions he has made to our understanding of its structure and function.

Scientifically, the history of the ribosome is quite old (see [1,2]). The word ‘ribosome’ (ribose-containing body) was coined nearly 60 years ago, after it had been discovered that the cytoplasms of virtually all cells contain large numbers of particles that are rich in RNA, but before the role of these particles in protein synthesis had been properly understood [3]. By the mid-1960s, the following facts had been established: (i) that the ribosome is a polymerase-like enzyme capable of catalysing the synthesis of proteins of any sequence whatever (more or less), (ii) that it consists of two, non-equivalent ribonucleoprotein subunits, one of which is about twice the mass of the other, (iii) that its substrates are aminoacyl tRNAs, (iv) that the large ribosomal subunit catalyses peptide bond formation, and (v) the sequences of the proteins it makes are determined by the interactions between aminoacyl tRNA anticodons and messenger RNA codons that are mediated by the small subunit.

Even though many aspects of the biochemistry of protein synthesis remained to be clarified, from the late 1960s on, the question became not so much what the ribosome does, but how it does it. A lot of progress was made, but as time went on, it became increasingly obvious that nothing decisive would emerge until and unless atomic-resolution structures were obtained for at least one species of ribosome. Thus, the publication in 2000 of atomic-resolution crystal structures for both subunits [4,5] was a turning point in the history of ribosome research. Not only did these first structures rationalize vast amounts of existing data, they made it possible to do experiments that would test inferences about the relationship between ribosome structure and function far more effectively than any that had been done before. Thus, no one was surprised when the 2009 Nobel Prize for Chemistry was awarded to Venki Ramakrishnan, Thomas Steitz and Ada Yonath, the three individuals who had done the most to make this happy turn of events possible. Since 2000, crystal structures have been obtained for ribosomes and ribosomal subunits from a wide variety of organisms, with and without tRNAs, mRNAs, protein factors and a host of antibiotic inhibitors bound. Virtually all of the research being done today on the ribosomal phase of protein synthesis is structure based.

All the above notwithstanding, it is important to realize that the contributions electron microscopists have made to our understanding of protein synthesis are every bit as significant as those made by crystallographers. Electron microscopy (EM) experiments done in the 1950s played a critical role in the discovery of the ribosome, and prior to approximately 1995, most of what was known about three-dimensional organization of the ribosome was obtained.
from EM images of negatively stained ribosomes (e.g. [6]). Furthermore, from the mid-1990s on, cryo-EM data began being used to produce models for the ribosome and its two subunits at ever increasing resolutions. These less than atomic-resolution structures led to important discoveries about the way the conformation of the ribosome changes during protein synthesis, and the manner in which it interacts with tRNAs and factors (e.g. [7]). Finally, there is a revolution underway today in structural biology that is being driven by technical advances in EM, and it is likely that cryo-EM will soon emerge as the method of choice for investigating ribosome structure. It has already provided atomic-resolution structures for several ribosome-related complexes that would have been incredibly difficult to address crystallographically because samples are so difficult to prepare (e.g. [8–10]).

This issue of the Philosophical Transactions begins with an essay by Harry Noller [11] on the history of the ribosome field that describes how the ribosome community became convinced that the ribosome is a ribozyme, which is to say an enzyme that depends on RNA for its catalytic power, rather than protein. By 1990, if not before, this had become the consensus in the field, in no small measure because of work done by Noller and his colleagues. What the atomic-resolution structures that began appearing in 2000 added to this story was irrefutable evidence that the ribosome is indeed an RNA enzyme.

Messenger RNA-directed protein synthesis has three stages: initiation, elongation and termination. The end products of initiation are complexes consisting of a ribosome bound to an mRNA at an appropriate start codon, which is invariably an AUG, with an initiator tRNA attached that is aminoacylated with a methionine residue. During elongation, the ribosome executes the same sequence of steps over and over again, each time adding an amino acid to the C-terminal end of a nascent peptide chain, and advancing down its mRNA by one codon. Elongation ends and termination begins when a stop codon is encountered. The now complete protein is released both from the ribosome and from the tRNA to which it is covalently bonded, the remaining ribosome–mRNA complex is (usually) broken up and the subunits of the ribosome returned to the pool.

Initiation is the topic of the three articles that follow Noller’s essay. Unlike the biochemistry of elongation, which is almost exactly the same in all organisms, the biochemistry of initiation varies a lot, especially between kingdoms. Compared to what happens in eubacteria, initiation in eukaryotes is baroque in its complexity, and all of the articles on initiation in this issue deal with its eukaryotic variant. One reason for this emphasis is that initiation is an important control point for gene expression in eukaryotes. Nenad Ban and Christopher Aylett’s [12] article provides a review of what has been learned about both the biochemistry and structural biology of eukaryotic initiation. Jamie Cate’s [13] paper focuses on a single initiation factor, eIF3, which in mammals is an enormous 13-subunit 800 kDa protein complex. It is essential for the binding of initiator tRNAs to partially assembled initiation complexes. The third article in this group, which was written by Jody Puglisi and co-workers [14], describes a form of initiation that enables eukaryotic ribosomes to bypass most of its normal complexity. It is triggered by the presence of internal ribosome entry site (IRES) sequences in mRNAs. This article is the only one in this issue that emphasizes kinetic data obtained using single-molecule methods, which are playing an increasingly important role in ribosome research.

The elongation phase of protein synthesis is discussed in the next three articles. The first, which is by Joachim Frank [15], describes what has been learned about the conformational changes that accompany the elongation cycle, concentrating particularly on what single-particle EM has contributed. Among the many advantages of EM is that it can, in principle, provide atomic-resolution descriptions of all of conformational states represented in a population of macromolecules, i.e. a ribosome preparation that is engaged in elongation. The paper that follows, which was written by Marina Rodnina et al. [16], focuses on the interactions that ensure the fidelity of translation. The emphasis is on kinetic experiments, both bulk and single-molecule, but some structural correlates are also discussed. The third article in this series is a contribution by Måns Ehrenberg et al. [17] that comments on the significance of two crystal structures relevant to the fidelity problem that appear to contradict each other. Here too the argument is guided by kinetic considerations.

Protein synthesis does not always proceed the way textbooks prescribe; it sometimes fails. For example, either spontaneous or enzymatically catalysed hydrolysis can truncate an mRNA, removing the stop codon that any ribosome attempting to translate it must encounter if protein synthesis is to terminate properly. A variety of systems have been identified in both prokaryotes and eukaryotes that catalyse the recovery of ribosomes from the stalled ribosome-mRNA complexes that result when defective mRNAs are translated. They are described in the contribution by Rachel Green and Alan Buskirk [18].

It is important to realize that the ribosome is far from being perfectly conserved across species boundaries. The ribosomes from different eubacterial species are all about the same, and they differ somewhat from those found in archaeal species, but within the Archea there does not seem to be much variation. It is the eukaryotes that are outliers in this regard. The RNAs in their ribosomes are much bigger than those found in prokaryotic ribosomes, and their ribosomes invariably contain a larger number of proteins. In addition, the higher the eukaryote, the bigger and more complicated its ribosomes. On top of that, mitochondria contain ribosomes that are distinctly different from cytoplasmic ribosomes, and the higher the eukaryote, the stranger and more deviant its mitochondrial ribosomes. What is conserved in all of these particles is the structure of their RNA components in the neighbourhood of the sites that constitute their functional hearts, i.e. the site on the large ribosomal subunit where peptide bonds form, and the site on the small ribosomal subunit where codon/anticodon interactions occur. The article by Marat Yusupov and Gulnara Yusupova [19] discusses the work they have done on the crystal structure of the 80S ribosome from yeast, and on the complexes this particle forms with antibiotics that block its function.

The issue closes with an article by Jamie Williamson and Joseph Davis [20] that discusses with the way ribosomes are assembled in prokaryotes. In 1968, it was demonstrated that the small ribosomal subunit from Escherichia coli can be reconstituted in vitro from mixtures of its components [21], and a few years later the same was shown for the large ribosomal subunit from E. coli [22]. Experiments done subsequently indicate that the order in which proteins add to assembling
Ribosomes in vivo is similar to the order in which they add in vitro. However, both in vitro processes are much too slow to explain the rate at which ribosomes are produced in vivo, and to the best of our knowledge, no one has ever managed to reconstitute a eukaryotic ribosome. Thus it has long been obvious that the in vivo process must be catalysed. A large number of protein factors have been identified in bacteria that facilitate ribosome assembly, and hundreds of factors have been discovered that play the same role in eukaryotes.

Ribosome assembly is a burgeoning area in molecular and cell biology, and Williamson and Davis’s article summarizes the state of that field in bacteria, which their innovative experiments have done so much to illuminate.

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References